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Patients, Pathogens, and Protective Immunity: The Relevance of Virus-Induced Alloreactivity in Transplantation

Brent Koehn,* Shivaprakash Gangappa,* Joeseph D. Miller,† Rafi Ahmed,† and Christian P. Larsen†,*

Successful transplantation requires the establishment of an ongoing state in which there is simultaneous inhibition of the undesired T cell-dependent rejection response and yet retention of the ability to develop effective cell-mediated primary and memory responses to pathogens. The complexity of achieving such a precarious state is underscored by the growing body of evidence that alloreactivity can be profoundly influenced by infections that occur before, concurrent with, or subsequent to an organ transplant. In this review, we explore the growing list of mechanisms that have been identified by which pathogen-host interactions might influence rejection, including the degeneracy of TCR recognition leading to cross-reactive immune responses, the effects of pathogens on innate immune mechanisms, and the potential impact of virally induced lymphopenia. The Journal of Immunology, 2006, 176: 2691–2696.

In recent years, organ transplantation has emerged as standard medical care for many forms of end stage organ failure. Successful organ transplantation requires treatment approaches that provide effective control of the undesired T cell-dependent rejection response while permitting the maintenance of protective immunity against pathogens. Given the potent nature of the rejection response (1–4) (high frequency of alloreactive T cells) and the need to respond to a diverse array of infectious agents, this is a conceptually daunting task—using either current immunosuppressive regimens or by tolerance induction strategies. In this brief review, we focus primarily on the latter, that is, on how viral infections and protective immune responses adversely influence our efforts to control or prevent alloimmune T cell responses and thus heighten the barrier for achieving tolerance to allografts.

Pathogens and transplantation: points of intersection

For decades the development of new immunosuppressive or tolerance strategies has largely been conducted in inbred rodents that are housed in specific pathogen-free conditions. It is now increasingly recognized that while these model systems offer a high degree of reproducibility and serve as powerful tools for mechanistic studies, the clean lab mouse is far removed in many respects from the critically ill patients presenting for transplantation. Much like the field of autoimmunity, recent attention in the field of transplantation has focused on the effects of pathogens and protective immunity in the transplant setting. There is evidence that both the type of pathogen and the timing of exposure with respect to transplantation can have a powerful effect on the fate of an allograft. In addition, individual pathogens may have effects in specific donor-recipient pairs (see more in cross-reactivity below). It is also pertinent whether a pathogen causes an acute infection, which is or has been cleared, establishes latency, or persists as a productive infection. Similarly, the effects of an encounter with a given pathogen can vary dramatically dependent on if the infection occurs concurrently with, before, or after transplantation. For example, mice challenged with strains of the lymphocytic choriomeningitis virus (LCMV)3 that cause an acute infection concurrent with a transplant can accelerate rejection and prevent tolerance induction (5, 6). In contrast, infection after transplant tolerance is established does not disrupt allograft survival. Prior infection with the LCMV Armstrong strain prevents tolerance induction in very small percentage (10–15%) of recipients (7), whereas prior infection with LCMV clone 13, which differs only by two amino acids but establishes a persistent infection, completely abrogates susceptibility to tolerance induction for the life of the recipient.

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2 Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; DC, dendritic cell.
mouse (8). Similarly, difficulties have also been seen for mice infected with the persistent parasitic pathogen, *Leishmania major* (9).

Prior infections with pathogens that are cleared can also have profound effects. In an effort to mimic the broadly exposed immune history of humans in an experimental tolerance model, mice that had been serially infected with multiple acute non-persistent viral pathogens (vaccinia, vesicular stomatitis virus, and LCMV Armstrong) were rendered highly refractory to tolerance induction (7). Finally, another consideration that may influence the effects of a pathogen on allograft survival is whether the agent infects the allograft itself. Examples of clinically important viruses that infect the transplanted organs include hepatitis B and C in liver transplantation and polyoma virus in kidney transplantation. In summary, there is mounting experimental evidence suggesting that a more detailed understanding how viral pathogens affect alloimmune responses will be crucial in the ongoing efforts to develop safer and more effective immunosuppressive strategies and ultimately tolerance induction protocols.

