Direct Visualization of Cross-Reactive Effector and Memory Allo-Specific CD8 T Cells Generated in Response to Viral Infections

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CD8 T cell cross-reactivity between heterologous viruses has been shown to provide protective immunity, induce immunopathology, influence the immunodominance of epitope-specific T cell responses, and shape the overall memory population. Virus infections also induce cross-reactive allo-specific CTL responses. In this study, we quantified the allo-specific CD8 T cells elicited by infection of C57BL/6 (B6) mice with lymphocytic choriomeningitis virus (LCMV). Cross-reactive LCMV-specific CD8 T cells were directly visualized using LCMV peptide-charged MHC tetramers to costain T cells that were stimulated to produce intracellular IFN-γ in response to allogeneic target cells. The cross-reactivity between T cells specific for LCMV and allogeneic Ags was broad-based, in that it involved multiple LCMV-derived peptides, but there were distinctive patterns of reactivity against allogeneic cells with different haplotypes. Experiments indicated that this cross-reactivity was not due to the expression of two TCR per cell, and that the patterns of allo-reactivity changed during sequential infection with heterologous viruses. The allo-specific CD8 T cells generated by LCMV infection were maintained at relatively high frequencies in the memory pool, indicating that memory allo-specific CD8 T cell populations can arise as a consequence of viral infections. Mice previously infected with LCMV and harboring allo-specific memory T cells were refractory to the induction of tolerance to allogeneic skin grafts. The Journal of Immunology, 2003, 170: 4077–4086.
LCMV infection was not a general bystander effect but was instead a specific activation (31). HY-TCR transgenic mice have an extremely limited T cell repertoire, with 30–50% of the CD8 T cells expressing transgenic α- and β-chains and the remaining cells expressing the transgenic β-chain with an endogenously expressed α-chain (32). CD8 T cells from HY-TCR transgenic mice responded well against both H2b- and H2d-expressing cells in mixed leukocyte cultures. However, after infection with LCMV, only H2b-specific responses were stimulated, suggesting that cross-reactive Ags and not a bystander effect drive the allo-reactive responses (31). Overall these results suggest that LCMV-specific CD8 T cells cross-react with allo-MHC molecules, though the identity of the cross-reactive viral peptides is not known.

We have used intracellular IFN-γ staining to visualize and quantify allo-specific CD8 T cell responses directly ex vivo from mice infected with LCMV. Infection of B6 mice with LCMV generated relatively high frequencies of virus-induced allo-specific CD8 T cells that dramatically altered the allo-specific CTL repertoire in the memory pool. We directly demonstrate that these allo-specific CD8 T cells cross-react with multiple viral epitopes and were not restricted to an individual LCMV-derivative peptide. The cross-reactivity between alloantigens and LCMV did not require the expression of dual TCR by individual T cells and was modulated by sequential virus infections. Finally, we show that the generation of allo-specific memory CD8 T cells by virus infection may have an important consequence for the host by being associated with a reduced ability to tolerize animals to allogeneic skin transplants.

Materials and Methods

Mice

Male B6 mice (H2b), BALB/c (H2d), DBA/2 (H2b), CBA/J (H2k), C3H (H2c), and B6.129S2-TercnullMomo were purchased from The Jackson Laboratory (Bar Harbor, ME) or the National Cancer Institute (Frederick, MD) at 4–5 wk of age. Mice expressing a single TCR α-chain were generated by crossing TCR α-chain knockout mice (B6.129S2-TercnullMomo) with wild-type B6 mice, resulting in F1 progeny that can have only a single α-chain gene per cell. All experiments were done in compliance with institutional guidelines as approved by the Institutional Animal Care and Use Committee of the University of Massachusetts Medical School (Worcester, MA).

Viruses and cells lines

LCMV, strain Armstrong, and PV, strain AN3739, stocks were prepared in baby hamster kidney cells (BHK21) as previously described (9). For the preparation of acute virus-specific T cell responses (7–9 days postinfection), mice were inoculated i.p. with 5 × 10^5 PFU of LCMV. To generate PV-immune mice, naive mice were inoculated with 2 × 10^6 PFU of PV that was purified through a sucrose gradient and diluted in HBSS. Mice were considered immune 6 wk or longer after virus infection. The RMA cell line (H2b), a Rauscher virus-induced T cell lymphoma, and the P815 cell line (H2a), a DBA/2 derived, methylcholangrene-induced mastocytoma, were maintained in RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with 10% FBS, 100 U/ml penicillin G, 100 μg/ml streptomycin sulfate, and 2 mM L-glutamine, and 2 mM L-glutamine. L929 (H2b), a continuous liver cell line derived from C3H mice were maintained in MEM (Life Technologies) supplemented as described above.

