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New Insights into Graft-Versus-Host Disease and Graft Rejection

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Abstract

Allogeneic transplantation of foreign organs or tissues has lifesaving potential, but can lead to serious complications. After solid organ transplantation, immune-mediated rejection mandates the use of prolonged global immunosuppression and limits the life span of transplanted allografts. After bone marrow transplantation, donor-derived immune cells can trigger life-threatening graft-versus-host disease. T cells are central mediators of alloimmune complications and the target of most existing therapeutic interventions. We review recent progress in identifying multiple cell types in addition to T cells and new molecular pathways that regulate pathogenic alloreactivity. Key discoveries include the cellular subsets that function as potential sources of alloantigens, the cross talk of innate lymphoid cells with damaged epithelia and with the recipient microbiome, the impact of the alarmin interleukin-33 on alloreactivity, and the role of Notch ligands expressed by fibroblastic stromal cells in alloimmunity. While refining our understanding of transplantation immunobiology, these findings identify new therapeutic targets and new areas of investigation.

INTRODUCTION

In patients with failing organs or tissues, modern medical practice relies on transplantation procedures as an important clinical resource with lifesaving potential. Transplanted organs and tissues are defined as allogeneic when harvested from donors who are genetically nonidentical to the recipients, which is the case for the vast majority of donor-recipient pairs (with the rare exception of identical twins). Immune responses directed against foreign tissue antigens (or alloantigens) mediate key unique complications of allogeneic transplantation. These complications include graft rejection, which occurs after transplantation of allogeneic organs (e.g., heart, lung, liver, small intestine, or kidney), tissues (e.g., bone marrow or pancreatic islets), or composite grafts (e.g., limb or facial structures). Conversely, graft-versus-host disease (GVHD) can occur after transplantation of grafts containing large amounts of donor immune cells, most commonly bone marrow, mobilized peripheral blood, or cord blood. Graft rejection involves immune reactivity of the recipient against transplanted allografts, while GVHD is triggered by the reactivity of donor-derived immune cells against allogeneic recipient tissues.

Clinical Importance of Alloimmune Complications

An improved understanding of the cellular and molecular mechanisms mediating graft rejection and GVHD is essential, because both complications are major medical problems that cause significant morbidity and mortality, limiting the success of transplantation procedures. Historically, T cells have been considered the dominant cellular subset mediating graft rejection and GVHD, and most efforts to prevent or treat these complications have focused on interventions that target T cell reactivity. To prevent graft rejection, transplantation recipients routinely receive lifelong immunosuppression with calcineurin inhibitors, with or without additional agents. Although this strategy supported major progress in modern transplantation medicine, especially in controlling acute rejection, it is linked to significant problems, including drug toxicity, increased risks of opportunistic infections, and an increased incidence of malignancy (posttransplant lymphoproliferative disorders and other cancers). In addition, current immunosuppressive regimens insufficiently control chronic rejection, a distinct immunopathological syndrome leading to steady attrition in the viability of transplanted allografts over time. As a result, many patients experience a need for retransplantation, which can be medically challenging, limited by low organ availability, and particularly problematic in recipients of life-sustaining allografts (e.g., heart or lung). B lineage cells as well as other non–T cells are thought to play a major role in chronic rejection and chronic GVHD. As an alternative to immunosuppression based on calcineurin inhibitors, preclinical and early clinical research efforts have attempted to achieve states of true tolerance to the transplanted organ that allow allograft survival in the absence of lifelong immunosuppression. Strategies to induce tolerance currently under investigation include targeting costimulatory pathways and establishing allogeneic hematopoietic chimerism via nonmyeloablative hematopoietic cell transplantation, followed by organ transplantation. Although promising in principle, the full real-life clinical potential of these strategies remains to be established.

Risks and Benefits of Alloimmunity in Allogeneic Hematopoietic Cell Transplantation

Alloimmune rejection is uniformly detrimental after solid organ transplantation, but a delicate balance between immune complications and benefits needs to be considered after allogeneic bone marrow, cord blood, or mobilized peripheral blood transplantation [jointly referred to here as allogeneic hematopoietic cell transplantation (allo-HCT)]. In this setting, graft rejection is relatively

rare, except in patients with preexisting autoimmune or alloimmune reactivity. In contrast, GVHD is the prevailing clinical problem, with the potential to induce life-threatening, immune-mediated damage to target organs, such as the gut, skin, liver, thymus, and lung. Similar to chronic allograft rejection, chronic GVHD is a distinct entity that affects a large fraction of patients, can cause major lifelong morbidity, and remains poorly responsive to current treatments. In parallel to these complications, however, transplanted T cells and other immune cells induce beneficial anticancer effects referred to as graft-versus-tumor (GVT) activity. Because the majority of allo-HCT procedures are performed for patients with hematological malignancies (e.g., leukemias or lymphomas), and only a minority for benign disorders, it is essential to identify strategies to control GVHD that still preserve potent GVT activity. Another important problem unique to allo-HCT is the occurrence of delayed immune reconstitution and poor immune function, a prevalent problem often associated with chronic GVHD.

Evolving Concepts in the Pathogenesis of Alloimmune Injury

To understand the pathogenesis of alloimmune complications of transplantation, it is useful to consider elements borrowed from the immune system's responses to conventional antigens, as well as features that are unique to the artificial conditions induced by transplantation. Unlike most conventional exogenous antigens, alloantigens are broadly expressed either in the transplanted allograft (rejection) or in the allo-HCT recipient (GVHD), and they are persistent in the sense that they can never be completely eliminated. In this regard, alloimmunity shares important features with autoimmunity and chronic viral infections. Nevertheless, much remains to be learned about the molecular nature, tissue distribution, and cellular presentation of alloantigens. Another determinant of alloimmune reactivity is the delivery of context-dependent innate signals during the priming of the immune response. For example, ischemic injury during the harvest and processing of the allograft exerts major effects on the induction of alloimmunity via exposure to damage-associated molecular patterns, for example, DNA, RNA and other molecules released by damaged cells. Microbe-associated molecular patterns have also been reported to influence alloreactivity, with a recent focus on the role played by the microbiome in both solid organ rejection and GVHD. During allo-HCT, damage to recipient tissues is caused by myeloablative or, to a lesser extent, nonmyeloablative conditioning regimens involving total body irradiation or chemotherapy. In turn, tissue damage generates inflammatory signals that enhance the adaptive immune response to recipient alloantigens. Another key feature of GVHD pathogenesis is the preeminence of the gut, as both a site of immune priming and a target of the disease, with the potential for a self-reinforcing pathogenic loop. Although basic and clinical researchers have been focusing on this question for years, recent work has brought new insights into a complex cross talk among innate lymphoid cells (ILCs), intestinal epithelial cells, microbiota, and T cells that regulates the onset of GVHD. Finally, nonhematopoietic cells in secondary lymphoid organs (SLOs) and other tissues have recently come into focus as a source of alarmins [e.g., interleukin (IL)-33] and innate signals (e.g., Notch ligands) that control key aspects of alloimmunity during graft rejection and GVHD. Thus, although there is no doubt that T cells are essential in alloimmunity, T cell function is influenced by interactions with a complex microenvironment and by the unique conditions induced by transplantation procedures.

In this review, we discuss recent advances in the field that have identified new cellular subsets and new molecular pathways that regulate alloimmunity, with an impact on both graft rejection and GVHD. To organize our review, we focus on the identification of key cellular subsets other than T cells and other than professional antigen-presenting cells (APCs) that recently emerged as critical players of alloimmunity in graft rejection and GVHD, including nontraditional cellular

sources of alloantigens, ILCs, microbiota, intestinal epithelial cells, and nonhematopoietic stromal cells in SLOs. Although they are important, B lineage cells are not discussed extensively here due to space limitations. Progress across the field provides us with a refined understanding of the complex processes underlying alloimmunity, while identifying new essential molecular pathways, potential therapeutic targets, and areas of future investigation.

ANTIGEN PRESENTATION IN GRAFT-VERSUS-HOST DISEASE AND ALLOGRAFT REJECTION

After allogeneic transplantation, APCs stimulate alloreactive T cells through the provision of alloantigens and costimulatory signals. In recent years, intense scrutiny has been devoted to define the mechanisms and the cellular subsets involved in alloantigen presentation (**Figure 1**). Importantly, both hematopoietic and nonhematopoietic APCs can be activated by innate immune stimuli that are associated with the transplantation procedure, including ischemic damage, tissue damage secondary to myeloablative conditioning, and exposure to signals from the microbiome. In a positive feedback loop, signals from activated alloreactive T cells drive further activation of professional APCs, as well as increased antigen presentation capacity in other APCs. These mechanisms, as well as the nature and distribution of alloantigens, determine how alloreactive T cells get activated and mediate the immune complications of transplantation.

