ALTERATIONS OF IMMUNE T CELL RESPONSES BY HETEROLOGOUS IMMUNITY

The significance and characteristics of memory CD8 T cells in viral infections have been extensively studied. In many of these studies of T cell memory, experimental viral immunologists go to great lengths to ensure that their animal colonies are free of endogenous pathogens to design reproducible experiments. These experimental results are then proposed to provide the basis for our understanding of human immune responses to viruses. Although these findings can be enlightening, humans are not immunologically naïve, and they often have memory T cell populations that can cross-react with and respond to a new infectious agent or cross-react with alloantigens and influence the success of tissue transplantation. When activated, these cross-reactive T cells can modulate the immune response and outcome of subsequent heterologous infections, a phenomenon we have termed heterologous immunity.

Reports of pathogen-specific memory CD8 T cells recognizing cross-reactive epitopes on different proteins of the same pathogen or proteins from closely related or totally unrelated pathogens are increasing.1 Perhaps it is not surprising to observe cross-reactive T cell responses directed at evolutionarily conserved sites within virus groups, such as different strains of influenza virus2–4 or dengue virus5,6 or conserved sites between different members of the same virus group, such as hantaviruses,7 arenaviruses,8 and flaviviruses.9 However, examples of cross-reactive T cell responses involving completely unrelated viruses such as lymphocytic choriomeningitis virus (LCMV) and vaccinia virus (VV),10,11 influenza virus and hepatitis C virus (HCV),12 influenza virus and Epstein-Barr virus (EBV),13 influenza virus and HIV,14 and human papillomavirus and coronavirus,15 have now also been shown. These cross-reactive T cell responses are more frequently observed once memory T cell populations have been generated as a result of increased frequency and higher activation state of memory T cells.16–18 When cross-reactive immune responses are present, they can alter T cell dynamics and have considerable consequences on the pathogenesis of infection and either inhibit or enhance the replication of a newly encountered heterologous virus.19–22 They may have a significant impact on autoimmune diseases that have historically been associated with viral infections.23 They can also have a significant impact on allospecific T cell activity before and after transplantation.24,25 It is likely that an individual’s history of virus infections and the unique composition of the cross-reactive memory T cell pool may either initiate or reactivate T cells with alloreactive potential during transplantation.

CROSS-REACTIVITY, IMMUNODOMINANCE, AND T CELL RECEPTOR NARROWING

The CD8 T cell memory pool created after a virus infection has a distinct hierarchy of dominant and subdominant epitope-specific responses in a naïve host.26 This immunodominance hierar-
Public versus Private Specificity

T cells that are involved in many epitope-specific responses maintain distinct amino acid motifs in the TCR CDR3 between clonotypes and between different individuals, suggesting that these sites are required for the TCR to bind to the MHC-ligand structure. For example, in the human HLA-A2–restricted influenza A M1–58 Vb17 response, the amino acid motif IRSS is common,26 and in the allospecific H2-Kb–restricted HLA-CW3 Vb10 response in DBA/2 mice, SxG in the first three positions of the CDR3 region is a common motif.29 These similarities between individuals in Vβ usage and amino acid motifs, as well as conservation of immunodominance hierarchies, can be thought of as the public specificities of epitope-specific T cell responses. However, within these public motifs, there can be tremendous diversity in the TCR repertoire between individuals.29–32

The TCR on the antigen-specific T cell clones are unique to the individual, and these unique regions have been referred to as the “private specificity” for that epitope-specific response. This variation is probably a consequence of the random stochastic process of TCR rearrangement in the thymus, which results in variations in the naive peripheral TCR repertoire, and of the random stochastic process whereby a T cell encounters an antigen-presenting cell presenting its cognate ligand.32

Cross- Reactive Memory T Cells Alter Subsequent Immune Repertoires

Because of their high frequency and enhanced activation state, cross-reactive memory CD8 T cells have an advantage over naïve T cells, leading to an alteration in the hierarchy of T cell responses, as seen in sequential heterologous virus infections of mice with distantly related arenaviruses.8 Individual LCMV-immune mice challenged with VV varied in proliferative expansions of T cells specific to three different LCMV epitopes: NP_205 to 212, GP_34 to 41, and GP_118 to 125.10 This finding reflected the private specificities of the memory TCR repertoires that are unique to each individual mouse. T cell cross-reactivity involving two epitopes can select for a very small subset of the cross-reactive T cell population, leading to a substantial narrowing of the TCR repertoire (Figure 1).34 This narrowing of the repertoire had different patterns between individuals, reflecting the private specificities of the immune system that developed after the primary infection.