Synthetic peptides

Synthetic peptides listed were generated by either Genemed Synthesis (San Francisco, CA) or American Peptide (Sunnyvale, CA) and were purified with reverse phase-HPLC to 90% purity. Final products were analyzed by mass spectroscopy. Peptides used included the following: LCMV-NP369-404 (FQPQNGQFI), LCMV-GP33-41 (KAVYNFATC), LCMV-GP276-286 (SGVENGPGGYC), and LCMV-NP205-212 (YTVKYKNPL).

Assay for cell-mediated cytotoxicity

The 51Cr-release assay for quantitation of virus-specific CTL was based on previously published methods (14) and has been described in detail elsewhere (33). To prepare peptide-pulsed target cells, 3T1-crated RMA cells were incubated with 100 μM synthetic peptide for 1 h at 37°C and then washed three times. Allogeneic target cells were prepared by labeling with 51Cr for 1 h at 37°C. For effector cells, single cell suspensions were prepared from spleens, and erythrocytes were removed by lysis using a 0.84% NH4Cl solution. Effector cells were then added in graded E:T ratios, as indicated in the figures. The amount of radioactivity released in the supernatants was quantitated in a liquid scintillation counter (Wallac, Turku, Finland). Percent specific lysis was calculated as (E – S)/(M – S) × 100, where E equals the cpm released from targets incubated with lymphocytes, where S equals the cpm released from target cells incubated with no lymphocytes, and where M equals the cpm released from cells following lysis with 1% Nonidet P40 (USB, Cleveland, OH).

In vitro splenocyte cultures

Splenocyte cultures were maintained in RPMI 1640 supplemented with 10% FBS, 100 U/ml penicillin G, 100 μg/ml streptomycin sulfate, 2 mM L-glutamine, 1% (v/v) nonessential amino acids solution (Sigma-Aldrich, St. Louis, MO), 50 μM 2-ME, 1 mM sodium pyruvate, 10% (v/v) T-Stim culture supplement (BD Biosciences, Bedford, MA), and 50 mM α-methyl-d-mannoside. T cells were cultured in the presence of either P815 or L929 cells treated with mitomycin as previously described (34). Generally, after three passages in vitro the cultures were 90–95% CD8-positive.

Intracellular IFN-γ staining

IFN-γ-producing CD8 T cells were detected using the Cytofix/Cytoperm Kit (BD Pharmingen), as previously described (15). Splenocytes (2 × 10^6 cells) were incubated with either 5 μM synthetic peptide or the indicated cell lines (5 × 10^5 stimulator cells per sample) in the presence of 10 U/ml human recombinant IL-2 (BD Pharmingen), and 1 μl/ml GolgiPlug for 5 h at 37°C. Peritoneal exudate cells (PEC) from syngeneic or allogeneic mice were also used to stimulate CD8 T cells, as described for cell lines. PEC were isolated by peritoneal lavage from the indicated mice that had been injected i.p. with thioglycolate medium (1 ml) 3 days previously. Recovered PEC were gamma-irradiated (2000 rad) and then stored frozen at −70°C until needed. CD8 expression on the thawed, irradiated PEC populations was negligible. Alternatively, T cells were stimulated by a combination of 50 ng/ml PMA (Sigma-Aldrich) and 500 ng/ml ionomycin (Sigma-Aldrich). Following the incubation splenocytes were stained for cell surface markers. Samples were then fixed and permeabilized with Cytofix/Cytoperm solution and stained with either anti-IFN-γ (clone XMG1.2; BD Pharmingen) or an IgG1-isotype control (clone R3-34; BD Pharmingen). The samples were analyzed using a BD Biosciences FACSCTulor (BD Biosciences) and CellQuest Software (BD Biosciences). When shown, error bars are representative of SEM.

