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Notch signaling and alloreactivity

Vedran Radojcic¹ and Ivan Maillard^{2,3,*}

¹Division of Hematology-Oncology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA

²Life Sciences Institute, University of Michigan, Ann Arbor, MI, USA

³Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, USA

Abstract

Solid organ and allogeneic hematopoietic cell transplantation have become standard therapeutic interventions that save patient lives and improve quality of life. Our enhanced understanding of transplantation immunobiology has refined clinical management and improved outcomes. However, organ rejection and graft-versus-host disease remain major obstacles to the broader successful application of these therapeutic procedures. Notch signaling regulates multiple aspects of adaptive and innate immunity. Preclinical studies identified Notch signaling as a promising target in autoimmune diseases, as well as after allogeneic hematopoietic cell and solid organ transplantation. Notch was found to be a central regulator of alloreactivity across clinically relevant models of transplantation. Notch inhibition in T cells prevented graft-versus-host disease and organ rejection, establishing organ tolerance by skewing CD4⁺ T helper polarization away from a proinflammatory response towards suppressive regulatory T cells. Notch ligand blockade also dampened alloantibody deposition and prevented chronic rejection through humoral mechanisms. Toxicities of systemic Notch blockade were observed with γ -secretase inhibitors in preclinical and early clinical trials across different indications, but they did not arise upon preclinical targeting of Delta-like Notch ligands, a strategy sufficient to confer full benefits of Notch ablation in T cell alloimmunity. As multiple clinical grade reagents have been developed to target individual Notch ligands and receptors, the benefits of Notch blockade in transplantation are calling for translation of preclinical findings into human transplantation medicine.

Introduction

Since early successes of transplantation medicine in the mid-twentieth century,^{1,2} solid organ and hematopoietic cell transplantation have become mainstream therapeutic interventions. Almost 150,000 allogeneic transplants are performed annually in the world, including more than 115,000 solid organ³ (Global Observatory on Donation and Transplantation, produced by the World Health Organization and The Spanish National Transplant Organization collaboration, <http://www.transplant-observatory.org/Pages/Data-Reports.aspx>) and ~30,000 allogeneic hematopoietic cell transplants (allo-HCT).⁴ Our

*Corresponding author: Ivan Maillard, MD-PhD, Life Sciences Institute 6382A, 210 Washtenaw Avenue, Ann Arbor, MI 48109, USA. Tel: 734-763-3599, imaillard@umich.edu.

understanding of transplantation medicine has improved dramatically since initial concepts were first laid out. However, a major hindrance to a broader and more successful application of the procedures in their respective fields remains the lack of improved therapeutic options for transplant-related immune complications: rejection after solid organ transplantation, and graft-versus-host disease (GVHD) after allo-HCT. While broad immunosuppression remains a major strategy for controlling transplant complications, its efficacy remains limited. Current approaches lead to increased rates of opportunistic infections. In addition, they contribute to significant end-organ toxicities, such as kidney injury from chronic use of calcineurin inhibitors. Novel strategies of immunomodulation are necessary to harness the full therapeutic benefits of transplant procedures. Here, we review preclinical evidence identifying a role for Notch signaling in adaptive immune responses that defines the outcome of allo-HCT and solid organ transplantation, and we propose to consider Notch as a new therapeutic target in this area of unmet clinical need.

The Notch signaling pathway

Notch is a highly conserved cell-to-cell surface signaling pathway with pleiotropic roles in multiple developmental processes, tissue homeostasis and disease.^{5–7} In mammals, Notch signaling is initiated by the interaction of Notch receptors (Notch1–4) with ligands of the Jagged (Jagged1–2) and Delta-like families (Dll1, Dll3 and Dll4) (Figure 1). Extensive O-fucose glycosylation of the multiple EGF-like repeats on the extracellular domain of Notch receptors is necessary for ligand recognition and thus for receptor function.^{8,9} Receptors are further modulated by the activity of Fringe family glycosyltransferases, which regulate their binding affinity to individual Notch ligands.^{10–12} Notch receptor-ligand interactions generate a physical force that unmask an extracellular domain proteolytic site, allowing for cleavage by a disintegrin and metalloproteinase (ADAM10).^{13–15} This process rapidly leads to proteolysis within the transmembrane domain by the γ -secretase complex, releasing intracellular Notch (ICN).^{16,17} ICN translocates to the nucleus where it binds CSL/RBP-Jk (CBF-1, Suppressor of hairless, Lag-1; also called RBP-Jk and encoded by the *Rbpj* gene).¹⁸ ICN acts as a transcriptional activator that interacts with CSL/RBP-Jk and recruits a Mastermind-like family transcriptional coactivator (MAML), leading to target gene activation.^{19–22} To ensure tight Notch regulation and short-lived signals, the C-terminal PEST domain of ICN and other mechanisms target active Notch for rapid degradation.^{23–25} Altogether, Notch signaling connects cell surface signals induced by multiple Notch ligands and receptors to a common pathway of “canonical” transcriptional activation mediated by ICN, CSL and MAML at the core of a large multiprotein complex.