Nature of Alloantigens

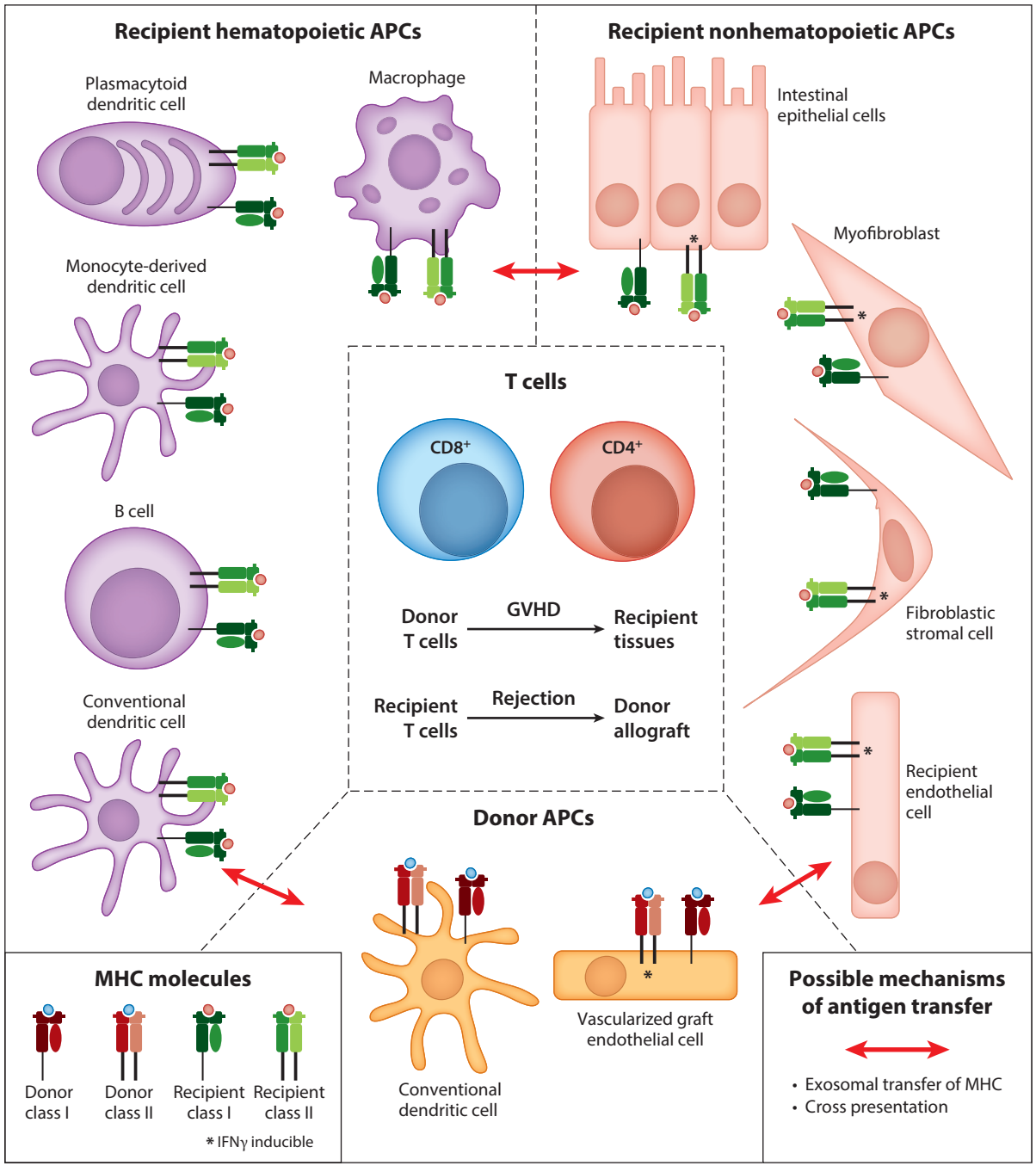
During microbial infections, naïve T cells encounter peptide antigens loaded on major histocompatibility complex (MHC) molecules. Costimulatory and coinhibitory signals regulate whether antigen encounter leads to productive immune responses or tolerance. Individual cellular subsets have variable capacities to provide these signals to T cells, but hematopoietic conventional dendritic cells (cDCs) appear to be the critical professional APCs that prime naïve T cells in SLOs in response to most infections. $CD8^+$ T cells recognize endogenous molecules presented on MHC class I, while $CD4^+$ T cells recognize exogenous molecules processed after uptake of extracellular pathogens and presented on MHC class II. However, it is now clear that overlap exists between endogenous and exogenous pathways, allowing presentation of exogenous peptides on MHC class I (cross presentation), and endogenous peptides on MHC class II.

In allogeneic transplantation, genetic polymorphisms between donor and recipient can exist both within and outside the MHC locus. Critical differences exist between T cell activation in MHC-matched and MHC-mismatched transplants. In MHC-mismatched allogeneic responses, a large proportion of T cells are thought to react with polymorphic regions of allogeneic MHC, irrespective of which peptide is loaded (1, 2). Alternatively, allogeneic MHC loaded with specific endogenous peptides can function as a molecular mimic of self-MHC loaded with foreign peptides (3). MHC-mismatched transplantation induces strong alloimmune responses, and as a result, lethal

Figure 1

Potential inflammatory or tolerogenic alloantigen-presenting cells after allogeneic transplantation. (*Center*) After allogeneic hematopoietic cell transplantation, donor T cells become activated by recipient alloantigens, leading to graft-versus-host disease (GVHD). After solid organ transplantation, recipient T cells become activated by donor alloantigens, leading to allograft rejection. (*Left*) Potential recipient hematopoietic antigen-presenting cells (APCs). (*Right*) Potential recipient nonhematopoietic APCs. (*Bottom*) Potential donor APCs. All nucleated cell types express major histocompatibility complex (MHC) class I. Only some cell types constitutively express MHC class II, while others express MHC class II in response to IFNγ (inducible), denoted by the asterisk. Red arrows indicate potential mechanisms of alloantigen transfer: exosomal transfer of intact allopeptide-MHC complexes and endocytosis of cellular material followed by cross presentation of peptide alloantigens by another cellular subset.

GVHD and acute rejection of skin transplants can be driven by single polymorphisms in either MHC class I or class II (4). In solid organ transplantation, MHC matching increases allograft survival but is difficult to achieve in practice (5). In contrast, allo-HCT is often fully MHC matched, although several types of mismatched allo-HCT are performed when MHC-matched donors are not available.



In MHC-matched transplants, polymorphisms at non-MHC loci encode alloantigens that are processed and presented on syngeneic MHC [minor histocompatibility antigens (mHAs)]. Similar to microbial antigens, mHAs are recognized by rare T cell clones with T cell receptors cognate to processed mHA loaded on MHC molecules. For example, H-Y mHAs encoded by the Y chromosome cause higher risks of alloimmunity when female T cells encounter male target cells (6). Among hundreds of potential mHAs in human transplantation, only a few are known to date. However, analysis of single nucleotide polymorphisms encoding putative mHA epitopes demonstrated that the degree of genetic mismatch correlates with the incidence of severe GVHD after allo-HCT (7). mHAs that are restricted to the hematopoietic compartment can selectively drive GVT over GVHD (8), presumably because they induce responses against residual hematological malignancies but not target epithelial organs. In mouse models, potent alloimmune responses can also be driven by virally encoded endogenous mHAs that act as superantigens (9), although the role of similar mechanisms in human transplantation is unknown.

Antigen-Presenting Cells in Graft-Versus-Host Disease

Unlike most responses to pathogens in which specialized hematopoietic APCs prime T cells, in allo-HCT both recipient hematopoietic and nonhematopoietic cells have independently been shown to be sufficient to prime alloreactive T cell responses in distinct mouse GVHD models (10, 11). Recipient-derived APCs are essential to initiate acute GVHD; however donor APC subsets can amplify later disease states. For example, cDCs derived from the donor bone marrow restimulate alloreactive $CD4^+$ T cells in target GVHD organs (12, 13). After T cells are initially primed by recipient cells, donor $CD103^+$ cDCs seed the gut, capturing alloantigens and becoming activated by innate inflammatory signals before migrating to mesenteric lymph nodes, driving a potent positive feedback loop of T cell alloactivation through the provision of recipient alloantigen, IL-12, IL-6, and CD40 costimulatory signals (14, 15). Further dysregulation of donor-derived APCs after allo-HCT sustains the seemingly paradoxical autoimmunity and immunosuppression observed in chronic GVHD (16, 17).