Tetramer staining

Peptide loaded H2Kb and H2Db tetramers were prepared as previously described (35). Splenocytes were first stimulated in vitro for 5 h with allogeneic cells and 1 μM/ml GolgiPlug was added for the final hour. For staining, cells were then blocked against nonspecific binding with anti-CD16/CD32 (Fcγ III/I receptor, clone 2.4G2, BD Pharmingen) and unlabelled streptavidin (Molecular Probes, Eugene, OR). Samples were washed and then coated with the indicated peptide-loaded tetramer and anti-CD8 (clone 53-6.7; BD Pharmingen) for 1 h at 4°C. A potential issue for tetramer-staining CD8 T cells that have been stimulated with their cognate ligand is that the TCRs are down-regulated from the cell surface, making staining with tetramer difficult as previously described (8). To circumvent this problem, brefeldin A was added to the samples for only the last hour of the 5-h incubation, which would allow normal trafficking of the TCR and result in enhanced tetramer staining on stimulated CD8 T cells. After washing twice with FACS buffer, samples were stained for intracellular IFN-γ as described above.

Skin transplantation

Thymectomized, adult B6 mice were infected with LCMV and then allowed to rest for at least 6 wk. Athymic LCMV-immune or athymic naive B6 mice were tolerated and then engrafted with allogeneic skin as previously described (36, 37). Briefly, 7 days before grafting, recipient mice were given i.v. 10^7 adult donor splenocytes (donor-specific transfusion (DST)). Recipient mice also received four doses of anti-CD154 mAb (0.25 mg) administered i.p. over the course of 2 wk beginning at the time of DST. BALB/c or C3H skin grafts (1–2 cm in diameter) were transplanted onto the dorsal flanks of the recipient B6 mice that had received DST from the same haplotype. Graft rejection was defined as the first day that the entire graft was rejected.
The method of Kaplan and Meier (38) was used to compare the graft survival among groups. The log rank statistic was used to test the equality of allograft survival distributions for LCMV-immune and uninfected mice (39). Values of \( p \leq 0.05 \) were considered statistically significant.

Results

Detection of allo-specific CD8 T cells

In Fig. 1A splenocytes from B6 mice infected with LCMV were used in a standard cytotoxicity assay to demonstrate the magnitude of both virus- and allo-specific CTL responses generated by infection. Target cells pulsed with either LCMV-NP396 or LCMV-NP205 were efficiently lysed by splenocytes from LCMV-infected mice, and the allogeneic P815 (H2\(^d\)) and L-929 cell lines (H2\(^k\)) were lysed to levels substantially above the background levels for the syngeneic RMA cell line. Splenocytes from uninfected B6 mice did not display cytotoxicity against any targets (Fig. 1B).

To quantify the magnitude of the allo-specific CD8 T cell responses generated by LCMV infection, the intracellular IFN-\( \gamma \) assay was used (40, 41). The effectiveness of the intracellular IFN-\( \gamma \)-assay to detect allo-specific T cells was examined by stimulating allo-specific splenocyte cultures (90–95% CD8 positive) with allogeneic cell lines in vitro, as described in Materials and Methods. As shown in Fig. 2A, H2\(^d\)-specific splenocyte cultures were specifically stimulated to produce IFN-\( \gamma \) when incubated with the H2\(^d\) cell line P815, but not with the H2\(^b\) cell line RMA. In addition, H2\(^k\)-specific splenocyte cultures produced IFN-\( \gamma \) when stimulated with the H2\(^k\) cell line L-929, but not with the RMA cell line (Fig. 2B). Both populations of CD8 T cells produced IFN-\( \gamma \) when stimulated with a combination of PMA and ionomycin, and background staining of these samples with an isotype-control Ab was negligible (Fig. 2). These results indicate that intracellular IFN-\( \gamma \) staining is an effective technique to quantify allo-specific CD8 T cells.

LCMV infection elicits IFN-\( \gamma \)-producing, allo-specific CD8 T cells

Splenocytes from B6 mice infected 8 days previously with LCMV were stimulated for 5 h directly ex vivo with either LCMV-derived peptides, PEC from allogeneic mice, or allogeneic cell lines and then stained for cell surface molecules and intracellular IFN-\( \gamma \) (Fig. 3). Stimulation with either LCMV-NP396 or LCMV-NP205
revealed the expected levels of IFN-γ-producing cells (Fig. 3A). Both allogeneic PEC (Fig. 3B) and allogeneic cell lines (Fig. 3C) also stimulated CD8 T cells to produce IFN-γ above the levels stimulated by syngeneic cells (B6 PEC or RMA). Splenocytes from naive B6 mice were not able to produce IFN-γ following stimulation with either viral peptides or allogeneic target cells in this short 5-h assay (Fig. 3D and data not shown). The kinetics for the induction of allo-specific CD8 T cell responses following LCMV infection were then compared with virus-specific CD8 T cell responses. Splenocytes from B6 mice infected 6, 8, 10, or 12 days previously with LCMV were examined for NP396-specific (Fig. 4A) or allo-specific (Fig. 4, B and C) CD8 T cell responses by intracellular IFN-γ assay. The allo-specific responses mirrored the development of the NP396-specific CD8 T cell response, with the total number of responding T cells peaking at days 8 and 10.

