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Evolving Paradigms that Deterine the Fate of an Allograft

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Abstract

Despite the many advances in both immunological knowledge and the practical application of clinical immunosuppression, the holy grail of indefinite graft survival with immune tolerance in clinical solid organ transplantation remains a distant dream. The tremendous progress made in understanding the molecular and cellular basis of allograft rejection has not been translated into durable modalities that have advanced clinical care and outcomes. Indeed, currently used drugs and treatment protocols, largely directed at inhibiting alloreactive T cells, have not optimally improved allograft survival or function. A shift in emphasis, focusing on under appreciated immune pathways must now be considered to make further improvement. We highlight 3 areas of recent interest, complement, NK cells and lymphatics, which reinforce the concept that the transplant community must direct attention on how the immune system as a whole responds to a transplant. The current challenge is to integrate molecular, cellular, and anatomic concepts to achieve the equivalent of a unified field theory of the immune response to organ transplants.

Introduction

The current paradigm in transplant immunology is that in the absence of intervention, alloreactive T cells primed by alloantigens presented by donor and/or recipient antigen presenting cells (APCs) function as core mediators of the pathogenic rejection response via cytotoxicity and cytokine-mediated inflammation. Secondary involvement of B cells, antibodies, and macrophages contribute to graft destruction through a variety of effector pathways. While this paradigm continues to hold truth, basic advances over the past decade indicate innumerable intricacies and complexities that have altered our thinking about why an allograft might be accepted or rejected. Molecular modulators of the innate immune system, including Toll-like receptors (TLRs), cytokines, chemokines and complement impact the strength and character of the alloimmune response and independently contribute to graft injury. While dendritic cells (DCs) and macrophages are important contributors to graft injury and immune tolerance, recent work has shown that B cells as well as mast cells, basophils, eosinophils and natural killer (NK) cells exert control over alloimmunity and the decision to reject or accept an allograft. In addition, the transplant research community is only beginning to understand how various components of the immune repertoire interact in vivo in response to a transplant—where and when cells interact partially determine outcome.

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In this minireview, we highlight 3 areas that demonstrate that we must rethink our understanding of how the immune system as a whole responds to a transplant. Moving from molecules to cells to whole organism interactions, we will review recent experimental findings in complement biology, NK cell function and the physiology of lymph nodes (LNs) and lymphatics that influence transplant outcome. It is hoped that through an improved understanding of these interactive mechanisms, we will ultimately better devise incisive experimental approaches to prevent rejection and induce durable transplant tolerance, first in animal models and ultimately in the clinic.

Complement

Complement is part of the innate immune system. Complement activation is initiated through the classical, alternative or mannose binding lectin pathways which converge at the production of the C3 convertase. Cleavage of C3 and then C5 initiates formation of the membrane attack complex to yield soluble and surface bound split products that serve as chemoattractants, activators of innate immune cells and opsonins. Classical pathway activation functions as a key antibody-initiated, effector mechanism. Because unregulated complement activation has the potential to damage self cells, the host produces soluble and cell surface complement regulatory proteins. Decay accelerating factor (DAF) is one cell surface expressed regulator that functions by accelerating the decay of C3 convertases, preventing cascade amplification and limiting downstream complement activation (1). Other inhibitors include CD46 and CD59.

Traditional thinking regarding complement in transplantation is that the primary function of serum complement (liver-derived) is as an effector mechanism which underlies antibody-initiated vascular injury (2). Experimental work published over the last decade has revealed expanded roles for complement in transplantation. Complement can function as a “danger signal” and therefore contributes to ischemia reperfusion (IR) injury (3). IR injury is abrogated in animals deficient in C3 or factor B (but not C4) (3–5), and IR injury is exacerbated in animals deficient in DAF(6).

Paradigm shifting studies performed by Sacks and colleagues documented that the effects of complement on IR injury and graft rejection are dependent upon kidney-derived, not serum complement (3). Prolonged survival of C3 deficient kidneys was observed in allogeneic recipients with normal serum complement, and transplantation of C3-deficient kidneys into syngeneic hosts prevents IR injury. In contrast, wild type kidneys transplanted into syngeneic recipients with normal serum complement develop post-ischemic acute renal failure equivalent to controls. (3). This novel insight, that an extravascular pool of complement generated locally by the kidney graft is pathogenic in IR injury, raised the possibility that blocking local complement activation might be useful therapeutically. As proof of concept, therapeutic overexpression of a complement regulatory protein on graft endothelium limited the extent of IR injury following transplantation (7). Whether these findings can be translated into prevention of IR injury in human allograft recipients remains to be studied.