Notch signaling in adaptive immunity

In the adaptive immune response, highly differentiated cellular elements arise after antigenic exposure in peripheral lymphoid organs. Adaptive immunity provides targeted and long-lasting protection, while memory formation facilitates rapid and focused responses upon subsequent antigen encounters. Antigen processing and presentation by professional antigen-presenting cells (APCs) to a limited number of mature T cells is the initial step in formation of adaptive immunity. The final T cell differentiation and effector response depend on the nature of the antigen and on the local priming environment, with regulatory

impact from cytokine signals. Moreover, the field has been probing other inputs, including from the Notch signaling pathway, to account for the full spectrum of immune diversity and helper T cell (Th) plasticity.²⁶

Notch has long been recognized to play a key role in early T cell development.^{27,28} In addition, immunologists have pursued investigations into Notch's role in multiple aspects of adaptive immunity. Thus, a multifaceted impact of Notch signaling on adaptive immunity has surfaced over the past decade, including identification of its enabling role in many arms of Th polarization (including Th1,^{29–33,34} Th2,^{30,35–39} Th9,⁴⁰ and Th17^{41,42}); its effects in terminal effector vs. memory precursor CD8⁺ T cell differentiation^{43–46}; its effects on regulatory T cell function⁴⁷; and its synergy with B cell receptor signaling to promote class switching and terminal B cell differentiation and antibody production.^{48–50} How Notch mediates such a broad range of immune effects is progressively being elucidated.

Initial studies of Notch regulation in adaptive T cell responses reported upregulated expression of Delta-like but not Jagged ligands in APCs cultured in a Th1 polarizing environment.³⁰ In vitro studies identified Notch signaling as a positive regulator of Th1 differentiation, with key inputs from TLR-Myd88 signaling.^{30,51–53} Notch1 and Notch2 receptors were identified as critical for Th1 polarization in some but not all models of Th1 differentiation.³³ Forced expression of ICN or Dll ligands, or CD4⁺ T cell exposure to Dll1-Fc fusion protein promoted Th1 differentiation,^{29–32,53} which was inhibited by γ -secretase inhibitors (GSI).³¹ Notch was proposed to directly regulate *Tbx21*, encoding the master Th1 transcription factor Tbet.³¹ In this study, Notch1 was shown to directly bind to the *Tbx21* locus and systemic Notch blockade with GSI alleviated experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis driven in part by pathogenic Th1 responses. A subsequent study, relying on Notch blockade via GSI and genetic pan-Notch inhibition with dominant negative MAML (DNMAML) expression also reported *Tbx21* as a direct target of Notch signaling in addition to linking Notch to direct regulation of *Ifng* transcription.³⁴ Other *in vitro* and *in vivo* studies, however, did not document *Tbx21* regulation by canonical Notch signaling,^{54,55} despite the fact that in many cases Notch inhibition decreased IFN- γ production through other mechanisms.^{35,37,54–57} In addition, noncanonical effects of Notch1/2 receptors may exist in the regulation of IFN- γ production, although the significance of this finding remains unclear.³³ How exactly Notch regulates Th1 responses remains to be fully clarified and could be context-dependent, with the bulk of current evidence supporting its role in Th1 function, rather than initial polarization.