The results above demonstrate that IFN-γ-producing, allo-specific CD8 T cells were generated by infection with LCMV during the acute phase of the response. We next examined whether these allo-specific cells were maintained in the memory CD8 T cell pool. Splenocytes from LCMV-immune mice (15 wk postinfection) were stimulated with either LCMV-derived peptides or with allogeneic cell lines and then stained for intracellular IFN-γ. The expected frequencies of NP396-specific and NP205-specific memory CD8 T cells were detected in the LCMV-immune mice (Fig. 5A). Interestingly, significant levels of allo-specific CD8 T cells generated during the acute phase of the response against LCMV were also maintained in memory (Fig. 5B). The allo-specific CD8 T cells from LCMV-immune mice expressed high levels of CD44 and CD11a on the cell surface (data not shown), consistent with a memory phenotype. Splenocytes from naive animals did not respond to either viral peptides or allogeneic target cells (data not shown and Fig. 3D). Thus, infection with LCMV elicits allo-specific CD8 T cell responses that form a stable memory population.

Mechanism for the generation of allo-specific CD8 T cells by infection with LCMV

The induction of allo-specific CD8 T cells by LCMV infection could be explained by virus-specific T cells cross-reacting with alloantigens. To assess potential cross-reactivity of LCMV-specific CD8 T cells, splenocytes from mice infected with LCMV were stimulated in vitro with allogeneic target cells in short 5-h assay (Fig. 3D and data not shown). The kinetics for the induction of allo-specific CD8 T cell responses following LCMV infection were then compared with virus-specific CD8 T cell responses.

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tetramer, 10% binding the GP33 tetramer, 3% binding the GP276 tetramer, and 3% binding the NP205 tetramer (Fig. 6B). The proportions of tetramer-positive T cells reacting with H2a cells differed with each peptide specificity. For example, one-seventh of the NP396-positive CD8 T cells reacted with the P815 targets whereas one-twenty-sixth of the GP276-positive CD8 T cells did. Approximately 50% of the total number of P815-reactive cells could be accounted for by cross-reactivity with these four LCMV-specific epitopes. CD8 T cells stimulated to produce IFN-γ by in vitro culture with the NCTC-929 cell line (H2k) also showed a distinct pattern of costaining with LCMV-specific tetramers, with ~11% of the H2k-reactive T cells binding the GP33 tetramer, 9% binding the NP205 tetramer, and no distinguishable population binding to either the NP396 or the GP276 tetramer (Fig. 6C). The staining of allo-specific CD8 T cells with LCMV-specific tetramers directly demonstrates cross-reactivity between LCMV and allogeneic Ags. The cross-reactivity is not exclusively focused on individual epitopes, as it involves a variety of LCMV-derived peptides in distinct hierarchies. Moreover, cross-reactive CD8 T cell lines can be generated from LCMV-immune mice by culturing splenocytes with alternate in vitro stimulation with LCMV and allogeneic cells. One line is >90% specific for the LCMV-GP33 peptide with 60% of the CD8 T cells reacting with H2a-expressing cells (figure not shown).

Dual TCR expression is not required for the induction of allo-specific T cells after LCMV infection

One explanation for the ability of epitope-specific CD8 T cells to cross-react with allogeneic antigens is that some T cells may express two TCR-α-chains which are not subject to as effective allelic exclusion as are β-chains (42). This can result in the presence of two TCR with varying specificities on the cell surface. To examine this possibility, mice capable of expressing only a single TCR-α-chain were generated as described in Materials and Methods. As shown in Fig. 7A, F1 mice infected with LCMV were able to generate NP205-specific responses and allo-specific responses comparable to the wild-type B6 mice, as determined by intracellular IFN-γ staining. LCMV infection of F1 mice also elicited NP205-specific and allo-specific CTL responses that were similar to infected B6 mice (Fig. 7B). Thus, the ability to express two TCR-α-chains, and therefore two distinct TCRs, is not necessary for the induction of allo-specific CD8 T cells following infection with LCMV. These findings suggest that the CD8 T cells cross-reacting with allogeneic cells and LCMV express a single TCR.