During MHC-mismatched allo-HCT, $CD4^+$ T cells or, to a lesser extent, $CD8^+$ T cells can induce lethal GVHD regardless of whether target epithelial tissues express MHC class II or MHC class I, respectively (18). Consistent with the rapid kinetics of disease, MHC-mismatched allo-HCT likely triggers an inflammatory cytokine storm that induces disease irrespective of alloantigen expression in target cells. In these models, cDCs appeared sufficient to prime alloreactive T cells. This was based on an experimental add-back strategy in which recipients genetically lacking the ability to present allogeneic MHC alloantigens received cDCs with an intact antigen presentation machinery just prior to transplantation. However, the authors of this study transferred unirradiated cDCs into recipients conditioned with myeloablative regimens, which may not accurately recapitulate the state of endogenous conditioned recipient cDCs (19). While cDCs or plasmacytoid dendritic cells (DCs) were sufficient to drive GVHD in this context, neither appeared necessary, as profound depletion of cDCs, plasmacytoid DCs, and B cell subsets did not protect recipients from MHC-mismatched $CD4^+$ T cell–driven GVHD (19–21). Due to the ubiquitous distribution of alloantigens and high frequency of alloreactive T cells in MHC-mismatched hematopoietic cell transplantation, a single APC subset may not be critical to prime alloreactive T cells in these models. In fact, recipient cDCs can exert tolerogenic functions in GVHD; newer studies using multiple different genetic methods to deplete cDCs showed that recipients lacking cDCs displayed accelerated GVHD in both MHC-matched and mismatched models (11, 22). Similarly, expansion of recipient $CD8\alpha^+$ cDCs with recombinant Flt3L protected mice from GVHD (23).

In MHC-matched GVHD, Shlomchik et al. (10) used a $CD8^+$ T cell–driven allo-HCT model to show that eliminating MHC class I presentation in the recipient hematopoietic

compartment prevented GVHD. As in MHC-mismatched GVHD, cDCs were inferred to be the critical hematopoietic APC subset, although this was not formally proven in vivo with cell-specific loss-of-function experiments (24). Subsequent work indicated that MHC class I expression in nonhematopoietic tissues was also necessary to drive disease (25), which suggests that reexposure to mHA in target tissues drives $CD8^+$ T cell–mediated GVHD pathology in MHC-matched models of GVHD. In $CD4^+$ T cell–driven models of MHC-matched GVHD, conflicting evidence implicates both hematopoietic and nonhematopoietic APCs as the critical priming subset. In one model, recipient mice lacking MHC class II in nonhematopoietic cells had similar or worse tissue GVHD compared to controls (25). More recently, other groups showed that MHC class II expressed only on nonhematopoietic cells (11) or that alloantigen mismatch only in the nonhematopoietic compartment (26, 27) is sufficient to drive $CD4^+$ T cell–mediated GVHD in several mHA models, while depletion of recipient cDCs prior to allo-HCT actually worsened GVHD (11). Furthermore, when MHC class II was eliminated from the hematopoietic compartment, recipient mice lacked the ability to expand regulatory T cells (Tregs), suggesting tolerogenic roles for recipient hematopoietic APCs (28). Together, these findings suggest that nonhematopoietic APCs can be key stimulators of alloreactive $CD4^+$ T cells, while hematopoietic APCs can prime pathogenic $CD8^+$ T cell and both pathogenic and protective $CD4^+$ T cell responses in MHC-matched allo-HCT. However, it remains unknown which nonhematopoietic cellular subsets serve as important APCs in $CD4^+$ T cell–driven GVHD and if they reside in target tissues or in SLOs, where classical priming is thought to occur.

Expression of MHC class II and other molecules important for exogenous antigen presentation in both hematopoietic and nonhematopoietic cells is regulated by the class II transactivator (*CIITA*) (29). *CIITA* expression itself is controlled by several promoters that are differentially active in various cellular subsets. Professional APC subsets, such as cDCs and B cells, drive *CIITA* from constitutively active promoters, while nonhematopoietic tissues utilize the IFNγ-inducible promoter IV of *CIITA* (30). This regulation pattern suggests that inducible MHC class II could participate in GVHD pathogenesis after alloreactive T cells or other immune cells release IFNγ. Additionally, nonhematopoietic cells may respond to local damage and microbe-associated danger signals after myeloablative conditioning by upregulating costimulatory molecules. In fact, nonhematopoietic cells may be the critical responders to innate signals in GVHD pathogenesis, as recipient mice genetically lacking signal transduction machinery downstream of all Toll-like receptors in their hematopoietic compartment were not protected from GVHD (31).

In the small intestine and colon, two critical GVHD target organs, lamina propria myofibroblasts have been implicated as key subsets that upregulate MHC class II and costimulatory molecules to prime alloreactive $CD4^+$ T cells after allo-HCT (11). Human colonic myofibroblasts have been reported to stimulate as well as suppress $CD4^+$ T cells through programmed death–ligand 1 (PD-L1) and PD-L2 in vitro (32, 33). Intestinal epithelial cells have also been shown to express MHC class II on their basolateral surface, with upregulated expression during GVHD, although they typically have been considered to have tolerogenic functions through DC-independent expansion of Tregs (34–36). The differential regulation and microanatomical localization of MHC class II in intestinal epithelial cells, underlying lamina propria myofibroblasts, and other cell types may be critical in determining tolerogenic versus inflammatory $CD4^+$ T cell priming during homeostasis and in intestinal GVHD.

Sites of T Cell Priming in Graft-Versus-Host Disease

Where alloreactive T cells first get primed in GVHD remains debated. Naïve T cells typically traffic to SLOs, such as spleen and lymph nodes. Consistent with a role for SLOs in priming alloreactive T cells, splenectomized *aly/aly* mice that lacked lymph nodes had delayed and blunted

GVHD (37). Nonhematopoietic cells in SLOs, including blood endothelial cells, lymphatic endothelial cells, and fibroblastic reticular cells (FRCs), harbor MHC class II and upregulate its expression during adaptive immune responses (38). Antigen presentation from these cell types appears to be tolerogenic in many contexts (39, 40). However, in the context of allo-HCT, FRCs and other fibroblastic stromal cells were shown to drive GVHD through presentation of Delta-like Notch ligands within the first 48 h after transplantation (41), suggesting that these cells can be proinflammatory. FRCs were also shown to display peptide-loaded MHC class II from exosomes released from cDCs, although in this context it was tolerogenic (42). One intriguing possibility is that, prior to their elimination by myeloablative conditioning, recipient hematopoietic APCs may transfer intact MHC molecules to non-hematopoietic cells such as FRCs for presentation to alloreactive T cells. Fibroblasts infected with lymphocytic choriomeningitis virus were also shown to prime $CD8^+$ T cells in the absence of effective cross presentation by hematopoietic APCs but only in the setting of SLOs (43). High-endothelial venules, FRCs, and likely follicular DCs also appear to be direct targets of acute GVHD, which in turn contributes to the dysregulation of humoral immunity during chronic GVHD (44).

Antigen-Presenting Cells in Solid Organ Transplantation

After solid organ transplantation, donor cDCs in the graft, termed passenger leukocytes, were originally thought to migrate to recipient SLOs and activate a large pool of recipient T cells through direct presentation of allogeneic MHC molecules (via the direct pathway of allorecognition) (45). Alternatively, a smaller pool of recipient T cells can be activated by recipient hematopoietic APCs that internalize, process, and cross present donor alloantigens via the indirect pathway (46). While either pathway can trigger acute rejection depending on the type of allograft, the direct pathway appears important for strong short-lived alloresponses (47, 48). In contrast, the indirect pathway is critical for generating a long-lived $CD4^+$ T cell response supporting B lineage cell–derived alloantibody production and chronic rejection (49).

While the indirect pathway utilizes abundant recipient APCs to endocytose, process, and present exogenous donor allopeptides, donor APCs operating in the direct pathway are finite and far less abundant, suggesting that they may not be necessary per se (50, 51). Instead, a semidirect or cross-dressing pathway has been suggested, in which more abundant recipient APCs can capture exosomes containing intact donor MHC from migratory donor APCs or from exosomes released directly from the graft into the blood or lymphatics. This allows for stimulation of direct pathway alloreactive T cells by recipient APCs (52). The existence of this pathway was documented recently in heart, skin, and pancreatic islet allografts, while ruling out the presence of donor passenger leukocytes in recipient SLOs (50, 51). A similar mechanism may operate in allo-HCT to accentuate GVHD through the transfer of intact MHC from short-lived recipient hematopoietic APCs to donor APCs or nonhematopoietic SLO fibroblastic stromal cells (53). Cross-dressed recipient APCs displaying both intact donor MHC and donor allopeptides on recipient MHC acquire a unique ability to prime $CD8^+$ and, potentially, $CD4^+$ alloreactive T cells through the semidirect pathway, while also presenting processed allopeptides to alloreactive $CD4^+$ T cells through the indirect pathway. This combination of alloantigen presentation may be crucial to provide $CD4^+$ T cell help to $CD8^+$ T cells that are activated by intact donor MHC (54).