**The T cell repertoire, diversity, specificity, and degeneracy in the recognition and response to pathogens and allografts**

To defend against an ever-changing panoply of pathogens, the mammalian immune system has evolved the capacity to generate an extraordinarily diverse repertoire of T cells-bearing receptors, which are capable of discriminating foreign peptides presented in the context of self-MHC. Although the theoretical upper limit of diversity may exceed $1 \times 10^{13}$, it is estimated that the actual number of different clonotypes expressed is closer to $2 \times 10^6$ in mice and $2.5 \times 10^7$ in humans (reviewed in Ref. 10). While the specificity of TCR recognition is often emphasized as a hallmark of adaptive immunity, if each TCR could recognize only a single MHC-peptide complex, then the actual repertoire would be incapable of expressing enough unique TCRs to cover the entire "antigenic universe" (10). Experimental evidence indicates that, in fact, TCRs show a considerable degree of degeneracy in their recognition of MHC-peptide complexes (11–13). Similarly, mathematical modeling estimates suggest that one TCR might recognize $>10^6$ distinct peptide/MHC complexes (10).

While degeneracy clearly is important, specificity is nonetheless a cardinal feature of the adaptive immune response. During pathogenic challenge, T cells bearing appropriate TCRs are activated, undergo rapid clonal expansion, and differentiate into effector cells, which bring about clearance or control of the infection. Upon resolution of the infection, $>90\%$ of the expanded T cell population undergoes apoptosis, whereas $<10\%$ is retained as a pool of Ag-specific memory cells. Memory cells are an important feature of the immune system allowing for rapid response and clearance of pathogens that have been encountered previously (14–16). Memory T cells are phenotypically distinct from their naive counterparts and have a lower activation threshold that facilitates a rapid recall response. Thus, paradoxically both specificity and cross-reactivity appear to be evolutionarily advantageous and integral characteristics of T cell-Ag recognition.

Although the immune system did not primarily evolve to reject transplanted tissues, it is nonetheless apparent that immune responses against tissues from nongenetically identical individuals within a species are particularly potent. This is due, at least in part, to the high frequency of T cells that respond to this allogeneic stimulus (1–4). Tissue or organ allografts can be recognized by recipient TCRs with affinity for donor-derived peptides on self-MHC molecules. This is known as indirect presentation in the transplant parlance. In addition to this conventional route of Ag presentation, a surprisingly high frequency of recipient T cells bears TCRs that directly recognize donor MHC-peptide complexes displayed on the surface of the donor APCs. In fact, in experimental systems, it has been found that 0.1–10% of an individual’s T cell repertoire is capable of reacting with alloantigens expressed by a given donor despite there being no history of prior exposure, a figure that is at least 200-fold greater than the estimated precursor frequency of virus-specific responses in naive individuals (1–4, 17, 18). By combining the estimates of the number of distinct clones in the repertoire noted above with an intermediate estimate of frequency of alloreactivity (~1%), mice and humans would bear ~20,000 and 250,000 distinct alloreactive clones, respectively (13).

**The cross-reactive immune response: virus infection-mediated enhancement of alloreactivity**

In recent years, there has been considerable interest in the influence of cross-reactive T cell responses on viral immunity and pathogenesis, autoimmunity, as well as transplantation. Welsh and colleagues have contributed a significant body of work demonstrating that T cells specific to previously encountered viruses participate in the immune responses to even unrelated viruses that are subsequently encountered (19–23). Importantly, these heterologous immune responses can contribute to either protective immunity or immunopathology.

It is now appreciated that even peptides with dissimilar sequences can still stimulate the same T cell; hence, cross-reactivity probably depends more on three-dimensional structures than on sequence homology, making it exceedingly difficult to predict which epitopes will be cross-reactive (24–27). In keeping with these observations, recent work has revealed that LCMV-immune mice are not only protected when challenged with a related Pichinde virus (19), but even show immunity toward an unrelated vaccinia virus (20, 21). Furthermore, heterologous infections have been shown to alter both the epitope hierarchy and the tissue distribution of memory T cells (22, 23). Several reports also provide evidence that cross-reactivity can be observed for unrelated human viral pathogens (28–31).