Another recent insight is that kidney-derived complement can participate in tubulointerstitial injury (3,5,8). C3 deficient kidneys are resistant to adriamycin-induced tubular damage and progressive renal failure, even when transplanted into wild type recipients (8). In C3a receptor (C3aR) deficient mice, adriamycin induced less kidney injury with lower expression of interstitial type 1 collagen and α-smooth muscle actin. The data support the concept that chronic tubulo-interstitial injury can be modulated by complement activation and that complement is locally derived. Whether complement activation contributes to the

pathogenesis of human chronic transplant injury, including interstitial fibrosis and tubular atrophy, is unclear and will require further investigation.

A final recent insight into nontraditional mechanisms of complement injury in transplantation is that T cells and APCs produce and employ complement to optimally function. The Heeger and Medof labs (9–13) showed that during cognate T cell/APC interactions costimulation upregulates alternative pathway complement components (C3, factor B, factor D). The resultant cleavage products, C3a and C5a, bind to their respective receptors, C3aR and C5aR, expressed by the T cells and act as essential downstream mediators of the costimulatory signals required for activation of naive CD4 and CD8 T cells. Signals transmitted by C5aR and C3aR activate phosphoinositide 3-kinase gamma leading to phosphorylation and activation of AKT, which then upregulates the anti-apoptotic gene Bcl2 and downregulates the pro-apoptotic molecule Fas resulting in an augmented effector T effector repertoire. Locally produced C3a and C5a also bind to and impact the activation of C3aR/C5aR-expressing APCs, inducing release of innate cytokines (e.g. IL-12, IL-23) and upregulating costimulatory molecules (e.g. CD80), which together amplify the effector T cell response (9–13). Studies using bone marrow chimeric animals revealed that these effects on T cell immunity are entirely mediated by immune cell-derived complement and are independent of serum complement (10–12).

Complement dependent effects on alloreactive T cell immunity and IR injury explain in part the fact that murine kidney allografts genetically deficient in C3 exhibit long term survival in wild type hosts (14), while those deficient in DAF have worse outcomes 9,12). Whether and how complement impacts the function of alloreactive T cell memory has not been assessed.

Selected studies suggest that immune cell-derived and/or graft-derived complement contributes to human transplant rejection. The quantity of mRNA for alternative pathway complement components and complement receptors is higher in human transplant tissue with histologic evidence of rejection compared to non-injured control tissue (15,16). Gene expression profiling of human kidney transplants reveals higher expression of several complement genes in deceased donor grafts with longer ischemic times, complement gene upregulation correlates inversely with early and late renal function (17), and donor kidney expression of a specific polymorphic variant of C3 is associated with worse posttransplant outcomes (18). These findings support diverse roles for complement in mediating human allograft injury.

What outstanding issues require further study in this field? Deciphering molecular mechanisms underlying the effects of complement on T cell immunity and testing whether complement blockade impacts T cell memory and human transplant survival should be high priorities. Whether and how complement, and in particular, immune cell-derived or graft-derived complement impact allograft tolerance remain incompletely understood.

NK cells

Natural Killer (NK) cells are innate immune cells involved in protective immunity and immune surveillance. They efficiently kill viral infected and transformed autologous cells without antigen priming (19). In contrast to T and B cells whose activation is triggered by stimulation of their clonotypic antigen receptors plus costimulatory signals, NK cell activation is controlled by a balance of signals transmitted via an array of stimulatory and inhibitory cell surface receptors. There are two structurally distinct NK receptors identified thus far, which include the Killer-cell Immunoglobulin-like Receptors (KIR) and the C type lectin-like family (Ly49, NKG2A-F, CD94). Self MHC class I molecules are the dominant ligands for inhibitory NK receptors. As a consequence, the absence or aberrant expression of