Th2 responses, which are critical for parasite immunity and allergic responses, have also been studied for their regulation by Notch signaling. The initial observations of Th2 impairment upon Dll-driven Notch signaling³⁰ have been further expanded to identify a key role for Notch signaling in development of Th2 responses *in vivo*, in part through direct regulation of *Il4* and *Gata3*.^{35,36} In Th2 disease models, including infectious diseases, genetic Notch inhibition (*Notch1/2* inactivation or DNMAML expression) and pharmacological interventions led to failure of Th2 polarization, reducing disease symptoms (asthma),^{38,39} or impairing protective Th2 responses (*Trichuris muris*).³⁵ Conversely, forced Notch stimulation via Jagged, but not Dll ligands supported Th2 development.^{30,38,53,58}

Regulatory T cells (Tregs) are major modulators of immune responses and maintain peripheral tolerance. Treg dysregulation and/or loss are associated with multiple disease states, in particular autoimmunity.⁵⁹ While multiple studies have probed the effects of Notch on Treg homeostasis using in vitro assays and gain-of-function strategies, key information was obtained in recent studies using loss-of-function approaches in vivo. Charbonnier et al⁴⁷ documented a direct antagonistic role of active Notch on Treg stability and function. Selective Notch inhibition only in Tregs allowed for emergence of a “super-regulatory” phenotype which was sufficient for long term protection in a GVHD model, whereas forced Notch activation impaired intrinsic Treg function. In addition, Notch overexpression decreased Treg suppressive capacity in a colitis model. While the destabilized and Th1 skewed Treg phenotype was predominantly the result of canonical Notch signaling, non-canonical Rictor-mediated effects contributed to the phenotype as well.⁴⁷ The role of Notch signaling induced by Dll1/4 ligands in Treg homeostasis has been probed in various disease models, including experimental autoimmune encephalomyelitis and type 1 diabetes, supporting a role of Dll4-mediated Notch signals in decreasing Treg numbers and antagonizing their function.^{58,60,61} Given these emerging data, it is unclear how to interpret the positive regulatory role of Notch in Treg homeostasis that had been previously suggested, and it is possible that these findings were contextual or that they were artefacts of gain-of-function approaches.^{62–67}

Notch ligand engagement was documented to impact cytotoxic CD8⁺ T cell differentiation, with Dll1 ligand enhancing Granzyme B production and cytotoxic effector responses.^{43,44,68} Notch was reported to regulate *Eomes*, which is required for the stability of CD8⁺ T cell memory and controls the expression of prototypic effector molecules such as IFN- γ and Granzyme B.⁶⁹ Notch has been increasingly studied in adaptive CD8⁺ T cell immunity as well. In infectious disease models (*Listeria monocytogenes*, Influenza), Notch activity was critical to ensure short-lived effector CD8⁺ T cell differentiation, in part due to direct regulation of *Il2ra* expression.^{45,46} Upon Notch blockade, the acquisition of effector molecules (IFN- γ , Granzyme B, and Perforin) and the ultimate functional impact was variably affected in different models.^{45,46} Data on the role of Notch signaling in CD8⁺ T cell memory responses are more limited, however Notch appeared to favor effector CD8⁺ T cell responses over memory formation.⁴⁶

While the proposed impact of Notch signaling in immunity is broad, the exact mechanisms of Notch-mediated effects remain uncertain. Moreover, Notch signaling inputs, when it comes to ligand sources and precise receptor-ligand interactions still remain uncertain. A possible explanation for the versatility of Notch effects in Th differentiation has been proposed by Bailis et al.³⁴ This study identified a facilitating Notch effect on Th polarization that rested upon its ability to simultaneously target multiple transcriptional Th programs, while sensitizing T cells to exogenous cytokines.³⁴ Based on this model, Notch only enhanced Th lineage commitment defined by environmental cues, but did not act as lineage-defining switch. Further evidence supporting this facilitator role in adaptive immunity, focusing on the very early events after antigen exposure, was recently brought forward by Laky et al.⁷⁰ In this study, antigen-specific responses were probed using transgenic T cells recognizing model antigens. A key facilitator role for active Dll4-driven Notch signaling emerged in shaping of CD4⁺ T cell priming, where Notch worked in concert with CD28-

driven co-stimulation, augmenting the effects of the PI3K/Akt pathway.⁷⁰ Notch enhanced T cell activation and proliferation, IL-2 production, and optimized the metabolic machinery, ultimately providing enhanced adaptive immune response. Altogether, these effects were reminiscent of costimulatory signals, suggesting that Notch may function as a new type of costimulatory modulator. In addition to early regulation of antigen-specific responses, metabolic Notch signature was also reported recently to sustain memory CD4⁺ T cells.⁷¹