There is considerable evidence to suggest that heterologous immunity may be one of the principle mechanisms by which viral or other infections alter the alloimmune response. Given the extraordinary precursor frequency of alloreactive T cells and the degeneracy of T cell recognition, it is perhaps not surprising that alloreactive T cells become activated following viral infection (Fig. 1). It has been known for some time that T cells reactive to a wide variety of alloantigens may be activated during an immune response to a virus in rodents (32–34). Since then, broad-based cross-reactivity has been described in more detail in LCMV-immune B6 (H-2b) mice; four unique LCMV epitopes that were stained by tetramer were found to have a subset of alloresponsive T cells against either H-2b or H-2k (35). Furthermore, through the use of $\alpha$-chain–/– mice that are incapable of secondary $\alpha$-chain rearrangement, the authors found that dual TCR expression couldn’t account for their observations (35).
The effects of cross-reactivity could be operative for infections that occur before, at the time of, or subsequent to the transplant. Protective immune responses to certain viruses such as LCMV can be generated independently of CD28 and CD40 costimulation. In the setting of an LCMV infection that occurs concomitantly with a transplant, tolerance induction protocols are unsuccessful. T cells with dual specificity for donor alloantigens and viruses that would normally be rendered tolerant by a CD28 and/or CD154 blockade transplant tolerance induction protocol in the absence of a virus become activated and mediate rejection in a CD28/CD154-independent manner (5, 6).

**Heterologous immunity: good news for protective immunity but a challenge for transplantation**

The cumulative effects of prior exposures to viral or other pathogens can also have profound effects on the rejection or tolerance induction. For example, not only does acute infection with LCMV of B6 mice prime T cells that are reactive to BALB/c alloantigens during infection, but after the virus is cleared, these cells are retained as a long-term alloreactive memory population (5, 6). Memory T cells pose a unique barrier because of their lower threshold for activation (reduced requirements for CD28 and CD154), longevity, and increased frequency (compared with their naive parent clone). In B6 mice, serial exposure to acute viral infections leads to progressive accumulation of alloreactive memory T cells. When a critical threshold of memory cells is reached either by serial infection or by adoptive transfer of donor-specific memory cells, a potent barrier to tolerance induction is observed (7, 35). This is in accord with work from Welsh’s and Heeger’s groups showing that primed/memory T cells resist CD154 blockade-induced tolerance (36, 37).

The need to develop new approaches to address the barrier that memory T cells pose to tolerance is particularly acute as the immunomodulatory agents that are currently used or studied, such as rapamycin, cyclosporine, CD28 costimulatory blockade, or T cell depletion, are often ineffective at suppressing memory responses (38). Therefore, alternate strategies must now be developed to specifically inhibit allomemory T cells while maintaining protective immunity. Evidence for functional heterogeneity within the memory T cell compartment is becoming increasingly appreciated and appears to be largely dependent upon the initial priming conditions (i.e., type of pathogen, route of infection, size of inoculum), Th1- or Th2-polarizing conditions, and so on. It is likely that distinct approaches will be needed to overcome memory responses generated by prior exposure to donor Ags (transfusion, pregnancy or prior transplant) as opposed to cross-reactive memory induced by viral or other pathogens (39, 40). These therapeutic strategies might involve transient targeting of distinct or auxiliary costimulatory pathways used by memory cells, manipulation of survival/death pathways, cell cycle inhibitors, or via exploitation of Ag-specific regulation (41–43). As candidate approaches are developed, it will be critical to rigorously determine whether cross-reactive memory can be eliminated or controlled while retaining sufficient non-cross-reactive memory to preserve protective immunity.

There is evidence that cross-reactive responses elicited by environmental exposure are also likely to influence transplantation in humans. More than 15 years ago, Lombardi et al. (44) explored the contribution of naive and memory population to the allogeneic MLR in an article provocatively entitled “Are primary alloresponses truly primary?” Subsequently, direct evidence for cross-reactivity has been provided by studies showing that human clones specific for an EBV peptide presented in the context of HLA-B8 also recognize three common allogeneic HLA molecules (B14, B44, or B35) (45–47). Interestingly, HLA-B44 was identified as an “immunogenic” rather than a “permissive” mismatch for HLA-B8 renal allograft recipients on the basis of reduced allograft survival in large registry analysis (48). Further analysis has confirmed that the anti-EBV response is sufficiently broad to accommodate the specific loss of the alloxreactive subset while maintaining viral control (46). This suggests that approaches such as those developed by Burrows et al. (49, 50) describing the use an altered peptide ligand to an EBV epitope have the potential to antagonize the allospecific response without incapacitating EBV control.