autologous MHC class I molecules, which commonly occurs following a viral inflection or tumor transformation, contributes to NK activation. The stimulatory NK receptors include NKG2D, NKp46, NKp44 and NKp30, which recognize a variety of activating ligands (e.g., MICA, MICB, RAE-1, ULBP-1) expressed by infected, inflamed, or injured cells. While the strongest likelihood of NK activation occurs when activating ligands are produced by cells lacking self MHC class I molecules, the absence of MHC class I or presence of activating ligands alone can be sufficient to activate NK cells under certain conditions (20). Once activated, NK cells are highly cytolytic, but also possess other effector functions, including producing copious amounts of the proinflammatory cytokines IFN-γ and TNF-α, and amplifying inflammation and adaptive immunity by activating and acquiring APC function (21,22).

NK cells have been hypothesized to participate in allograft injury because: a) MHC disparate graft cells lack self MHC class I, and therefore fail to engage NK inhibitory receptors; b) ischemic injury and tissue inflammation upregulate graft derived activating ligands, providing NK activating signals (18); and c) cytolysis and release of proinflammatory cytokines by NK cells can cause tissue damage. Despite these facts, the contribution of NK cells to solid organ allograft injury remains controversial. While numerous studies indicate that NK cannot mediate injury of vascularized allografts in the absence of T or B cells (23) and NK cells are not routinely detectable in grafts undergoing rejection, recent work revealed that if pre-activated by treatment with IL-15, NK cells can reject skin allografts in the complete absence of adaptive immune cells (23). The implication of this finding is that NK cells can act as potent effector cells in allograft rejection if certain NK stimulating cytokines are present.

NK cells may also act to amplify the adaptive immune response, which then contributes to allograft injury. For example, CD28 deficient mice readily reject MHC mismatched heart allografts and graft rejection in this model can be prevented by depletion of host NK cells (24). NK cells may activate DCs by releasing proinflammatory cytokines such as IFN-γ and TNF-α, which then promote T cell priming and allograft rejection when costimulation is limited. NK cells can also kill Foxp3+ Tregs, depending on the activation status of NK cells (25). This raises the intriguing possibility that NK cells may indirectly contribute to graft injury through negatively regulating Tregs (25). In fact, very little is known about the contribution of NK cells to the induction of peripheral immune regulation in transplant models and studies in this area are needed. Another potentially important issue is that KIR and KIR ligand genes are polymorphic and whether and how these polymorphisms impact the development of acute, and perhaps more importantly, chronic allograft rejection is unknown and requires further investigation.

While traditionally considered innate immune cells, recent data indicate that NK cells display some features that are reminiscent of adaptive immunity. They can acquire antigen-specific memory (26); express transcription factors involved in T cell differentiation (e.g., T-bet, GATA3, RORγt etc.) (27), and are susceptible to tolerance induction to viral antigens (28). The relevance and the impact of these “adaptive” features of NK cells in transplant models are unclear and need to be carefully examined.

Our notions of the roles of NK cells in transplantation have been further expanded by the recent, surprising finding that NK cells are required for tolerance induction in several model systems. Depletion of NK cells (but not NKT cells) prevented tolerance to islet and skin allografts induced by costimulatory blockade (29,30). Mechanistic experiments suggest that NK cell induced killing of graft-derived donor DCs limits priming of pathogenic anti-donor T cells, facilitating Treg dependent immune tolerance (30). Other very recent studies show that NK cells may produce IL-10 in order to inhibit or modulate local immune responses

(31). The precise in vivo conditions that control NK cell mediated injury versus tolerance induction have not been deciphered, but enhanced understanding of these issues could be of considerable importance in the design of therapies aimed at prolonging graft survival. The fact that NK cells can simultaneously perform both pro- and anti-inflammatory activities constitutes a major challenge in defining the exact role of NK cells in rejection and tolerance induction.

Together, these recent findings have expanded our understanding of NK cell biology and raise important testable questions about how NK cells impact transplantation. The data underscore the need to develop and test novel strategies aimed at modulating NK cells as adjuvant therapies to prevent graft injury and to facilitate tolerance induction.