Altering immunodominance of LCMV epitope-specific responses alters the hierarchy of allo-specific responses

The results above show that the T cell cross-reactivity between LCMV and allogeneic Ags. The cross-reactivity is not exclusively focused on individual epitopes, as it involves a variety of LCMV-derived peptides in distinct hierarchies. Moreover, cross-reactive CD8 T cell lines can be generated from LCMV-immune mice by culturing splenocytes with alternate in vitro stimulation with LCMV and allogeneic cells. One line is >90% specific for the LCMV-GP33 peptide with 60% of the CD8 T cells reacting with H2a-expressing cells (figure not shown).
were associated with alterations in the magnitude of the allo-specific responses. This finding is again consistent with the concept that the generation of allo-reactive CD8 T cells by infection with LCMV is driven by cross-reactive Ags and not a generalized bystander effect.

**Mice previously infected with LCMV resist tolerization to allogeneic skin grafts**

The results in Fig. 5 demonstrate that mice infected with LCMV have a stable population of readily activated allo-specific, memory CD8 T cells. Because allo-specific CD8 T cells are known to contribute to the rejection of allogeneic transplants (45, 46), we examined the impact of prior infection with LCMV on the maintenance of allogeneic skin grafts. B6 mice were thymectomized as adults, rested, and then infected with LCMV and rested for at least 6 wk. The athymic, LCMV-immune mice were then tolerized against alloantigen using anti-CD154 Ab and DST and were then engrafted with skin from either BALB/c or C3H mice, as described in Materials and Methods. This transplantation protocol has been previously shown to allow naive B6 mice to accept and maintain

**FIGURE 5.** LCMV-immune mice have a stable population of allo-specific CD8 T cells. Splenocytes from LCMV-immune mice (15 wk postinfection) were examined by intracellular IFN-γ staining directly ex vivo. Splenocytes were stimulated in vitro with either LCMV-specific peptides (A) or allogeneic cell lines (B). The value in the upper right quadrant represents the percentage of CD8 T cells producing IFN-γ. The results are representative of three separate experiments examining two to three mice per group.

**FIGURE 6.** LCMV epitope-specific CD8 T cells cross-react with allogeneic cells. Splenocytes from B6 mice acutely infected with LCMV (8 days postchallenge) were stimulated with RMA cells (A), P815 cells (B), or L-929 cells (C) and then stained with LCMV-specific tetramers and for intracellular IFN-γ, as described in Materials and Methods. For analysis, samples were gated on CD8-positive cells and then examined for tetramer and IFN-γ staining. The values represent the percent of CD8 T cells in each quadrant. The results are representative of three separate experiments examining two to three mice per group.
allogeneic skin grafts for extended periods (37). Fig. 9 shows that LCMV-immune mice rejected skin grafts from BALB/c mice (10 of 14 rejecting), and this rejection was statistically significant from that of naive B6 mice (p < 0.016, 0 of 5 rejecting). LCMV-immune mice receiving C3H skin grafts also rejected transplants (7 of 11 rejecting) to a greater extent than control mice (1 of 4 rejecting), though this did not reach statistical significance (p = 0.215), perhaps reflecting the lower frequency of H2k-specific vs H2d-specific CD8 T cells in LCMV-immune mice. These findings suggest that infection with LCMV establishes a memory population of allo-specific CD8 T cells that is refractory to tolerization and that can reject allogeneic transplants in mice treated with costimulation blockade.

Discussion

The degenerate nature by which T cells recognize Ag allows for individual T cells to recognize multiple targets (11, 47). This inherent degeneracy in the immune system reduces the number of individual T cells with unique specificities necessary to protect a host against a large and diverse array of potential pathogens. T cell cross-reactivity has been demonstrated between heterologous viruses (4, 5, 7, 9, 15, 26), between peptides with disparate amino acid sequences (3, 8, 48), between Ags derived from self and foreign proteins (16, 49), and between self and foreign MHC molecules (22, 50). Previous studies demonstrated that allo-specific CTL generated by infection with LCMV cross-react with LCMV-infected target cells, but the epitope-specificity of the cross-reactive CTL was not elucidated (24–26). In this study, we have quantified cross-reactivity between LCMV-specific CD8 T cells and allogeneic targets. Using MHC tetramers folded with LCMV-specific peptides, we directly demonstrate that allo-specific CD8 T cells cross-react with distinct LCMV-derived epitopes in a broadly based manner, with subsets of the peptide-specific populations cross-reacting with either of two allogeneic targets and with allogeneic targets cross-reacting with several peptide epitopes. Moreover, these allo-specific CD8 T cells activated during the acute response against LCMV are maintained in memory at significant frequencies. Such cross-reactivity between viral- and alloantigens suggests that an individual’s history of infections will greatly impact the T cell repertoire and have important ramifications for future attempts to transplant allogeneic grafts.