In mouse models, DCs appear to be the critical APC subsets for allograft rejection. Donor cDCs and donor cDC–derived exosomes participate in the direct and semidirect pathways, respectively. Recipient cDCs appear critical for presenting processed donor allopeptides in the indirect pathway and intact donor exosomal MHC in the semidirect pathway, although the role for

nonhematopoietic fibroblastic stromal cells in capturing donor exosomes has not been explored (50–52, 54). Additionally, after initial T cell priming, recipient DCs derived from circulating monocytes colonize the graft and help capture and represent alloantigens to effector T cells within the graft to maintain rejection (55). Conversely, plasmacytoid DCs can drive tolerance to vascularized solid organs through the expansion of Tregs (56).

Much of the experimental evidence accumulated in solid organ transplantation relies on mouse models, in which naïve alloreactive T cells require initial priming prior to driving alloimmunity. This may be the case for recipient mice housed in specific-pathogen-free conditions, which are not exposed to a high level of environmental antigens that drive the expansion of memory T cell pools. In contrast, humans have a large memory T cell pool (>50%), which could potentially cross-react with allogeneic MHC without requiring initial priming. Indeed, the frequency of pre-existing alloreactive memory T cells correlates with organ rejection (57). In solid organ rejection, circulating effector memory $CD4^+$ and $CD8^+$ T cells cross-reactive to allogeneic MHC could be directly reactivated in the allograft by donor hematopoietic or nonhematopoietic cells without the requirement of priming from professional APCs. This appears to be an important mechanism of acute rejection of vascularized grafts. With the use of a humanized model of arterial grafting in immunodeficient mice and transfer of human leukocytes, Abrahimi et al. (58) showed that genetic knockdown of MHC class II in the transplanted endothelium led to a significant delay in rejection. This was mediated through loss of $CD4^+$ T effector memory cell help for $CD8^+$ T effector memory cell responses. While endothelial cells do not typically express MHC class II at high levels, they can upregulate MHC class II through the IFNγ-sensitive promoter of *CIITA* (**Figure 1**, vascularized graft endothelial cell). Ischemic damage during transplant and initial alloimmune activation may create a powerful positive inflammatory feedback loop between the innate and adaptive immune response. This feedback loop could result in the increased reactivation of cross-reactive memory T cells and the rejection of vascularized grafts independent of donor passenger leukocytes and of priming from recipient APC subsets.

CROSS TALK OF EPITHELIAL TISSUES WITH INNATE LYMPHOID CELLS AT SITES OF ALLOIMMUNE INJURY

Damage to epithelial surfaces is a critical feature of GVHD that attracted the attention of many researchers, as it is linked to the most dangerous clinical features of the disease. Disruption of the intestinal epithelium plays a critical role at the onset of GVHD as a result of both conditioning-related toxicity and immune-mediated injury (59, 60) (**Figure 2**). However, epithelial damage in the gut is counterbalanced by repair and protective mechanisms regulated by ILCs, a family of lymphoid cells that do not express antigen receptors but have evolved to sense and respond to a broad range of innate signals (61). ILC subsets are defined by their transcriptional regulation and cytokine production, with analogies to mature $CD4^+$ T helper cell subsets (e.g., ILC1 and $CD4^+$ Th1 cells, ILC2 and $CD4^+$ Th2 cells, ILC3 and $CD4^+$ Th17 cells). In the intestine and in other target organs such as the thymus, ILC-derived signals such as IL-22 are emerging as important components at the center of a cross talk between the immune system and epithelial tissues that controls GVHD severity (62, 63).

Intestinal Epithelial Damage in Graft-Versus-Host Disease

In experimental models and in clinical allo-HCT, the intensity of radiation or chemotherapy-based conditioning regimens is linked to the incidence and severity of GVHD (59, 64). Although multiple direct or indirect effects could be involved, the use of higher intensity conditioning regimens is