In addition to T cell responses, Notch contributes to B cell immunity. Notch was reported to regulate the development of marginal zone B cells in the spleen in a dosage-specific manner,^{72–75} with recent data showing that continuous Notch signals are also required for the maintenance of these cells.⁷⁶ In addition, Notch was described to enhance follicular B cell activation and differentiation into antibody-producing cells.⁴⁸ Notch signaling was reported to induce B cell differentiation and class switching,⁴⁹ and in synergy with BAFF signaling promote survival of germinal center B cells via Jagged1-Notch1/2 interactions.⁷⁷

Beyond adaptive immunity, functions of Notch signaling are also being discovered in the regulation of myeloid cell subsets (including macrophages and dendritic cells), as well as innate lymphoid cells.^{78–87} The mechanisms and significance of these effects remain to be fully explored. Notch signaling can also play a role in the homeostasis of adult organs, as well as in tissue repair. For example, canonical Notch signaling is dispensable for the maintenance of adult hematopoietic stem cells,^{88,89} but Notch2 was reported to play a role in the speed of hematopoietic regeneration after transplantation.⁸⁹ In the intestine, a major target organ of GVHD, Notch signaling promotes differentiation into absorptive enterocytes and regulates intestinal stem cell homeostasis, while supporting epithelial regeneration after injury.^{90–92} Whether these effects and other potential functions of Notch signaling in tissue repair are relevant in the context of allogeneic transplantation remains to be fully investigated. So far, investigations into the immune functions of Notch signaling during transplantation have produced evidence supporting its consideration as a potential therapeutic target (Figure 2). Furthermore, targeting individual Notch ligands and receptors has emerged as a strategy to avoid the side effects of systemic pan-Notch inhibition, preserving intestinal and hematopoietic homeostasis and thus offering a therapeutic window.⁵⁷ Here, we will outline findings about the role of Notch signaling in allogeneic hematopoietic cell and solid organ transplantation, and propose new perspectives highlighting common features of Notch signaling in alloimmunity.

Notch signaling in alloimmunity

Notch in graft-versus-host disease

Mouse models of allo-HCT were instrumental in defining the critical role that Notch signaling plays in the development of alloimmune injury during GVHD, a major hindrance to the most widely used immunotherapeutic strategy in human medicine. After allo-HCT, transplanted naïve donor T cells recognize host antigens presented by APCs activated by conditioning injury and its cytokine storm.^{93,94} Additional co-stimulatory cues lead to T cell activation, proliferation, and acquisition of effector functions. Activated T cells exert anti-tumor responses, a major therapeutic benefit of allo-HCT. However, alloantigen recognition also leads to host tissue injury in the form of GVHD, blunting the therapeutic efficacy of the

procedure. The negative impact of GVHD is related not only to the severe morbidity and mortality of GVHD itself, but also to the need for global immunosuppression that increases risks of disease relapse, causes end-organ damage, and dramatically increases the odds of severe opportunistic infections in vulnerable hosts.^{93,94}