CROSS-REACTIVITY AND HETEROLOGOUS IMMUNITY: A BALANCE BETWEEN PROTECTION AND PATHOLOGY

Memory T cells that are cross-reactive with a heterologous virus can provide partial protective immunity and, in experimental models, can be the difference between life and death in the infected host.19,20,35,36 Experimental model systems have shown that T cells not only mediate protective immunity but also mediate substantial immunopathology.37–44 LCMV-immune mice displayed dramatically altered pathology upon VV infection, although viral load was decreased. LCMV-specific T cell infiltration and induction of panniculitis presenting as necrosis of visceral fat occurred in intraperitoneal infections19 and bronchiolitis obliterans, obstruction of bronchioles by fibrin and inflammatory cells, in respiratory infections.20 In humans, erythema nodosum, the most common form of panniculitis, and bronchiolitis obliterans are diseases of unknown cause but can be seen in some viral and bacterial infections and are also associated with autoimmune diseases.43–45 Erythema nodosum has been observed after vaccination for smallpox or hepatitis B. The development of bronchiolitis obliterans in lung allografts is associated with transplant rejection.45

Influenza-immune mice that had augmented viral replication upon LCMV or murine cytomegalovirus (MCMV) in-
infection also developed a severe consolidating mononuclear pneumonia with evidence of bronchiolization instead of the usual mild mononuclear infiltrate observed in acute MCMV infection of naïve mice. Bronchiolization involves bronchiolar-like cells’ replacing normal alveolar epithelium and is thought to be an indicator of lung repair.46

HETERLOGOUS IMMUNITY AND ALTERED PATHOLOGY IN HUMANS

Manifestations of heterologous immunity may therefore be a contributing factor in the variations observed in human disease pathogenesis thought previously to be affected only by genetic differences, the physiologic condition of the patient, or the inoculation route and dosage. Cross-reactive T cell responses and heterologous immunity remind us of the phenomenon of “original antigenic sin,” which was first described for B cell responses against influenza virus subtypes.47 Different strains and variants of influenza virus are cross-reactive at the T cell level, leading to speculations that these cross-reactive cells may be involved in the pathogenesis of influenza virus infections.5–4 Also, infection with one dengue virus serotype generates CD8 T cells with a higher avidity to a second and previously encountered dengue virus, suggesting that cross-reactive memory CD8 T cells preferentially expand over T cells with greater avidity to the serotype, causing infection.5,48 These lower avidity cross-reactive T cells may lead to a more severe disease outcome, such as hemorrhagic fever, observed in subsequent infections with different dengue virus serotypes.

EBV and Acute Infectious Mononucleosis

Many viral infections, such as measles, mumps, chickenpox, and EBV, present with more severe symptoms in teenagers and young adults than in young children. A massive CD8 T cell response is pathognomonic of infectious mononucleosis, and the difference between a clinical and an asymptomatic acute EBV infection is the magnitude of the T cell response, not the viral load.49 These older individuals have a longer history of infections and presumably a more complex pool of memory cells than young children.50 A subset of T cells directed against a major HLA-A2.1 restricted immunodominant EBV epitope, BMLF-1280, can cross-react with the invariant HLA-A2.1–restricted influenza A virus epitope M1.8.13 Activation of these cross-reactive T cells was observed in some but not all patients with acute mononucleosis, perhaps again reflecting private specificities in the host response.13 Because of the large size of its genome, EBV likely presents an extensive pool of potential CD8 T cell epitopes that could activate other cross-reactive memory CD8 T cells of different specificities.