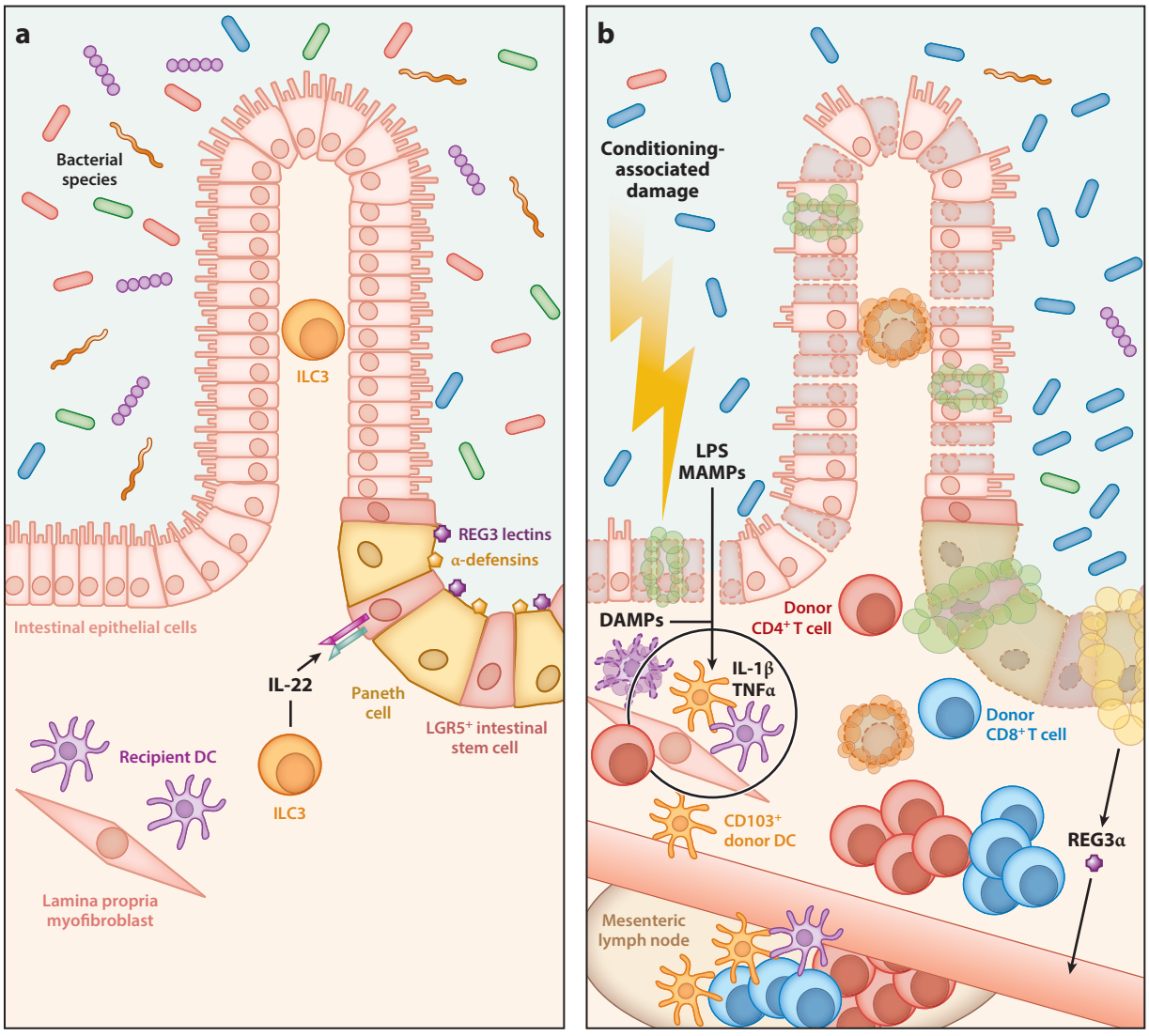


Figure 2

Loss of epithelial integrity and intestinal dysbiosis during acute GVHD. (*a*) Small intestine during homeostasis. The intestinal epithelium is constantly regenerated by LGR5$^+$ ISCs. ILC3s release IL-22 to support epithelial repair after injury. Paneth cells secrete α-defensins and REG3 proteins to regulate the intestinal microbiome. (*b*) Small intestine during acute GVHD. Damage from myeloablative conditioning, including irradiation and chemotherapy, injures the intestinal epithelium, including Paneth cells and ISCs, leading to loss of mucosal integrity. LPS and other MAMPs translocate across the gut epithelium, activating recipient and donor innate immune cells, which in turn release inflammatory cytokines (IL-1β, TNFα). Alloimmune activation of donor T cells occurs in the mesenteric lymph node and locally in the lamina propria, leading to alloimmune reactivity that further damages the intestinal epithelium. REG3α release into the blood is associated with intestinal GVHD and loss of Paneth cells. Recipient ILC3s are lost through a combination of conditioning-associated and alloimmune damage preventing IL-22-mediated maintenance of ISCs and leading to impaired regeneration of the gut epithelium. GVHD and intestinal injury also dysregulate the microbiome, leading to dysbiosis, which reinforces GVHD pathogenesis. Abbreviations: DAMPs, damage-associated molecular patterns; DC, dendritic cell; GVHD, graft-versus-host disease; IL, interleukin; ILC3, type 3 innate lymphocyte; ISC, intestinal stem cell; LPS, lipopolysaccharide; MAMPs, microbe-associated molecular patterns; TNFα, tumor necrosis factor alpha.

associated with increased histological evidence of gut epithelial damage after allo-HCT, increased abundance of lipopolysaccharide in the serum (suggesting defective intestinal barrier function and bacterial translocation), and increased serum levels of inflammatory cytokines such as TNFα and IL1β (consistent with a cytokine storm) (59, 65). Trafficking studies revealed evidence of early and prominent infiltration of the gut by donor-derived T cells within days after allo-HCT, only shortly after initial priming in SLOs (60). As discussed in more detail below, dysregulation of the intestinal microbiome is also a prominent feature of allo-HCT that plays a role in GVHD onset (65–68). Altogether, these studies have shown the intestine to be at the center of a self-reinforcing pathogenic loop leading to GVHD.

Recent work has revealed detailed features of intestinal epithelial damage after allo-HCT that appear important for GVHD pathogenesis. In mouse allo-HCT models, profound loss of $Lgr5^+$ crypt-based intestinal stem cells (ISCs) was detected at disease onset, suggesting that ISCs themselves can be targeted by the combination of conditioning and alloimmune injury (62, 69, 70). Both in mice and in human patients, acute GVHD was also associated with loss of intestinal Paneth cells (65, 71). Paneth cells are specialized epithelial cells that sit at the basis of intestinal crypts and have been reported to function as a niche for ISCs by producing agonists of the Wnt and Notch signaling pathways (72). Paneth cells produce a range of antibacterial peptides including α-defensins and REG3 family proteins that regulate the intestinal microbiome (73). Paneth cell loss in allo-HCT models was associated with dysbiosis even in the absence of irradiation-based conditioning (65). In patients, low Paneth cell numbers in intestinal biopsies at the onset of GVHD predicted a high risk for GVHD-related nonrelapse mortality (71). Interestingly, unbiased proteomic analysis identified serum levels of the C-type lectin REG3α as a sensitive and specific biomarker of intestinal acute GVHD with a role in risk stratification (74), possibly after release into the circulation upon Paneth cell injury. Thus, damage to ISCs and their niche may play a specific role at the core of GVHD pathogenesis.

Protective Role of Interleukin-22 and Innate Lymphoid Cells in Graft-Versus-Host Disease

The cytokine IL-22 is a member of the IL-10 family that recently emerged as a critical regulator of GVHD on the basis of its capacity to mitigate epithelial injury (62, 63). Whereas most cytokines mediate communication between immune cell subsets, IL-22 is produced by ILCs and other immune cells but predominantly targets nonhematopoietic compartments, such as epithelial and stromal cells (75). In mouse allo-HCT models, lack of recipient-derived IL-22 increased GVHD severity (62). Long-lived radiation-resistant IL-23-responsive $ROR\gamma t^+$ ILCs (type 3 ILCs) were identified as a critical source of IL-22 in both acute intestinal and thymic GVHD (62, 63). Both the frequency and absolute number of $IL\text{-}22^+$ ILCs were profoundly decreased during acute GVHD (62), suggesting that ILCs could be the targets of alloimmune reactivity. Interestingly, subsets of ILC3s that produce IL-22 have been shown to express MHC class II molecules and to be engaged in a cross talk with conventional T cells in the intestine (76). Some of these characteristics could make them vulnerable to the effects of activated alloimmune T cells.

Consistent with IL-22 exerting its key effects in the epithelial compartment, expression of the IL-22 receptor was detected in intestinal stem and progenitor cells, and direct effects of IL-22 occurred in isolated intestinal organoids exposed to recombinant IL-22 (70). The effects of IL-22 remained active in *ATOH*-deficient organoids lacking Paneth cells, suggesting direct effects of IL-22 on ISCs rather than indirect effects involving Paneth cells. After allo-HCT, administration of IL-22R agonists promoted intestinal epithelial regeneration, decreased intestinal GVHD scores, and improved survival (70). In the thymus, IL-22R expression was detected in cortical

thymic epithelial cells but not in thymocytes or other stromal elements (63). These findings parallel observations in the intestine, suggesting a dominant effect of IL-22 on epithelial compartments, with indirect effects on alloimmune cells. However, T cell–derived IL-22 and peri-transplant IL-22 administration were previously suggested to enhance GVHD (77, 78). It remains to be determined whether these findings can be reconciled with the effects of ILC-derived IL-22 on epithelial targets. When tested side by side genetically in allo-HCT models, protective effects of recipient-derived IL-22 appeared to dominate the impact of this cytokine (62).

Innate Lymphoid Cells in Organ Rejection

In contrast to the emerging wealth of information available about ILCs in the pathogenesis of GVHD, little is known about their potential involvement in allograft rejection. In the setting of intestinal or multivisceral transplantation, ILCs recovered from the transplanted gut demonstrate prolonged mixed chimerism with evidence of donor-derived cells persisting for many years after the procedure (79). Phenotypic characterization showed that, as compared to control intestine, $CD3^-$ cells with ILC features accumulated in biopsies from intestinal grafts (80), including $IFN\gamma^+$ cells with ILC1 features and $IL\text{-}22^+$ cells with ILC3 characteristics. The presence of these cells suggests that they could be involved in a cross talk with the epithelium and regulate aspects of allograft survival, although this remains to be investigated. It is tempting to speculate that donor- or recipient-derived ILCs could be involved in the outcome of other organ transplants, especially when ILCs are known to regulate immunopathology in the native organs (e.g., liver and lung). Additional investigations could identify new aspects of allograft rejection and new therapeutic interventions.

Altogether, ILCs represent an attractive area of future investigation in alloimmunity beyond just IL-22 production. Deep knowledge has been acquired already about the phenotypic, functional, and regulatory features of major ILC subsets, pointing at gene regulatory networks and effect functions that parallel those of major $CD4^+$ T cell subsets (61). On the basis of recent single cell profiling results, it is clear that new subsets of ILCs will be identified and studied functionally (81). More work is needed to fully unravel all elements of this cross talk in alloimmune injury.

THE MICROBIOME IN GRAFT-VERSUS-HOST DISEASE AND ALLOGRAFT REJECTION

The microbiome has recently come under intense scrutiny for its multiple roles in health and disease, including inflammation, metabolism, and cancer. A complex cross talk exists between the microbiome and the recipient organism, with local effects at sites of colonization (e.g., gut, lung, skin) and distant effects throughout the body. Large consortium projects such as the Human Microbiome Project and other initiatives have provided massive amounts of new information (82). Experimental models of disease have been useful to move beyond descriptive studies and test causal relationships between changes in the microbiome (dysbiosis) and specific biological outcomes. Although these studies are difficult, the field is now ripe for interventional trials that manipulate the microbiome in specific disease contexts.