Further evidence of the clinical importance of cross-reactive memory T cells has been provided by Heeger et al. (51), who have shown that a higher level of environmentally induced antidonor memory (i.e., not induced by prior transplant, transfusion, or pregnancy) is associated with a higher rejection rate in clinical renal transplantation. In summary, there is an increasing body of evidence to suggest that an individual’s past history of immune responses may influence the subsequent immune response to an allograft. This may be particularly true in adult human transplant recipients that have been exposed to multiple different pathogens over a lifetime.

**Influences of innate immune responses to pathogens on allograft survival**

It is increasingly recognized that, in addition to serving as the first line of host defense against pathogens, the innate immune system also plays a critical role in the programming of optimal adaptive antiviral immune responses. The early events in the innate response are mediated by myeloid cells and NK cells that sense pathogens or danger signals by using receptors for opsonized pathogens, germline-encoded pathogen pattern recognition receptors, and NK receptors for the detection of immune system also plays a critical role in the programming of optimal adaptive antiviral immune responses. The early events in the innate response are mediated by myeloid cells and NK cells that sense pathogens or danger signals by using receptors for opsonized pathogens, germline-encoded pathogen pattern recognition receptors, and NK receptors for the detection of immune
evasion strategies, such as MHC down-regulation (missing self receptors). Through these receptors, the cells of the innate immune system provide influential signals (rapid chemokine and cytokine production, enhanced Ag presentation, and up-regulation of costimulation molecule expression), which act in concert to enhance the efficiency and direct the character of the adaptive immune response. If infection occurs concomitantly with transplantation or perhaps even more importantly if infection occurs in the allograft, it is clear that these innate mechanisms may profoundly influence the strength and character of the alloimmune response.

Among the best characterized of the pattern recognition receptors are the TLRs. More than 11 distinct TLRs have been described, which detect an ever-expanding list of microbial associated molecular motifs (e.g., TLR4-LPS, TLR9-CpG DNA of bacteria and viruses, TLR7-sRNA, and TLR3-dsRNA) (52). Various TLRs are expressed by a wide variety of cell types, including granulocytes, monocytes, dendritic cells, NK cells, lymphocytes, endothelial cells, and stromal cells. Although a complete discussion of the effects on all of these cell types is beyond the scope of this review, we will emphasize briefly two mechanisms by which TLRs may allow viral infections to influence alloimmunity: 1) TLR and innate immune influences on cell recruitment and 2) TLR effects on dendritic cells (52).

The innate immune response to viral pathogens triggers rapid alterations in cell trafficking as an early event in host defense. In the setting of transplantation infection of the allograft, a virus (hepatitis B, hepatitis C, polyoma, CMV, EBV, and so on) may trigger a new wave or stronger recruitment of effector cells. TLR ligation on endothelial cells can up-regulate expression of selectins and whole host of chemokines, including IL-8, MCP-1, -2, and -3, MIP-1a and -1b, and RANTES, thereby enhancing the recruitment of a wide spectrum of inflammatory leukocytes. In addition, there is evidence that infections can specifically enhance the recruitment of T cells of the memory phenotype into sites of infection (53). Viral infections promote recruitment of memory populations into peripheral sites of infection regardless of specificity. Moreover, the local inflammatory environment promotes cytokine induced proliferation, though not overt activation of memory T cells without TCR stimulation (54). In one study, Ag-independent recruitment of memory T cells to the lung was found upon intranasal challenge, while naive T cell of the same specificity were excluded (55). Similarly, Bergmann and colleagues (56) reported that, during virus-induced encephalitis, memory CD8+ T cells with specificity for previously encountered unrelated pathogens comprise a significant proportion of CNS inflammatory cells. However, these cells were only transiently retained in the CNS in the absence of Ag recognition. Thus, viral infections of the allograft are likely to recruit polyclonal populations of memory cells that include virus-specific cells that are both cross-reactive with donor Ags and non-cross-reactive, creating an environment that lowers the threshold for T cell activation and making suppression more difficult.

Dendritic cells (DCs) play a pivotal role in the generation of T cell-dependent immune responses. Several distinct subsets of DCs act in concert as sentinels of the immune system by acquiring Ags in the periphery and transporting them to secondary lymphoid organs for presentation to T cells. During the process, DCs undergo a maturation process that involves alterations in chemokine receptor expression, up-regulation of MHC molecules and costimulatory molecules, and production of cytokines (e.g., IL-12). Virally derived TLR ligands provide critical signals for the generation of fully competent DCs (Fig. 2). For example, dsRNA derived from virally infected cells triggers signaling through TLR3 dramatically augmenting expression of CD40, CD80, CD86, IL-6, and TNF-α in CD8+ TDCs and promote efficient cross-priming of CTLs (57). Similarly, sRNA signals through TLR7 to activate plasmacytoid DCs. In contrast, activation of conventional DCs by sRNA is TLR3 independent (52).