Analyses of the M1.8 TCR repertoire from two individuals who experienced EBV-associated acute infectious mononucleosis revealed a substantially different hierarchy of TCR usage than in healthy influenza A immune donors. This suggests that a skewed subset of the M1.8–specific TCR repertoire, probably those cross-reactive with EBV, was being stimulated to proliferate. It is interesting that these cross-reactive T cells behaved differently in their functional responses to each ligand (Figure 2). Some cross-reactive cells bound both tetramers and produced TNF-α, IFN-γ, and macrophage inflammatory protein 1β (MIP-1β) to both ligands; some bound only one tetramer but produced TNF-α, IFN-γ, and MIP-1β to the alternate ligand; and some bound only one tetramer but were able to produce only MIP-1β to the alternate ligand. It seems that how a cross-reactive T cell interacts with its alternative ligand is highly variable and that functional patterns of T cell cross-reactivity are indeed heterogeneous. Multiple techniques are required to detect T cell cross-reactivity, including tetramer staining and different functional assays. A potentially important factor in TCR interaction with its ligand is TCR avidity. The cross-reactive interaction could be too weak to bind tetramer stably but be sufficient to induce a distinct hierarchy of cytokine production.13,51

HCV and Fulminant Hepatitis

There is extreme variability in the pathogenesis of HCV in humans, ranging from asymptomatic to fulminant and from sterilizing to persistent infections.52
HCV encodes an HLA-A2–restricted epitope (NS31073 to 1081) that shares six of eight amino acids with the influenza epitope (NA231 to 239), and T cells from influenza-immune individuals with no evidence of a past HCV infection can often respond to the HCV epitope in vitro.13 Many people may be partially immune to HCV as a consequence of this cross-reactivity. However, two patients who developed fulminant necrotizing hepatitis upon HCV infection were noted to have a highly narrowed focusing to this cross-reactive T cell response between influenza and HCV.59 This study demonstrates that, as in the mouse studies, cross-reactive T cell responses can be associated with enhanced immunopathology.

HETEROLOGOUS IMMUNITY AND TRANSPLANTATION

The principles of heterologous immunity are also applicable for the induction of immune responses against foreign or allogeneic antigens. The alloreactive T cell repertoire in humans who have never been exposed to alloantigens contains cells of both naive and memory phenotypes.54,55 The presence of these memory T cells suggests that alloreactive T cells are activated by past encounters with environmental antigens. Cross-reactivity is an important mechanism for heterologous immunity in the context of virus-specific immune responses and also contributes to the activation of alloreactive T cells by unrelated antigens.60,19,24,36,56–64 The unexpected activation of alloreactive T cell responses by heterologous immunity is a significant barrier for the transplantation of foreign organs and for the use of costimulation blockade protocols.65–67

RAPID IDENTIFICATION OF NAIVE ALLOREACTIVE T CELLS DIRECTLY EX VIVO

Current protocols to examine alloreactive T cell responses directly ex vivo measure effector functions such as IFN-γ production and cytotoxicity that are not detectable in naive T cells.54,68–72 Although these function-based assays are sensitive tools to identify effector and memory T cells, they are not optimal for the rapid detection of naive-phenotype alloreactive T cells. We have shown that naive T cells (CD11a<sub>low</sub> and CD44<sub>low</sub>) produce TNF but not IFN-γ within 4 h of TCR engagement.73 Using this unique cytokine profile (TNF<sup>+</sup>/IFN-γ<sup>−</sup>) as a marker, we were able to detect naive alloreactive T cells after a short in vitro stimulation with allogeneic cells.74 This rapid production of TNF was used for the reproducible quantification of naive alloreactive T cells from both mice and humans directly ex vivo, and the frequency of TNF-producing alloreactive T cells detected ex vivo correlated with the ability of mice to reject implanted allogeneic cells. Moreover, the TNF assay allowed naive phenotype T cells (TNF<sup>+</sup>/IFN-γ<sup>−</sup>/CD11a<sub>low</sub>) to be differentiated from effector/memory alloreactive T cells (TNF<sup>+</sup>/IFNγ<sup>+</sup>/CD11a<sub>high</sub>) that were generated by previous exposure to alloantigens and from tolerized alloreactive responses (TNF<sup>−</sup>/IFN-γ<sup>−</sup>).74 The clinical application of the TNF assay may allow the identification of transplant recipients who have low levels of T cell reactivity against a specific donor tissue and thereby minimize the requirements for long-term immunosuppression. In addition, this assay will provide us with unique insights into the alterations that occur in the alloreactive T cell repertoire after viral infections and the induction of tolerance by co-stimulation blockade.