Microbiome in Graft-Versus-Host Disease

Clinical and experimental data point to the microbiome as a major regulator of disease pathogenesis in both GVHD and allograft rejection. Pioneering studies were the first to report high-level protection from GVHD in germ-free mice subjected to allo-HCT (83). These observations had an impact on the broad implementation of gut decontamination and isolation strategies for allo-HCT

patients (84, 85). However, documenting the impact of these practices is complex and difficult to generalize because it can be profoundly influenced by features of the microbiome that change over time and in different locations around the world. In recent years, many studies facilitated by deep sequencing technologies have focused on individual bacterial species in the microbiome and, thus, more on the nature than the absolute numbers of microorganisms at specific sites. Both in mouse models and in human allo-HCT patients, massive shifts in the composition of the gut microbiome take place over the course of treatment, with loss of overall bacterial diversity and outgrowth of individual species (65–68) (**Figure 2**). Various factors are thought to contribute to dysbiosis after allo-HCT, including the use of conditioning agents with gastrointestinal toxicity, changes in food intake, use of specific prophylactic antibiotics, and administration of broad-spectrum antibiotics for infectious complications. Interestingly, different broad-spectrum antibiotics are associated with distinct changes in the microbiome and with variable rates of severe GVHD, in both mice and humans (68, 86). In addition, the occurrence of GVHD itself appears to influence changes in the microbiome, perhaps due to effects on the intestinal epithelium and on ILCs regulating the epithelium-microbiome interface (59, 62, 65, 70, 71). In turn, dysbiosis has the potential to alter the alloimmune response and potentiate GVHD—a self-reinforcing pathogenic loop.

Although much work remains to be done, new information on the microbiome in GVHD has begun to have a complex and potentially profound impact on the use of experimental allo-HCT models and on clinical practice. For example, Hill and coworkers (87) recently reported that acute GVHD in mice was regulated by an IL-17-sensitive microbiome. Recipient-derived IL-17A was critical to prevent GVHD, but cohousing of wild-type recipients with IL-17R-deficient mice was sufficient to transfer increased susceptibility to acute GVHD based on changes in the gut microbiome. Thus, acquired changes in the microbiome based on genetic defects have to be considered in experimental design and data interpretation. Past discordant results in different laboratories could also be related to differences in microbiome composition at different institutions. In clinical practice, some groups have started to implement changes in the use of specific prophylactic antibiotic regimens and broad-spectrum antibiotics (88). These and other interventions to manipulate the microbiome will have to be studied and validated carefully, as they may not always translate to multiple centers and their effectiveness may change over time. Another consideration in future studies of the microbiome in allo-HCT will be to include end points beyond GVHD itself. For example, anticancer effects of chemotherapy, radiation, and immunotherapy agents can be influenced by the microbiome (89, 90).

Microbiome in Organ Rejection

Clinical and experimental observations have also identified dysbiosis as a potentially significant factor in allograft rejection. Initial observations focused on recipients of nonsterile allografts, such as small bowel and lung, in which local immune effects of the microbiome were expected. Both organs also happen to be prominent GVHD targets, suggesting that features of GVHD-mediated damage and allograft rejection could be shared at these sites. After small bowel transplantation, significant shifts in bacterial taxa were observed in ileal effluents during episodes of rejection (91). After lung transplantation, several groups documented changes in airway microbiota after transplantation, and some suggested an association of specific microorganisms with bronchiolitis obliterans, a distinct syndrome associated with chronic rejection that can also be observed during chronic GVHD (92–94). Recently, longitudinal changes in the microbiota in stool and urine were also reported in patients undergoing kidney transplantation (95). More work is needed to evaluate to what extent dysbiosis is influenced by medical interventions associated with transplantation itself, with underlying chronic conditions, or both. Most importantly, it remains to be established if dysbiosis occurs as a cause or a consequence of rejection episodes.

In experimental models of allograft rejection, the introduction of artificial infections can break immune tolerance and precipitate acute rejection (96, 97). Increased graft survival was also reported using a skin transplant model in germ-free mice and in mice treated with broad-spectrum antibiotics (98). Fecal transplantation experiments suggested that the composition of microbiota rather than the absolute number of colonizing bacteria held the key to its effects on allograft survival in this model, in part through type I interferon–mediated effects (98). In a mouse model of orthotopic liver transplantation, gut-derived microbe-associated molecular patterns were found to reach the portal circulation and modulate ischemia/reperfusion injury, which itself can enhance alloimmune rejection (99). Mechanistically, future investigations should focus on the multiple pathways through which microorganisms could influence alloimmunity, especially since the nature of microorganisms involved is essential and suggests molecular specificity to the process. Innate stimuli are likely to be important. Molecular mimicry stimulating alloantigen-specific T cell responses has also been postulated. Recent reports have uncovered other mechanisms involving the secretion of chemicals such as butyrate that have epigenetic effects in Tregs and perhaps other immune cells (100–103). Thus, bidirectional interactions between the microbiome and the immune system could be complex in GVHD and allograft rejection.

ROLE OF THE ALARMIN IL-33 IN ALLOIMMUNITY

IL-33 is a member of the IL-1 cytokine family that was originally identified as a ligand of the orphan IL-1 receptor–related protein suppression of tumorigenicity 2 (ST2, also known as IL1RL1) (104). Although initial work focused on the role of IL-33 in Th2 responses, it is now clear that IL-33 exerts pleiotropic effects on multiple potential targets, including Tregs, activated T cells, ILC2s, and myeloid cells (105, 106). IL-33 qualifies as an alarmin because it lacks a signal peptide and an active secretion mechanism. Instead, IL-33 is normally found in the nucleus in a tight association with chromatin that is regulated by its N-terminal nuclear domain. IL-33 release into extracellular space is induced mostly by nonapoptotic cell damage. Its activity is enhanced after proteolytic processing of full-length IL-33 into a shorter IL-1-like C-terminal domain. IL-33 is primarily expressed in multiple nonhematopoietic cell types, including epithelial, endothelial, and stromal cells, which can be further enhanced by inflammatory stimuli. IL-33 binds to the ST2 receptor and to its heterodimeric partner IL-1 receptor accessory protein (IL-1RAcP), leading to MYD88- and IRAK1/4-dependent NF-κB and mitogen-activated protein kinase activation in target cells. As another important level of regulation, ST2 is expressed in two isoforms due to alternative splicing of its messenger RNA: a full-length membrane-bound form (mST2) that mediates signaling in target cells and a shorter secreted form (sST2) that functions as a decoy receptor and can effectively inhibit IL-33-mediated effects in $mST2^+$ target cells. Importantly, the impact of IL-33-mediated signals is influenced by the relative abundance of mST2 and sST2 in alloimmunity.

IL-33 and Organ Rejection

Initial insights into the role of IL-33 in alloimmunity were gathered in mouse models of heterotopic heart transplantation (107–109). In a model of MHC-mismatched transplantation, administration of IL-33 led to delayed rejection and was associated with enhanced accumulation of Th2-like T cells (107). In an MHC-matched mHA-driven model with features of chronic rejection, exogenous IL-33 prolonged allograft survival, increased the accumulation of Tregs and myeloid-derived suppressor cells, and decreased the deposition of antibodies in the graft (108). Mechanistically, the protective effects of IL-33 required recipient ST2 expression and were lost upon Treg depletion (109). IL-33 can induce direct effects in $ST2^+$ Tregs but also indirect effects via IL-33-responsive

DCs (110). Interestingly, elevated levels of sST2 in plasma are observed during episodes of acute cardiac and small bowel allograft rejection in patients, suggesting that IL-33/ST2 signaling might be dysregulated in human transplantation (111, 112).

IL-33 and Graft-Versus-Host Disease

After allo-HCT, proteomic studies of plasma biomarkers identified sST2 levels as a powerful indicator of steroid-refractory GVHD and nonrelapse mortality (113). Besides providing a new method for risk stratification of GVHD patients, these findings raised the possibility that sST2 release might function not only as a useful biomarker but also in the regulation of GVHD pathogenesis. In mice, exogenous IL-33 led to contrasting results depending on the model and administration schedule (114–116). Post-transplant administration of IL-33 in a highly inflammatory MHC-mismatched allo-HCT model enhanced the activity of conventional alloreactive T cells and worsened GVHD, while *il33*$^{-/-}$ recipients and recipients of ST2-deficient T cells were protected from GVHD (114). In contrast, more prolonged peri-transplant administration of IL-33 starting 10 days before allo-HCT expanded recipient Tregs and decreased GVHD severity in a Treg-dependent manner (116). The protective impact of adoptively transferred Tregs was also dependent on ST2 expression in Tregs.

Using another approach to interrogate IL-33/ST2 signaling, Paczesny and colleagues (115) developed an antibody-based strategy to inhibit ST2 systemically that predominantly affects circulating sST2 and blocks its ability to function as a decoy IL-33 receptor. This strategy effectively enhanced IL-33 availability to mST2-expressing cells during the peri-transplant period and resulted in the expansion of ST2^{+} Tregs and myeloid-derived suppressor cells, as well as in GVHD protection in multiple allo-HCT models. Furthermore, marked changes were observed in the relative expression of sST2 and mST2 during GVHD, with a profound increase in the sST2/mST2 ratio in the small and large intestine. Although the critical source of sST2 remains to be determined, abundant expression was observed in stromal and endothelial cells, as well as in activated T cells. More insights into this regulation will clarify how tissue damage and inflammation during GVHD are connected to IL-33 release and to its regulated availability for mST2-expressing target compartments.