Lymph nodes and lymphatics

Lymphatics transport antigen, lymphocytes, APC, and mediators such as cytokines and chemokines to draining lymph nodes (dLN) and the general circulation. Recent reviews of lymphatics describe morphologic structures, transcriptional regulators, and receptors that determine embryogenesis and development (31). These reviews also define aspects of lymphatic function in peripheral edema and tumor metastases. However, surprisingly little is known about cellular and molecular lymphatic function in inflammation, immunity, suppression and tolerance. There are several reasons why so little is known. First, there are very few lymphatic specific markers. Blood vascular endothelium and other cells, including leukocytes, share most markers. Second, it is extremely difficult to isolate purified lymphatic endothelial cells. Attempts to generate lymphatic vascular endothelial cell lines have not been repeatable, so that until recently there have been no lines available (32). Third, the advent of advanced imaging modalities has only lately permitted better analysis of cell structures and intercellular interactions in lymphatics and LNs. Several recent reports defined fibroblastic reticular cells and the reticular stromal network within LN that connect afferent lymphatics to the subcapsular space of the LN, and connect the subcapsular space to the deeper cortex (33). These structures are important for transport of soluble molecules and leukocytes. While these new reports are the first to define the morphology and function of these structures, their physiologic roles in the choice between immunity and tolerance remain mostly unexplored. For transplantation research, it will be important to devise drugs or methods to enhance or block these structures and determine their role in graft rejection or acceptance. For example, does the transient interruption of lymphatic drainage of vascularized grafts alter antigen presentation, immunity, and tolerance?

During acute and chronic inflammation new lymphatic vessels appear in inflamed tissues and the dLN. It is presumed that endogenous lymphatics undergo growth and sprouting, although there is little direct evidence to support this. Lymphatic vessels may also be derived from local inflammatory cells, such as CD11b+ macrophages. The local inflammatory infiltrate further contributes to lymphangiogenesis by the production of vascular endothelial growth factors (VEGFs) that promote tissue and LN lymphangiogenesis (34). In transplantation, there is de novo generation of lymphatics within chronically and acutely rejecting human kidney allografts (35). These lymphatic vessels are derived from both donor and recipient cells. It is not known, however, if the lymphatics participate in proinflammatory or suppressive events or both. Lymphangiogenesis is often accomplanied by the formation of ectopic or tertiary lymphoid organs (TLO), found in chronically rejecting grafts. The role of TLO is regulating rejection or acceptance is currently unknown.

Cellular and molecular analyses demonstrate that during inflammation lymphatics hypertrophy in the dLN, and B cells in the LN are required for this response. Lymphangiogenesis inside the LN then promotes DC mobilization from the surrounding

tissues into the LN, thereby enhancing immunity; so that blocking DC recruitment to afferent lymphatics can prevent CD8+ T cell responses, and the tissue lymphatics themselves can regulate DC recruitment in order to suppress immunity. Additional studies demonstrate synchronous changes in afferent lymphatic endothelial cells and high endothelial venules during inflammation that are regulated by DC, VEGFs, fibroblastic reticular cells, and lymphotoxin (LT) (36–39). Inhibition of VEGF receptors by mAb blockade suppresses these interactions and regulates immunity (40). Together, these recent studies demonstrate complex communication among peripheral tissues, afferent lymphatics, intranodal lymphatics, and dLNs. The roles of these interactions in graft fate are not currently known. Likewise, the molecular and cellular factors that regulate this complex communication are also not known, suggesting research opportunities that will yield important intellectual and therapeutic principles.

With regard to the role of LNs and lymphatics in the choice between immunity and tolerance, the results show a range of effects. For immunity, entry of tumor cells into afferent lymphatics and the dLN is important for antigen presentation and tumor immunity, and failure to enter these spaces can result in tolerance (41). Lymphatics have specialized endothelial junctions that regulate lymphocyte entry, and Bromberg and colleagues showed that the immunosuppressant FTY720 regulates T cell entry into afferent lymphatics and dLN (32). In allograft models, presentation of alloantigen in the dLN is important for immunity, and lack of presentation can result in immunological ignorance (42. On the other hand, transport of soluble antigen or migration of APC from afferent lymphatics into dLN results in suppression and/or tolerance, with generation of suppressive T cells (Treg) that function within the LN (43–47). NKT cells may participate in this regulatory process by recruiting specialized DC into the LN (46). The LN also contains specialized stromal cells that regulate T cell tolerance and responsiveness (47). The Bromberg lab demonstrated that in the setting of exogenous immunosuppression, tolerance occurs within the LN, while immunogenic events resulting in graft rejection occur outside the LN (48). Together these studies demonstrate that afferent lymphatics and LNs play key roles in both immunity and tolerance, but the regulatory mechanisms that determine the choice between these two divergent outcomes remain to be fully defined. What is needed now is research to define the controlling principles of the choice between immunity and tolerance in the LN, with the likelihood that such discoveries will lead to novel therapeutics.