VIRAL INFECTIONS ACTIVATE ALLOREACTIVE T CELL RESPONSES

The presence of memory alloreactive T cells in humans who have never been exposed to alloantigens may be attributed to past viral and bacterial infections.54,55 Studies from our laboratory have shown that C57BL/6 mice that are acutely infected with LCMV generate effector CD8 T cells that recognize a broad range of allogeneic haplotypes.74,62,75,76 The alloreactive CD8 T cells activated by LCMV displayed allospecific cytotoxic activity and produced IFN-γ after stimulation with alloantigens. Allospecific CD8 T cell cytotoxicity is also detectable in mice that are acutely infected with Pichinde virus (PV), VV, and MCMV and in humans who are infected with EBV and have developed acute infectious mononucleosis.58–62 Importantly, the alloreactive CD8 T cells that are activated during acute infection are maintained into memory, indicating that viral infections may account for the detection of memory alloreactive T cells in humans.24,62 The activation of alloreactive T cells by infection and their survival into memory have significant long-term implications for the transplantation of foreign tissues and for the induction of tolerance against alloantigens.

The virus-induced activation of alloreactive cytotoxic T cells suggests that an acute infection would precipitate the rapid rejection of foreign tissue grafts. In humans, herpes virus infections have been associated with the rejection of transplanted tissues.79,80 Using an in vivo cytotoxicity assay, we have shown that viral infections induce a CD8 T cell–mediated rejection of allogeneic implants.81 For evaluation of the rejection of allogeneic implants, carboxyfluorescein diacetate-succinimidyl ester (CFSE)-labeled allogeneic splenocytes (H2<sub>b</sub> and H2<sub>k</sub>) were adoptively transferred into either naive or infected C57BL/6 mice (H2<sub>b</sub>), and their survival relative to co-transferred syngeneic splenocytes was assessed 20 h later. Because the in vivo cytotoxicity assay also detects natural killer cell–mediated rejection of allogeneic splenocytes,81,82 studies to examine T cell–dependent mechanisms were done in mice that were depleted of natural killer cells. CD8 T cell–mediated rejection of H2<sub>b</sub> and H2<sub>k</sub> splenocytes was detectable as early as 1 d after infection with either LCMV or PV, and this virus-induced rejection of the allogeneic populations reached maximum levels in mice that were infected for 3 d.81 These results indicate that the alloreactive T cells that are activated by a viral infection will mediate the rapid rejection of allogeneic tissues.
Cross-Reactivity between Virus-Specific T Cells and Alloantigens

