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Efficient Induction of Primary and Secondary T Cell-Dependent Immune Responses In Vivo in the Absence of Functional IL-2 and IL-15 Receptors¹

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IL-2 and IL-15 are thought to be important cytokines for T cell-dependent immune responses. Mice deficient in IL-2, IL-2Rα, and IL-2Rβ are each characterized by a rapid lethal autoimmune lymphoproliferative disorder that complicates their use in studies aimed at investigating the role of these cytokines and receptors for immune responses in vivo. We have previously characterized a novel transgenic (Tg) mouse on the IL-2Rβ⁻/⁻ genetic background (Tg⁻⁻ mouse) that lacks autoimmune disease but still contains peripheral T cells that are nonresponsive to IL-2 and IL-15. In the present study, these mice were used to investigate the extent by which IL-2 and IL-15 are essential for T cell immunity in vivo. Tg⁻⁻ mice generated normal primary and secondary Ab responses to OVA, readily mounted first and second set allogeneic skin graft rejection responses, and developed primary and recall CD8 T cells responses to vaccinia virus. However, Tg⁻⁻ mice generated a slightly lower level of IgG2a Abs to OVA, exhibited a somewhat delayed first set skin graft rejection response with lower allo-specific CTL, and developed a significantly lower number of IFN-γ-producing vaccinia-specific CD8⁺ T cells. Thus, although T effector function is somewhat impaired, T cell immunity is largely functional in the absence of IL-2- and IL-15-induced signaling through IL-2Rβ. The Journal of Immunology, 2003, 170: 236–242.

Although IL-2 is the dominant cytokine for T cell responses in vitro, the requirement for IL-2 during in vivo immune responses is still not precisely defined. A direct assessment of the necessity for IL-2 might be expected to emerge by evaluating T cell-mediated immunity in IL-2-deficient mice. In this regard, IL-2⁻⁻ mice often developed immune responses in vivo, but sometimes disparate results were noted even when the same agent was used to elicit the immune response (4, 6, 15–19). One serious complication in evaluating immunocompetency in IL-2⁻⁻, IL-2Rα⁻⁻, and IL-2Rβ-deficient mice is that their rapid and extreme autoimmunity provides an abnormal environment that might potentially augment or suppress a specific antigenic challenge. Furthermore, recent studies have raised the possibility that the initial T cell proliferation to Ag challenge in vivo is dependent upon IL-15 (20). These experiments also showed that expression of IL-2 and IL-2Rα was detected from activated T cells only after 5–7 cell divisions, suggesting that IL-2 may function primarily during a later phase of the T cell response. A direct evaluation of immune responses by IL-15- and IL-15R-deficient mice showed that these mice mounted primary and memory virus-specific CD8-dependent T cell responses, albeit at somewhat lower levels than generated in wild-type mice (21, 22). Whether this response was due to IL-2 was not evaluated. Therefore, regardless of some of their nonoverlapping activities, it is still unclear whether IL-2 and IL-15 are mandatory cytokines for T cell-dependent immune responses in vivo.

An ideal scenario to investigate whether IL-2 and IL-15 are essential, but function redundantly, for T cell immunity is to immunologically challenge IL-2Rβ⁻⁻ mice that are deficient in responding to both cytokines without complications associated with severe systemic autoimmunity. Our laboratory has developed such a mouse model by expressing IL-2Rβ as a transgene in the thymus of IL-2Rβ⁻⁻ mice (23, 24). Peripheral T cells in these mice (designated transgenic (Tg)⁻⁻) remained nonresponsive to IL-2 and IL-15, yet Tg⁻⁻ mice did not exhibit autoimmunity and unlimited expansion.
immunodominant H-2d-restricted CD8+ T cell epitope from the spike protein (S100) of the JHM strain of mouse hepatitis virus (JHMV) (26). Secondary responses were induced by immunizing mice i.p. with 5 × 10^5 PFU of recombinant Sindbis virus (SIN/S100) expressing the same JHMV S510 epitope and challenging 4 wk later with 5 × 10^6 PFU of vJS510. SIN/S100 was delivered by inserting hybridized oligonucleotides encoding the S510 epitope (5’-CTAGATGTGTTCTCTTTGGAATGGGCCCCATTTGTGA-3’ and 5’-CTAGTCACAAATGGGGCCCATTCCAAAGAGAACACAT-3’) into the XhoI site of the parent SIN vector pTE3/21 (27). Stocks of SIN/S100 virus were generated via transfection of BHK-21 cells with infectious in vitro transcribed mRNA from XhoI linearized SIN/S100 and titered using BHK-21 cells (27). Mice were sacrificed at indicated time points and spleen and single-cell suspensions were prepared from the original lymph nodes and spleen.