Using multiple mouse models of allo-HCT, our group documented GVHD protective effects of genetic strategies to block Notch activity in mature T cells.^{55–57} In these studies spanning multiple MHC-mismatched and matched models of allo-HCT, recipients of Notch-deprived T cells (via DNAM1L expression vs. *Rbpj* or *Notch1/2* inactivation) experienced long-term GVHD-free survival.^{55–57} When compared to wild-type T cells, Notch-deprived T cells showed profound defects in multiple cytokines (including IFN- γ , TNF- α , IL-2, IL-17 and IL-4), but preserved *Tbx21* and enhanced *Foxp3* expression. Notch inhibition only had a minor impact on overall proliferation of allogeneic T cells in vivo, but it significantly enhanced Treg expansion. Most importantly, Notch deprivation preserved graft-vs-tumor effects, as DNAM1L T cells exerted potent antitumor activity against A20 host-type lymphoma.^{56,57} Other studies in alternate models of mouse allo-HCT replicated the GVHD-protective benefits of Notch ablation. Protective effects of Notch inhibition were also reported in a mouse model of aplastic anemia induced by an allogeneic response.⁹⁵ Sandy et al⁵⁵ showed that Notch-deprived allogeneic T cells acquired impaired Ras/MAPK and NF κ B activity and expressed multiple negative regulators of T cell activation. Subsequent studies dissected the role of individual Notch receptors and ligands to facilitate therapeutic targeting of Notch signaling in GVHD.^{57,96} Using humanized neutralizing antibodies to individual Notch ligands and receptors as well as genetic strategies, Tran et al identified key functions for Notch1 and Notch2 as well as Dll1 and Dll4 Notch ligands in the pathogenic functions of Notch signaling during acute GVHD.⁵⁷ The Notch1-Dll4 axis was identified as dominant. Pan-Notch inhibition, either via use of GSI or combined Notch1/2 receptor blockade with neutralizing antibodies, however, was accompanied by severe intestinal damage. This on-target toxicity of systemic pan-Notch antagonism was due to the impaired intestinal regeneration, with conditioning by total body irradiation exacerbating the milder phenotype seen with steady-state Notch1 blockade.⁹⁷ Combined blockade of individual Dll1 and Dll4 ligands, however, avoided gastrointestinal side effects and led to long-lasting GVHD protection, with immunophenotypic T cell changes mimicking the effects seen with genetic Notch ablation. Simultaneously, Notch blockade via Dll1/4 targeting had no adverse impact on hematopoietic reconstitution, while anti-tumor efficacy remained potent.⁵⁷ Thus, Dll1/4 blockade in the peri-transplant period emerged as a promising new therapeutic approach to prevent GVHD.

Sandy et al documented that enhanced Treg recovery after Notch blockade was due to the expansion of preexisting donor Tregs, although it remained unclear if these expanded Tregs were important to mediate the protective effects of Notch inhibition after allo-HCT.⁵⁵ The role for Tregs in GVHD-mediated pathology has been well documented across multiple mouse models, in which adoptive Treg therapies mitigate, and Treg depletion aggravates GVHD.⁹⁸ Tregs are major effectors of emerging GVHD preventative strategies,⁹⁹ and their depletion is well described during both acute and chronic GVHD.^{100–102} A recent study by Charbonnier et al.⁴⁷ provided additional insights into Notch regulation of peri-transplant Treg homeostasis. In their study, using a MHC-mismatched allo-HCT model, the authors

identified a critical inhibitory role of active Notch signaling on posttransplant Treg homeostasis. Despite unperturbed Notch signaling in conventional T cells, conditional Notch ablation in Tregs alone was identified as sufficient to provide GVHD protection. Proposed mechanisms included induction of an enhanced suppressive phenotype with increased stability in the inflammatory environment.⁴⁷ Interestingly, a dominant role of Notch-inhibited Tregs was not observed in past work using DNMAML-mediated pan-Notch inhibition, since co-transplantation of Notch-sufficient and Notch-deficient T cells failed to control GVHD.⁵⁶ The precise role of Notch signaling in posttransplant Tregs vs. conventional T cells thus remains to be clarified. Nevertheless, existing observations all highlight the robust therapeutic potential of Notch inhibition in acute GVHD.

Recent evidence suggesting a role for hematopoietic APCs in providing Notch ligands driving alloreactivity were provided by Mochizuki and collaborators.⁹⁶ This study identified a subset of host-derived Dll4-expressing dendritic cells (DCs) in recipient animals. The same group recently identified a putative human counterpart of mouse Dll4-expressing inflammatory DCs.¹⁰³ In both settings, these cells were found early after transplant (week 3 onwards in humans) and promoted *in vitro* Th1 and Th17 polarization more effectively than naïve DCs. While these data were suggestive of a role for professional APCs in driving Notch signaling in allogeneic T cells, it is difficult to confirm directly the importance of these cells as a source of Notch ligands *in vivo*. Using genetic approaches allowing for *Dll1/4* inactivation in specific compartments and complementary biochemical strategies, our group demonstrated that both host and donor hematopoietic APCs were dispensable sources of Notch ligands to drive GVHD.^{104,105} Indeed, transplant recipients lacking *Dll1/4* within the hematopoietic system were not protected from GVHD and remained susceptible to systemic Dll1/4 blockade, while nonhematopoietic elements^{106,107} emerged as key sources of Dll1/4. Moreover, *Dll1/4* inactivation in fibroblastic reticular cells replicated all the benefits of pan-T cell and systemic Notch blockade.¹⁰⁵ Thus, the cellular source of Dll1/4 in GVHD may overlap with recently identified fibroblastic niches in secondary lymphoid organs that provide critical differentiation inputs to marginal zone B cells, subsets of Notch-dependent DCs and follicular helper T cells.¹⁰⁸