One set of regulatory mechanisms for LNs and lymphatics involves the lymphotoxin (LT) family, a complex multi-ligand and multi-receptor subset of the TNF superfamily. T and B Lymphocytes express the cell surface LTα1β2 trimer that binds to LTβR on other cells, such as DC, blood and lymphatic vascular endothelial cells, and stromal cells of the LN and spleen. LTβR has another ligand, LIGHT, which may also be expressed by many leukocytes. LIGHT can also bind to HVEM and the decoy receptor DCR3. Other members of this family include membrane and secreted TNF, and secreted LTα3 trimer. These latter three ligands all bind to the TNF receptors TNFR55 and TNFR75. Additional members of this superfamily include CD160 and BTLA, and BTLA also binds HVEM (49).

LTα, LTβ, and LTβR regulate lymphoid organ embryogenesis and structure (49). These receptors and ligands also regulate immunity, so that the CCR7 ligands CCL19 and CCL21, crucial for LN migration, induce the expression of LTα1β2 on T cells; the interaction of T cells and B cells with LTβR is required for their survival; and the interaction of LTα1β2 with LTβR on DC is important for T cell priming and homeostasis. LTβR on the endothelial cells is also required for the remodeling of secondary lymphoid organs and blood and lymphatic structures that occurs during immunity (36,50), and remodeling depends on interactions between DC and endothelial cells mediated by VEGF (37). Together these data suggest intriguing interactions mediated by LT among a variety of leukocytes and

endothelial cells that are required for lymphatic responses within dLN during immune responses. These interactions likely regulate the choice between immunity and tolerance. Thus, there is increased expression of LTα, LTβ, and other members of the LT and TNF families by Treg (51); LIGHT/HVEM promotes immunity (52); while BTLA/HVEM is important for activated Treg effector suppression (53). Preliminary data (JSB, unpublished) confirm that: a) Treg express increased LTα and LTβ; b) $LT\alpha^{-/-}$ and $LT\beta R^{-/-}$ strains have altered development and migration of Treg; c) LTβR deficiency promotes Treg trafficking from blood to LN; and d) LTα blockade prevents Treg lymphatic migration and tolerance. Together these findings demonstrate an essential role for LT in the function of T cells, lymphatics and LN; and in the choice between tolerance and rejection. The general model that emerges is one where the anatomic structure of the immune system is the guiding determinant of the type of immune response that is initiated and sustained. What is needed now is further definition of the controlling principles of structure and function in order to create new therapeutic approaches.

Perspective

Immunity, regulation, graft rejection versus acceptance, and eventually tolerance have proven to be extraordinarily complex. Discoveries of new molecules, cells, functions or pathways have each led to the hope that the field has finally reached the point that reliable immune manipulation can now be achieved. History has taught us, however, that we have not discovered all the important guiding principles of immune regulation. The brief reviews here highlight unexpected and important roles for components that were thought already to have been thoroughly characterized, but now suggest that there are many layers of regulation and control yet to be discovered. One of the great challenges now is to integrate molecular, cellular, and anatomic concepts and come up with the equivalent of a unified field theory of the immune system. Once that perspective is gained, we may finally be poised to make the major leaps forward in clinical care and outcomes.

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Abbreviations

APC	antigen presenting cell
DAF	decay accelerating factor
DC	dendritic cell
dLN	draining lymph node
IR	ischemic reperfusion
KIR	killer-cell immunoglobulin-like receptors
LN	lymph node
LT	lymphotoxin
NK	natural killer cell
TLO	tertiary lymphoid organ
TLR	Toll-like receptor

Treg	regulatory suppressive T cell
VEGF	vascular endothelial cell growth factor

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