The seminal observations by Zinkernagel and Doherty (51) on the phenomenon of MHC restriction would on first consideration preclude the possibility for the recognition of allogeneic MHC by CD8 T cells. However, the recognition of Ag by the TCR has been demonstrated to be highly degenerate in nature, and CD8 T cell clones have been shown to efficiently recognize allogeneic MHC (52–55). Studies examining the contact between allogeneic MHC and the TCR of allo-specific CD8 T cell clones (Fig. 2C and BM3.3 clones) have indicated that the interaction mimics the diagonal binding orientation used when TCR bind to self MHC-presenting peptides and is similar to the recognition of self MHC
Although the overall orientation is similar, some portions of the cross-reactive TCR interact with polymorphic regions of the class I MHC molecules, indicating that the TCR interaction with allogeneic MHC has subtle distinctions from the engagement of self MHC molecules.

Our findings demonstrate that LCMV-specific CD8 T cells cross-react with allogeneic Ags with distinctive patterns. No single population of epitope-specific T cells elicited by LCMV infection could completely account for the T cells cross-reacting with either H2d or H2k cell lines. Using the NP396, GP33, GP276, and NP205 tetramers we accounted for ~50% of the CD8 T cells responding against H2d and 20% of the CD8 T cells responding against H2k. The remaining three known LCMV epitopes restricted by H2b (GP34-41, GP118-125, and GP92-102) are currently under evaluation for the ability to cross-react with allogeneic Ags. The results with single α-chain-containing mice presented in Fig. 7 indicate that the recognition of allogeneic cells occurs with CD8 T cells expressing a single TCR, although we cannot exclude the possibility that wild-type mice may have some cross-reactive T cells expressing two TCR. Cross-reactivity between LCMV-specific CD8 T cells and alloantigens was further confirmed by the observation that by altering the hierarchy of the virus-specific T cell response, the magnitude and specificity of the allo-specific response was also changed. In PV-immune mice infected with LCMV the normally subdominant NP205-specific response becomes a dominant response, while the frequency of CD8 T cells specific for the normally immunodominant peptides, such as NP396, which strongly cross-reacts with H2d, is reduced (15). This alteration in the hierarchy coincided with an increased frequency of H2b-specific T cells following LCMV infection of PV-immune mice in comparison to infected nonimmune mice.

Our results in Fig. 9 suggest that generation of memory allo-specific CD8 T cells by LCMV infection impedes the use of co-stimulation blockade to induce tolerance to allogeneic skin grafts.
Recent evidence has demonstrated that memory allo-specific CD8 T cells generated by allogeneic skin transplant are refractory to costimulation blockade and reject subsequent allogeneic transplants (56). The potential resistance of memory T cells to the induction of tolerance coupled with the heightened ability of virus-specific memory CD8 T cells to respond against Ag (57) suggests that previous viral infections may hinder allogeneic transplantation strategies. Interestingly, a recent study has demonstrated that prior infection with *Leishmania major* generated allo-specific CD4 T cells (58). Although the authors did not directly demonstrate cross-reactivity between CD4 T cells specific for *L. major* and allografts, mice infected with *L. major* at least 8 wk previously could not be tolerated with anti-CD154 and DST treatment to maintain allogeneic skin grafts. Thus, memory T cell responses generated by previous exposure to pathogens can be cross-reactive with allogeneic cells and may influence rejection of allogeneic transplants.

Virus-specific CD8 T cells elicited during acute viral infections are capable of directly rejecting allogeneic skin grafts. Acute infections with LCMV can break the tolerance established by costimulatory blockade, resulting in the rejection of allogeneic skin grafts (59, 60), and CD8 T cells specific for the LCMV-epitope GP33 efficiently reject skin grafts from transgenic mice that express the GP33 peptide in the skin (61). Acute infections with EBV, CMV, and HSV have been associated with allograft rejection in humans (62), and cross-reactivity has been shown between EBV-specific CTL clones and allografts (29, 30). The broad-based cross-reactivity of LCMV peptide-specific CD8 T cells with allogeneic cells demonstrates the degeneracy of Ag recognition by the TCR, and this ability of virus-specific CD8 T cells to cross-react with multiple Ags causes alterations in the T cell repertoire with each new infection. Thus, the capacity of individuals to respond to pathogens and allografts is determined by their past history of infections (4, 15).

References