NOTCH SIGNALING IN ALLOIMMUNITY

Notch signaling has emerged as a key regulator of T cell alloimmunity, in the setting of both GVHD and allograft rejection (41, 117–123). Recent data identified nonhematopoietic radioresistant fibroblastic stromal cells as the critical source of Notch ligands at the onset of GVHD (41). Thus, as for IL-33/ST2 signaling, Notch ligand-receptor interactions represent newly identified molecular signals that mediate communication between T cells and nonhematopoietic elements of their microenvironment, with a profound impact on the pathogenesis of alloimmunity.

Overview of Notch Signaling

Notch signaling is a highly conserved cell-to-cell communication pathway mediated by Notch ligand-receptor interactions between adjacent cells (**Figure 3*a***) (124). Four Notch receptors (Notch1–4) and five Notch ligands of the Jagged (Jag) and Delta-like (Dll) families (Jag1/2 and Dll1/3/4) have been identified in mammals. Among Delta-like ligands, only Dll1 and Dll4 have agonistic properties. Specific interactions between Notch ligands and receptors expressed in adjacent cells induce regulated proteolytic activation of the receptor by an ADAM family metalloprotease and then by the γ-secretase complex. γ-Secretase-mediated intramembrane proteolysis

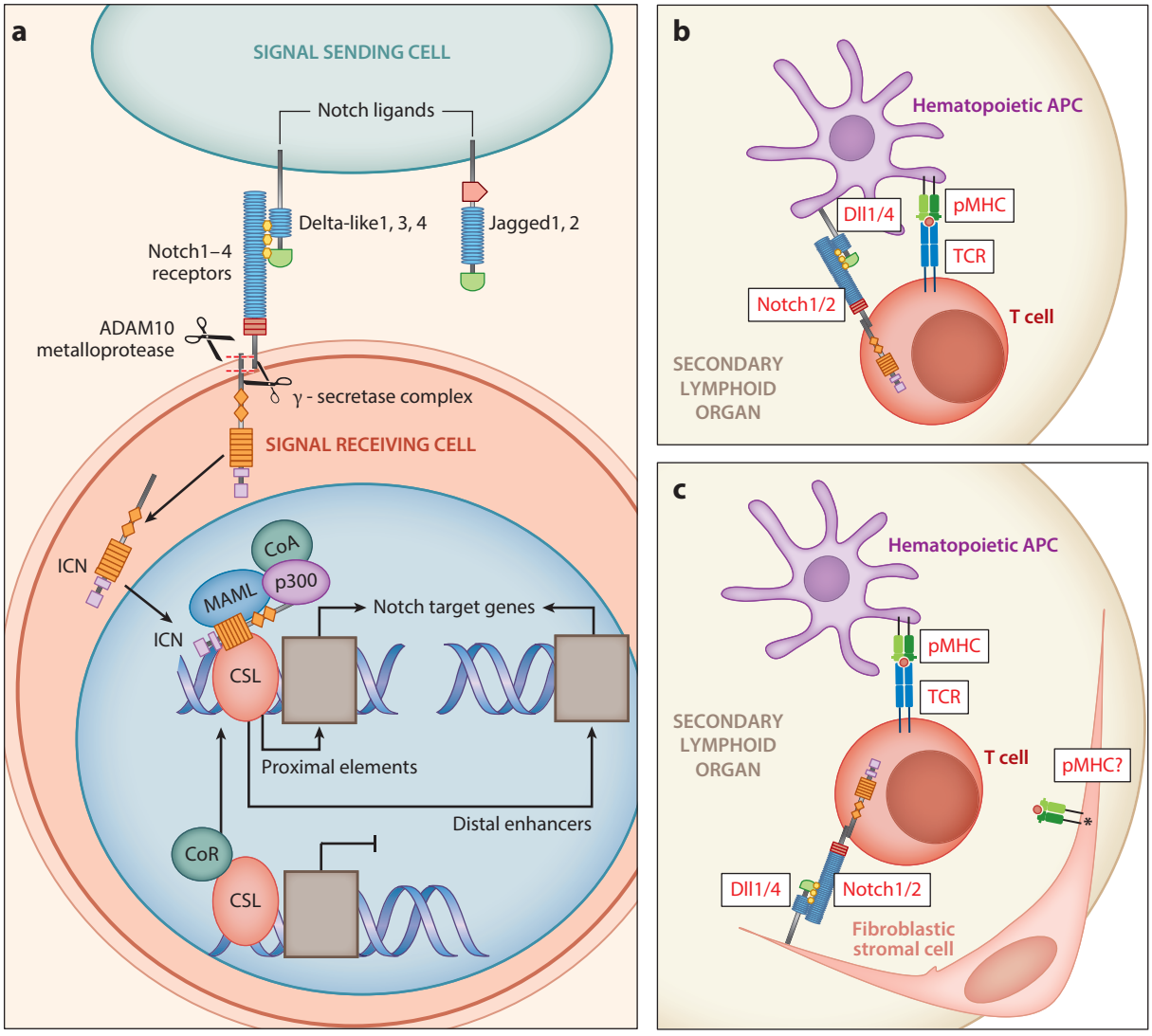


Figure 3

Overview of Notch signaling and sources of Notch signals in alloimmunity. (*a*) Notch signaling occurs via the physical interaction between Notch receptors (Notch1–4) and Notch ligands (Delta-like 1,3, and 4; Jagged 1 and 2). Ligand-receptor binding allows two cleavage events to occur, which are mediated by the ADAM10 metalloprotease and the γ-secretase complex, releasing ICN into the cytosol. After entry into the nucleus, ICN forms a transcriptional activation complex with the transcription factor CSL, a MAML coactivator, and other partners including p300. ICN/CSL/MAML transcriptional complexes can regulate Notch target genes at promoter proximal regions or through binding at distal enhancer sites. (*b*) Classical model of Notch involvement in T cell alloactivation. A naïve T cell is activated by a hematopoietic APC that provides both antigen-specific signals and Notch signals through Delta-like1/4;Notch1/2 interactions. (*c*) New model of Notch signaling and T cell alloactivation. Delta-like1/4 Notch signals are derived from nonhematopoietic fibroblastic stromal cells such as fibroblastic reticular cells or follicular dendritic cells in secondary lymphoid organs. Fibroblastic stromal cells may also present alloantigens through the expression of allopeptide-loaded MHC complexes. Abbreviations: APC, antigen-presenting cell; CoA, coactivator; CoR, corepressor; CSL, CBF1/suppressor-of-hairless/Lag-1; Dll1/4, Delta-like 1,4; ICN, intracellular Notch; MAML, mastermind-like; pMHC, peptide-loaded major histocompatibility complex; TCR, T cell receptor.

releases intracellular Notch (ICN) into the cytoplasm. ICN migrates into the nucleus where it partners with the DNA-binding transcription factor CSL (CBF1/suppressor-of-hairless/Lag-1), also called RBP-Jκ and encoded by the *Rbpj* gene. ICN and CSL become part of a large transcriptional activation complex in association with a mastermind-like (MAML) family coactivator and multiple other proteins that cooperate to mediate transcriptional activation of Notch target genes. The majority of Notch's well-documented effects in the immune system are mediated by canonical ICN/CSL/MAML-dependent transcriptional activation, although noncanonical mechanisms of Notch action have also been reported (117, 119, 120, 122, 125, 126). Progress is being made in identifying key transcriptional targets of Notch signaling that mediate downstream biological effects of the pathway in specific contexts, especially in Notch-driven cancers (127, 128). Many Notch targets are regulated via Notch activity at enhancer regions and via cooperation with other context-specific transcription factors. More work is needed to systematically uncover the transcriptional network regulated by Notch in immune cells.

In the hematopoietic system, Notch was first identified for its essential roles at early stages of T cell development in the thymus via interaction of Dll4 ligands in thymic epithelial cells with Notch1 receptors in T lineage progenitors (129–131). Notch signaling also regulates the differentiation, maintenance, or function of distinct subsets of B cells, DCs, macrophages, and ILCs (132). In some of these cases in which the source of Notch ligands was investigated, defined fibroblastic niches in SLOs function as the critical cellular source of Notch ligands presented to Notch-dependent populations in vivo (133). Moreover, a growing body of literature identified specific context-dependent roles for Notch in the regulation of mature $CD4^+$ and $CD8^+$ T cell function through interaction of Notch receptors in T cells with Notch ligands in their environment (41, 117, 119–122, 125, 133–139).