The promiscuous nature of antigen recognition by the TCR enables virus-specific CD8 T cells to cross-react with antigens derived from unrelated pathogens and immunogens. Numerous studies have demonstrated that virus-specific CD8 T cells directly recognize alloantigens, and this cross-reactivity may account for the activation of allospecific T cells after infection. Experiments in our laboratory have shown that short-term LCMV-specific CD8 T cell clones that are generated from infected mice recognize both LCMV-infected cells and allogeneic cell lines. This finding is in agreement with previous and more recent studies demonstrating that CD8 T cell lines specific for influenza virus, Sendai virus, and vesicular stomatitis virus (VSV) for mice and human CD8 T cell lines specific for EBV and HSV recognize alloantigens. Recent experiments have also shown that human CD4 T cell lines specific for CMV or EBV cross-react with allogeneic MHC class I. In detailed studies of cross-reactivity during an acute infection of C57BL/6 mice (H2b), we showed that LCMV-specific CD8 T cells defined by MHC-tetramer staining produce IFN-γ after in vitro stimulation with allogeneic cell lines but not with syngeneic cell lines (Figure 3). This cross-reactivity was broad based in that a proportion of each of the four epitope-specific responses (GP33, GP276, NP205, and NP396) examined recognized H2d antigens. However this cross-reactivity also showed selectivity in that different proportions of the epitope-specific cells recognized H2d antigens. The selective nature of cross-reactivity was also demonstrated in the recognition of H2k antigens that again was broad based but more restricted as only two of the four epitope-specific populations were activated by H2k. Together, these results indicate that virus-specific T cells broadly cross-react with alloantigen, but the selective nature indicates that this cross-reactivity is an antigen-driven phenomenon and is dictated by the diversity of TCR expressed by antigen-specific T cells.

**Figure 3.** Cross-reactivity between virus-specific CD8 T cells and alloantigens. (A) CD8 T cells were recovered from B6 mice that were infected 8 d earlier with LCMV. (B) CD8 T cells were incubated with allogeneic cell lines (either H2d or H2k) for 5 h and then examined for the production of IFN-γ by intracellular cytokine assay. Alloreactive T cells produced IFN-γ after stimulation with alloantigen. (C) Virus-specific CD8 T cells were identified by staining with MHC class I tetramers loaded with LCMV-derived peptides. Cross-reactive cells were defined as both IFN-γ and tetramer positive.

Heterologous Immunity and Transplantation Tolerance

The prolonged survival of foreign tissues in transplant recipients requires a long-term state of generalized immunosuppression. As an alternative to the use of immunosuppressive therapies in transplant patients, novel protocols that specifically induce tolerance against donor antigens have been developed. The productive activation of T cells requires both TCR engagement (signal 1) and co-stimulation (signal 2), and therefore a blockade of co-stimulatory signals during exposure to antigen will specifically tolerate the responding T cells. The blockade of co-stimulatory signals, such as CD28:CD80 and CD40L:CD40 pathways, induces tolerance to alloantigens and allows long-term allograft survival. Co-stimulatory blockade establishes antigen-specific tolerance by the physical deletion of alloreactive T cells, by the induction of anergy, and by the induction of immunoregulatory mechanisms. Recent work from our laboratory and elsewhere has demonstrated that both viral and bacterial infections have unexpected consequences for the use of co-stimulation blockade to induce tolerance against alloantigens.

Acute Viral Infections

Acute infection of C57BL/6 with LCMV simultaneously with the initiation of co-stimulation blockade completely abrogated the induction of tolerance and resulted in the rapid rejection of allogeneic tissues. The viral infection activated alloreactive T cells, stimulating the proliferation of these cells with a corresponding increase in cell number. The use of co-stimulation blockade to induce tolerance to alloantigens was also unsuccessful in mice that were persistently infected with LCMV. These results suggest that the success of co-stimulation blockade is extremely susceptible to inflammatory conditions that are present during the initial phase of tolerance induction. The negative effects of a viral infection at this early time point of tolerance induction were mimicked by exposure to TLR agonists.
Coadministration of agonists to TLR2, TLR3, TLR4, or TLR9 with co-stimulation blockade rescued alloreactive T cells from cell death and resulted in the rejection of transplanted tissues. Together, these findings indicate that activation of the innate immune system during the earliest stages of co-stimulation blockade will bypass the requirement of CD40L:CD40 signaling in the antigen-mediated activation of T cells and result in rejection of transplanted tissues.