In vitro T cell functional assays

Spleenocytes from virus-challenged mice were assayed for cytolytic activity either directly ex vivo or following 6 days of culture. For in vitro cultures, 1 × 10^6 splenocytes were placed in a 75 fl tissue culture flask with 40 ml of RPMI 1640 medium supplemented with 2 mM glutamine, 25 μg/ml gentamicin, 1 mM sodium pyruvate, 5 × 10^-3 M 2-ME, nonessential amino acids, 10% FCS, and 1 × 10^5 PFU SIN/S100 peptide. CTL assays were performed as described previously (28). Briefly, EL-4 (H-2b) and P815 (H-2b) target cells were labeled with 100 μCi Na^251CrO_4 (NEN, Boston, MA) at 37°C for 1 h and washed three times before use. For virus-specific CTL, EL-4 cells were stimulated with recombinant Sindbis virus (SIN/S100) at a concentration of 10^7 PFU/well and effector cells were transferred to 96-well plates in the presence or absence of 1 μM SIN/S100 peptide. For ex vivo allosppecific or redirected CTL, lymph node effector cells were directly incubated with P815 targets (1 × 10^5/well). After 4 h of incubation, supernatant (100 μl) was removed and specific ^51Cr release was determined. Specific lysis was defined as 100 × (experimental release − spontaneous release)/(detergent release − spontaneous release). Maximum spontaneous release values were <10% of the total detergent release values in all experiments.

S510 peptide (CWSLWNGPHL) used in functional and proliferative assays is derived from the immunodominant JHMV S510 CD8+ T cell epitope in H-2d mice (26, 29).

In vitro T cell proliferative responses were performed as previously described (23). In brief, unfractonated lymph node cells (1 × 10^6/well) or spleen cells (2 × 10^6/well) from skin-grafted or virus-infected mice were cultured with PMA (10 ng/ml), IL-2 (50 U/ml), IL-4 (10 ng/ml), IL-7 (10 ng/ml), or anti-CD3 (1 μg/ml) for 48 h. 3HThymidine was added during the last 6 h of culture.

ELISPOT assays were performed as described previously (28). Briefly, 3.3-fold dilutions of spleen cells from virus-challenged mice were plated in triplicate and stimulated with irradiated (25 Gy) spleenocytes from naive wild-type mice (4 × 10^5/well) in the presence or absence of 1 μM SIN/S100 peptide. Cells were incubated for 48 h at 37°C in the presence of plate-bound anti-IFN-γ mAb (10 μg/ml, R4.6A2; BD Pharmingen). Captured IFN-γ was detected by an anti-biotin mAb (BD Pharmingen) followed by consecutive incubations with streptavidin-peroxidase and diaminobenzidine as a substrate (Sigma-Aldrich, St. Louis, MO). Spots from two mononuclear cell dilutions (n = 6) were counted for each sample.

FACS analysis

CyChrome-anti-CD8 and FITC-anti-IFN-γ were obtained from BD Pharmingen. The DP7S10 tetramer has been described previously (30). For detection of intracellular IFN-γ, spleen cells were stimulated for 6 h in RPMI supplemented with 10% FCS and Gloglistop (BD Pharmingen) in the presence or absence of 1 μM SIN/S100 peptide. Cells were then stained with anti-CD8 mAb, permeabilized with Cytofix/Cytoperm reagents (BD Pharmingen) as per the manufacturer’s recommendations, and then stained with anti-IFN-γ mAb. FACS analysis was performed using a BD Biosciences FACScan and CellQuest software (BD Biosciences, Mountain View, CA). Typically 100,000 viable cells were analyzed per sample based on forward vs side scatter gating.