Early Insights on Notch Signaling in Alloimmunity

Early studies relying heavily on artificial gain-of-function strategies were the first to draw attention to a potential role of Notch in tolerance and alloreactivity (140–143). Adoptive transfer of DCs engineered to overexpress the Notch ligand Jag1 induced antigen-specific T cell hyporesponsiveness to a house dust mite antigen (141). Similar observations were then made when studying T cell responses to alloantigens or viral antigens using Jag1-transduced Epstein–Barr virus–transformed B lymphoblastoid cell lines as APCs (142, 143). In a heart allograft model, Dallman and colleagues (140) reported a $CD8^+$ T cell–dependent tolerogenic effect of adoptively transferred L cell fibroblasts overexpressing the Notch ligand Dll1 and allogeneic MHC molecules. Taken together, these studies suggested that inducing artificially high levels of Notch signaling could create a state of antigen-specific T cell tolerance. However, interpretation was difficult given possible non-cell-autonomous effects of overexpressed Notch ligands, the lack of direct genetic demonstration, and the artificial nature of the gain-of-function experimental systems. More recently, several laboratories used in vivo loss-of-function strategies to evaluate the role of Notch signaling in alloimmunity (41, 117–123, 139). These studies reached concordant conclusions that Notch functions as a major proinflammatory signaling pathway promoting pathogenic alloreactivity, in both GVHD and transplant rejection. Thus, the actual in vivo function of Notch signaling in alloimmunity turns out to be opposite from that initially suggested by artificial gain-of-function strategies.

Notch and Graft-Versus-Host Disease

In multiple mouse models of allo-HCT and GVHD, genetic inhibition of canonical Notch signaling in T cells led to profoundly decreased GVHD severity and GVHD-associated mortality (117, 119, 121). The effects of Notch signaling were dependent on Notch1/2 receptors in T cells

and Dll1/4 ligands in the recipient, with dominant effects of Notch1 and Dll4 (119, 123, 139). Transient systemic inhibition of Dll1/4 with neutralizing antibodies in the peri-transplant period was sufficient to confer long-term protection (41, 119). These findings suggested the existence of an early pathogenic pulse of Notch signaling in alloreactive T cells during their priming and initial activation. Upon Notch inhibition, protection from GVHD was associated with decreased production of multiple inflammatory cytokines, including IFNγ and TNFα, as well as increased expansion of preexisting Tregs. Upon in vivo priming in the absence of Notch signaling, alloreactive T cells acquired a state of acquired hyporesponsiveness to restimulation through their T cell receptor and CD28 coreceptor (121). Yet, some aspects of T cell function were preserved in Notch-deprived alloreactive T cells, including in vivo proliferation and expansion in lymphopenic recipients and expression of cytotoxic molecules. Ex vivo cytotoxicity and in vivo anticancer activity were also preserved. As a result, Notch inhibition allowed long-term posttransplant survival without severe GVHD and without recurrent cancer in mouse models of allo-HCT and leukemia. The relative importance of conventional T cells and Tregs in mediating Notch's effects remains to be determined, as do the precise mechanisms of Notch action in T cells. Genetic data point at canonical CSL/MAML-dependent transcriptional effects of Notch signaling as the critical effectors of Notch signaling in alloreactive T cells, although functionally essential transcriptional targets need to be identified (117, 122). In addition, Notch may also exert important effects in B cells during chronic GVHD (144).

Notch and Organ Rejection

Several groups investigated the impact of Notch signaling in allograft rejection using mouse models of vascularized heterotopic heart transplantation (118, 120). Riella and coworkers (118) first reported a protective effect of antibody-mediated Dll1 inhibition in the peri-transplant period. Anti-Dll1 antibodies delayed allograft rejection when applied together with genetic or pharmacological costimulatory blockade (in *Cd28*-deficient recipients or with CTLA4-Ig treatment). Protection in this model was associated with a STAT6-dependent shift from Th1 to Th2 cytokine production. To assess the overall impact of Notch signaling in T cells during allograft rejection, Wood et al. (120) used a genetic approach to block all canonical Notch signals in T cells through expression of the pan-Notch inhibitor dominant negative MAML1 (DNMAML). *Cd4-Cre;ROSA26*^{DNMAML} mice showed delayed rejection of heart allografts. Protection was most pronounced upon concomitant CD8$^+$ T cell depletion, which generates a model with features of both acute and chronic rejection. As in GVHD models, protection was associated with an increased ratio of Tregs to effector T cells and decreased production of proinflammatory cytokines. Furthermore, systemic inhibition of Dll1 and Dll4 Notch ligands led to prolonged allograft survival to a larger extent than T cell–specific Notch inhibition. Mechanistically, Dll1/4 blockade decreased both T cell– and B cell–dependent aspects of the rejection process, suggesting a broader impact of Notch signaling than just its T cell effects. Altogether, these in vivo loss-of-function studies provided concordant data indicating that Notch operates as a major proinflammatory pathway in both GVHD and allograft rejection.

Cellular Sources of Notch Ligands in Alloimmunity

Early studies of Notch in T cell immunity documented that professional APCs, such as DCs, increased Notch ligand expression in response to inflammatory stimuli and established the potential of these cells to function as a source of Notch ligands to T cells (135). Subsequent studies in alloreactivity and other immune contexts postulated that Notch ligands are derived from hematopoietic

APC subsets, although most of these studies were based on correlation and ex vivo coculture systems without in vivo genetic data (123, 136, 145). Radtke and collaborators (133) were the first to identify a nonhematopoietic cellular source of Dll1/4 Notch ligands for the differentiation of Notch-dependent T follicular helper cells, ESAM^{hi} DCs, and marginal zone B cells in SLOs. In mouse models of acute GVHD, Chung et al. (41) discovered that hematopoietic sources of Dll1 and Dll4 Notch ligands were not essential for disease pathogenesis (**Figure 3*b***). Instead, a population of nonhematopoietic fibroblastic stromal cells lineage traced with a *Ccl19-Cre* transgene was the critical source of Dll1/4 ligands at GVHD onset, with essential Notch signals delivered within days after allo-HCT (41) (**Figure 3*c***). *Ccl19-Cre* is expressed in the fibroblastic stromal cell compartment of spleen and lymph nodes as well as in Peyer's patches (146). After irradiation-based conditioning and allo-HCT, *Ccl19-Cre* activity was detected predominantly in a population of FRCs with high CD157 expression and in follicular DCs of spleen and lymph nodes, as well as, to a minor extent, in lymphatic endothelial cells (41). These findings identify a fibroblastic niche that plays a critical role in the pathogenesis of acute GVHD through the delivery of Notch signals.

Altogether, Notch is emerging as a critical regulator of alloimmunity via T cell–stromal cell interactions in SLOs. Whether fibroblastic cells also function as the dominant source of Notch ligands in nonmyeloablative allo-HCT, in allograft rejection, and in other types of immune response remains to be determined. In the thymus, expression of *Dll4* and chemokines in thymic epithelial cells is coregulated via *Foxn1* through an evolutionarily conserved pathway (147). In SLOs, CCL19 and DLL1/4 expression appears to coincide in a subset of fibroblastic stromal cells, perhaps generating a functional unit to attract T cell targets and expose them to Notch signaling (41). Upstream regulators of DLL1/4 expression in these cells remain to be identified. Another important question is how the delivery of Notch signals is coordinated with other immune signals, including presentation of alloantigens, costimulatory ligands, and innate stimuli.

CONCLUDING REMARKS

We discussed here only a selected number of key recent discoveries in transplantation immunology, with a focus on the involvement of new cell types other than T cells and professional APCs. Our review is not exhaustive and could have included additional important areas of progress. For example, chemokines play an important role in immune cell trafficking and have become the target of therapeutic interventions (148). B cells and antibody-producing plasma cells are emerging as critical regulators of both rejection and chronic GVHD (149–152). As for T cells, the interaction of B lineage cells with the recipient involves a complex cross talk with other immune cells and with stromal elements in SLOs. Different subsets of myeloid cells also play an essential role in the control of alloreactivity (153, 154). Finally, important progress continues to be made by investigating the activation of conventional T cells and Tregs that mediate or control alloimmune injury.

As a common theme among the discoveries highlighted in this review, alloreactive T cells engage in a complex cross talk with multiple cell types beyond traditional professional APCs. These interactions involve new cytokines, alarmins, and signaling pathways that change our understanding of transplantation immunobiology and nominate new potential therapeutic targets. In turn, discoveries made in the field of transplantation could have general significance in the regulation of other types of immune responses.

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