The death of alloreactive T cells occurs rapidly after the initiation of co-stimulation blockade, with a significant proportion of cells being deleted within 24 h. Despite this massive loss of alloreactive T cells, acute infection of mice with either LCMV or PV within 1 to 15 d after engraftment disrupted the induction of tolerance and resulted in the rejection of skin allografts (Figure 4C). In contrast to infection with LCMV or PV, injection of the TLR3 agonist poly(I:C) at the time of engraftment did not result in the rejection of skin allografts, suggesting that once the alloreactive T cell repertoire has been reduced by cell death, activation of the innate immune response is not sufficient to abrogate tolerance in the absence of viral antigens.

**Memory Alloreactive T Cells Generated by Viral Infection**

In humans, the pretransplantation frequency of memory phenotype alloreactive T cells, as determined by IFN-γ enzyme-linked immunosorbent spot assay, correlates with the risk for development of an acute rejection episode after transplantation. The results described previously indicate that memory alloreactive T cells are generated by previous viral infections, but the ability of these memory T cells to respond against a subsequent exposure to alloantigens and to mediate rejection is an unresolved issue.

In vitro experiments have indicated that memory CD8 T cells generated by viral infections will proliferate in vitro when cultured with allogeneic cells and that at least a proportion of these responding T cells are virus specific, further supporting a role for cross-reactivity. Studies by our group have shown that GP33-specific memory CD8 T cells derived from LCMV-immune mice proliferate in vitro when stimulated with H2d cell lines. Both CMV- and EBV-specific CD8 T cells from human PBMC proliferate after in vitro stimulation with irradiated HLA-mismatched lymphocytes. These studies indicate that under optimal conditions in vitro, alloantigens stimulate the division of virus-specific memory CD8 T cell and suggest that the cross-reactive memory T cells will respond against foreign tissues in vivo. We have observed that LCMV-specific memory CD8 T cells participate in the immune
response against skin allografts in vivo (M.A.B. et al., unpublished data, 2006). In these experiments, splenocytes from LCMV-immune C57BL/6 mice were labeled with CFSE and transferred into congenic hosts that subsequently received a skin allograft. In mice that were engrafted with allogeneic skin, virus-specific CD8 T cells were found to proliferate and to increase in number. Together, these experiments suggest that memory alloreactive T cells generated by previous infections actively respond to alloantigens and represent a long-lived barrier to the transplantation of foreign tissues.

Memory alloreactive CD8 T cells that are laid down after a viral infection present a specific impediment for the induction of tolerance by co-stimulation blockade. Memory T cells are less dependent on co-stimulatory signals to generate functional recall responses, and memory alloreactive T cells produced by previous exposure to alloantigens are refractory to the induction of tolerance by co-stimulation blockade.25,110–113 Memory alloreactive T cells generated by a single infection with LCMV are also resistant to co-stimulation blockade and will rapidly reject skin allografts.24 Moreover, mice infected sequentially with heterologous viruses have increased frequencies of alloreactive memory T cells and show an enhanced resistance to tolerance-inducing protocols.24,25 These findings demonstrate that memory T cells generated by previous viral infections will present a significant obstacle for the use of co-stimulation blockade to induce tolerance to allografts in patients who have been exposed to pathogens throughout their lifetime.

CONCLUSION

The immune system has evolved such that multiple diverse antigen-specific memory TCR repertoires accumulate over a lifetime. Memory T cells that are specific to previously encountered pathogens but that also cross-react with a newly encountered pathogen are preferentially maintained or expanded, such that the T cell repertoire specific to the previous pathogen becomes permanently altered. These activated cross-reactive memory T cells play a role in heterologous immunity by modulating the T cell immune hierarchy and the private specificity of individual antigen-specific TCR repertoires, leading to an alteration in the balance between protective immunity and immunopathology. Virus-specific T cell responses that are cross-reactive with alloantigens can also alter the memory allospecific T cell pool and influence graft rejection and tolerance induction. Thus, getting a certain infection in a host with a particular MHC and at the wrong time in a sequence of other infections might have significant detrimental consequences for the host. To understand the fine role that memory T cells can play in balancing the induction of protective immunity versus pathology, we need to learn more about the consequences of heterologous immunity and cross-reactive T cell responses.

DISCLOSURES

None.

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