A recent elegant study by Sporri and Reis e Sousa (58) sought to determine whether indirect activation of DC via host-derived inflammatory signals could substitute for triggering of pathogen recognition receptors. Although DCs that were activated indirectly were capable of up-regulating costimulatory markers and inducing T cell proliferation, they were unable to produce IL-12 or to drive differentiation of Th1 effectors like TLR-competent DCs. In contrast, DCs activated directly (cis) via TLR signals produced large quantities of IL-12 and were fully competent stimulators of T cell activation, proliferation, and differentiation. Although administration of poly(I:C) alone is not sufficient to reproduce the effects of LCMV infection in a model of virally induced abrogation of tolerance (6), it seems likely that pathogen pattern recognition/TLR-dependent mechanisms may contribute to heightened alloimmune responses that are observed with concurrent infections or those involving the allograft itself. In keeping with this notion, Zinkernagel’s group very recently reported that TLR ligation can convert quiescent autoreactivity to overt autoimmune disease (59). While there has been limited study on the role of TLR signals in models of viral infection and transplantation, Goldstein’s group has reported that the TLR-MyD88 pathway plays a role in the rejection of skin graft across minor Ag barrier even in the absence of viral infection (60).

NK cells are also potent mediators of innate immunity that are capable of influencing the rejection of allogeneic tissues. NK...
cells respond to viral pathogens through TLR and NK cell receptor systems that detect altered balances of self-MHC molecules and activating ligands. Human NK cells express functional TLR3, TLR7, and TLR8 receptors, which trigger IFN-γ production and cytotoxicity. Brehm et al. (61) studied the effects of viral infection on NK cell and CTL alloreactivity in vivo. They observed rapid increases in both CTL- and NK-dependent killing of allogeneic targets after LCMV infection in B6 mice.

Virtually induced lymphopenia and homeostatic proliferation

Viral infections are not only associated with stimulation of innate immune mechanisms but also with transient lymphopenia (62, 63). An emerging concept in the literature on both autoimmunity and alloimmunity is that lymphopenia (either clinically or virally induced) and the subsequent homeostatic proliferation can portend an increased risk of immunopathology (autoimmunity or rejection). Previous studies have shown that under lymphopenic conditions naive CD8 T cells can acquire characteristics of memory T cells in the absence of stimulation with specific Ag simply by the process of homeostatic proliferation (64). During this Ag-independent T cell differentiation pathway, expression of several memory markers (CD44, CD122, and Ly6C) became stably expressed, and these cells also became more responsive functionally to specific Ag. The authors predicted that these findings might have relevance in cases of disease or treatment-induced lymphopenia in transplant recipients or for autoimmunity because homeostatic proliferation of naive T cells requires interaction with self-peptide plus MHC molecules.

In the case of organ transplantation, T cell depletion is commonly used as therapy (65). Recent findings from Wu et al. (66) have implicated homeostatically dividing T cells in the development of resistance to tolerance induction. Furthermore, Pearl et al. (67) have shown recently that, following rigorous T cell depletion, effector-type memory T cells that are resistant to depletion are readily found in the periphery and even more significantly that they are uniquely associated with the graft itself at the time of rejection. Therefore, it is possible that virally induced lymphopenia may also adversely influence alloimmunity. Overcoming the barrier posed by lymphopenia-induced “memory” cells will require a better understanding of the functional properties of the costimulatory molecules and cytokines used during and after expansion as well as defining which aspects of this altered state are transient and which become fixed properties of T cells exposed to conditions of profound lymphopenia.

Conclusions

Patients, pathogens, and protective immunity have been closely intertwined since the beginning of transplantation more than 50 years ago. Before the advent of better diagnostics and antiviral therapies, infections with pathogens such as CMV and EBV were often fatal. Despite the tremendous advances in our understanding of viral pathogenesis and improved therapeutic options, our struggle with pathogens in transplantation is ongoing and ever changing. As we venture to apply the principles developed from immune tolerance research to clinical transplantation, it is increasingly apparent that further studies on the interactions of pathogens and protective immunity will be crucial in our effort to develop new immunosuppressive and ultimately tolerance regimens.