Our studies in multiple models of acute GVHD identified a narrow timing of posttransplant Notch activity that was essential for GVHD initiation. We found that Notch blockade conferred protective benefits when pursued within the 2-day immediate posttransplant period (¹⁰⁵ and unpublished). Together with the fact that hematopoietic cells are dispensable as a source of Notch ligands in GVHD,^{96,103–105} this finding provides an impetus to study the regulation of Notch ligand expression in nonhematopoietic cells, and their early interactions with incoming T cells. Mechanistically, another important line of investigation will be to identify the relevant transcriptional targets responsible for the broad immune effects of Notch signaling in allogeneic T cells, as well as in other immune contexts. The importance of putative non-canonical effects of Notch signaling remains unclear, given that the full functional benefits of Notch blockade are observed upon interference with the Notch transcriptional complex.^{55–57} Altogether, it is unlikely that non-canonical Notch effects play a major role in alloreactivity, as all major effects of Notch inhibition were observed upon interference with the ICN-CSL-MAML canonical transcriptional activation complex.^{55–57}

Published work identified active Notch as a master regulator of acute GVHD in mouse models of allo-HCT. Recent evidence of active Notch signaling in pathogenic T cells mediating GVHD in Rhesus macaques¹⁰⁹ adds to the growing knowledge supporting a translational potential for therapeutic Notch inhibition after allo-HCT. Whether there is a role for Notch ligands other than Dll1/4 in GVHD, and if active Notch contributes to posttransplant events beyond acute GVHD, remains to be seen. Chronic GVHD is another major cause of late morbidity and mortality after allo-HCT that is characterized by multifaceted immune dysfunction. Virtually all arms of Th immunity, humoral immunity, innate immune activation, as well as tissue responses, have been described to contribute to chronic GVHD pathogenesis.^{110–114} Given that a footprint of Notch activity has been identified to function in all these elements, future studies will help define the effects of Notch signaling in this area of unmet clinical need.

Notch in solid organ rejection

Acute cellular and chronic humoral rejection remain a major hindrance to the broader successful application of solid organ transplantation. If untreated, they can cause life-threatening organ failure that may require repeat organ transplantation into vulnerable patients. Treatment options bring limited success and are associated with high rates of opportunistic infections and can cause significant organ damage, both major contributors to the increased morbidity and mortality, and possible transplant failure. Key immunological events in acute and chronic rejection can be compared to the pathogenesis of acute and chronic GVHD, respectively. The role of Notch signaling in solid organ rejection was probed in mouse models of heterotopic heart allograft transplantation. Early findings in this field relied on artificial gain-of-function approaches and paradoxically suggested a role of Notch signaling as an inducer of transplant tolerance.^{64,68,115,116} However, recent observations using in vivo loss-of-function approaches have uncovered a proinflammatory effect of Notch signals in vivo that bears many similarities to its effects in GVHD.^{117,118}

To test the role of Notch in solid organ rejection, Riella et al¹¹⁷ used the BALB/c→C57BL/6 MHC-mismatched model of heart transplantation. The authors identified upregulation of Dll1 in professional hematopoietic cells and targeted Dll1 systemically with neutralizing antibodies. Dll1 inhibition, particularly when combined with co-stimulation blockade (use of CTLA4-Ig or *Cd28* knockouts), significantly prolonged allograft survival. The effects of Dll1 blockade were associated with Th skewing towards Th2 responses and decreased cytotoxic T cells, and they were dependent upon intact IL-4 production and STAT6 signaling.

Our group applied strategies of Notch blockade that had shown benefit in models of allo-HCT.^{56,57} In the same BALB/c→C57BL/6 model used by Riella et al,¹¹⁷ genetic pan-Notch inhibition in mature T cells significantly prolonged heart allograft survival, an effect significantly enhanced by peri-transplant CD8⁺ T cell depletion.¹¹⁸ In Notch-deprived recipients, a dramatic decrease of graft-infiltrating effector T cells, and in situ, but not systemic Treg expansion was noted. We tested the individual contributions of Dll1 and Dll4 ligands. Individual Dll1 or Dll4 neutralization via peri-transplant antibody-mediated blockade induced protective effects indistinguishable from those seen with genetic pan-

Notch inhibition in T cells. Dual blockade conferred enhanced benefit and improved organ survival beyond that seen with genetic Notch ablation with DNMA1L. Systemic Dll1/4 blockade conferred the same T cell modulating benefits by decreasing graft infiltration and skewing in situ T cell responses towards Tregs, but also significantly delayed humoral alloimmune responses. While DNMA1L recipients still developed delayed organ rejection accompanied by donor-directed antibody generation and in situ complement deposition, this effect was profoundly inhibited with systemic Dll1/4 blockade and correlated with decreased plasmablast and germinal center formation.¹¹⁸ These effects suggested that Notch inhibition may exert beneficial effects both on cellular and humoral mechanisms of transplant rejection.

In solid organ transplant models, much less is known about cellular sources of Dll1/4 than in allo-HCT. However, our preliminary data suggest a role for fibroblastic reticular cells as a source of Notch ligands, as seen in GVHD (Maillard, unpublished). Whether the temporal requirement for active Notch signaling via Dll1/4 is as narrow as seen in allo-HCT, remains to be seen. Altogether, our studies established key role for Notch ligands Dll1/4 in mediating solid organ rejection via effects that extend beyond T cell modulation, thus opening the door to new translational strategies.

Conclusions

Notch plays an essential role in alloimmune responses driving GVHD and solid organ rejection (Figure 2), 2 complications that limit the success of transplantation medicine. Notch-driven alloreactivity is controlled by Dll1/4 Notch ligands provided through cellular sources that extend beyond conventional professional APCs. Therapeutic targeting of Notch signaling in mouse models of allo-HCT and solid organ transplantation provides durable benefits, contingent upon early peri-transplant intervention. The therapeutic potential of Notch blockade is substantial, with early and transient targeting allowing for long-term protection without additional immunosuppression in preclinical models. This could provide new strategies to pave the way towards transplantation medicine free of calcineurin inhibitors, a long desired goal in the field.

Abbreviations

Allo-HCT	allogeneic hematopoietic cell transplantation
APC	antigen-presenting cell
CSL	CBF1/Suppressor of Hairless/Lag-1
Dll	Delta-like ligand
DC	dendritic cell
DNMA1L	dominant negative Mastermind-like
GVHD	graft-versus-host disease
HCT	hematopoietic cell transplantation

ICN	intracellular Notch
MAML	Mastermind-like family transcriptional coactivator
Th	helper T cell
EAE	experimental autoimmune encephalomyelitis
Treg	regulatory T cell

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Figure 1. The Notch signaling pathway

Four Notch receptors (Notch1–4) and 5 Notch ligands of the Jagged (Jagged1–2) and Delta-like (Dl1,3,4) families have been identified in mammals. Notch receptors are modified by Fringe glycosyltransferase, which modulates their interaction with Notch ligands. Receptor-ligand binding leads to extracellular cleavage of the receptor by the ADAM10 metalloprotease, followed by the intramembrane proteolysis via γ -secretase. Cleaved intracellular Notch (ICN) translocates into the nucleus. ICN associates with CSL (CBF1/Suppressor of Hairless/LAG-1; also known as RBPJ), a Mastermind-like (MAML) family

coactivator, and other transcriptional co-activators (CoA) to displace co-repressors (CoR) and build a large multiprotein complex that activates target gene transcription.

Figure 2. Effects of Notch signaling in the alloresponse

Inflammation and use of conditioning regimens in allo-HCT activate dendritic cells (DC) and nonhematopoietic cells, which express Dll1 and Dll4 Notch ligands. In concert with TCR and costimulatory molecules, Notch activates naïve T cells, leading to proliferation, Th polarization, proinflammatory cytokine production, and acquisition of effector functions, thus promoting GVHD or solid organ rejection (left). In addition, T cell help and Notch engagement in B cells facilitate alloreactive antibody production, contributing to tissue deposition and enhanced alloimmune tissue injury. Upon genetic or biochemical inhibition of Notch signaling (right), cytokine production is decreased and Treg expansion enhanced,

despite preserved overall T cell activation and proliferation. Decreased antibody production by B cells adds to an environment of enhanced tolerance characterized by diminished GVHD and long term solid organ transplant survival.