Results

T cell-dependent Ab responses by Tg-/- mice

Several distinct agents were used to challenge the Tg-/- mice to evaluate their capacity to mount T cell-dependent immune responses. Initially, the induction of primary and secondary Ab responses to OVA was assessed. When compared with control mice, Tg-/- mice readily elicited primary and secondary responses to OVA.
OVA (Fig. 1). Because induction of IFN-γ by Tg−/− T cells is impaired in vitro, we also examined the level of OVA-specific IgG1 and IgG2a in the serum after a secondary challenge (Fig. 2). Although there was some individual variation, most Tg−/− mice produced lower levels of IgG2a, but this difference was not statistically significant (p = 0.09) when compared with titers from control mice.

**Allogeneic skin graft rejection by Tg−/− mice**

Control and Tg−/− mice received a fully allogeneic BALB/c skin graft. Both groups of mice readily rejected the skin graft, although the rejection by the Tg−/− mice was significantly (p = 0.008) delayed (Fig. 3A). Mean rejection time was 9.8 ± 0.6 days for control littersmate mice and 12.2 ± 0.4 days for Tg−/− mice. Ex vivo analysis of CTL activity to H-2d alloantigens by T cells from lymph nodes of mice that received BALB/c skin graft 7 days previously indicated that alloantigen-specific CTL were detected from control, but not Tg−/− mice (Fig. 3B). No CTL activity was detected against either H-2b EL4 or H-2d P815 targets when the T cells were obtained from untreated normal mice (data not shown). By 14 days after receiving the skin graft, no ex vivo CTL activity was observed by lymph node T cells from either control or Tg−/− mice (data not shown). It is also important to point out that lymph node T cells from the grafted control mice, but not untreated normal or grafted Tg−/− mice, readily proliferated when cultured in exogenous IL-2, further confirming the absence of IL-2Rβ function by the Tg−/− T cells (Fig. 3C). Furthermore, when these mice received a second graft, they exhibit no difficulty in mounting a second set rejection response, which was slightly more rapid in the Tg−/− mice (Fig. 3A). Histological assessment indicated that extensive and comparable infiltrates were observed just before rejection in control and Tg−/− transplant recipients (data not shown).

Therefore, despite the absence of detectable cytolytic activity and proliferation to IL-2, Tg−/− mice still successfully rejected the allogeneic skin graft, perhaps by CD4 effector cells, as reported by others (31–34).

**Anti-CD3-induced CTL activity in Tg−/− mice**

The failure to induce detectable allospecific CTL from Tg−/− T cells in vivo and in vitro (23) pointed to the importance of IL-2Rβ in the generation of cytotoxic effector cells. However, in vitro IL-4 redundantly functioned to promote CTL (23), indicating that there is not a strict requirement for IL-2Rβ to generate CTL in culture. To test whether a redundant pathway might function in vivo, control and Tg−/− mice were injected with anti-CD3. Two days later, lymph nodes were examined ex vivo for their ability to lyse P815 targets due to redirected lysis (Fig. 4). We reasoned that the use of anti-CD3 would afford the greatest chance to induce a potential redundant activity for CTL in the Tg−/− mice. Under these conditions, a similar level of CTL activity was observed for control and Tg−/− T cells. No CTL activity was detected when normal untreated T cells were used as the effector cells (data not shown). Again, exogenous IL-2 induced proliferation of lymph node cells from control, but not Tg−/− mice (23) (data not shown). Thus, this experiment demonstrates that Tg−/− T cells indeed have the capacity to develop into CTL in vivo.

**Induction of antiviral responses in Tg−/− mice**

To investigate primary antiviral responses, control and Tg−/− mice were infected with a recombinant vaccinia virus (vJSS10) expressing the immunodominant JHMV S510 CTL epitope, thereby permitting a direct assessment of virus-specific CD8+ T cell expansion via staining with the D+SS10 tetramer. To assess secondary responses, mice were primed with a recombinant Sindbis virus expressing the JHMV S510 epitope (SINJS510) and then challenged 4 wk later with vJSS10. Following both primary and secondary viral challenges, D+SS10 tetramer CD8+ T cells were detected in both spleen and inguinal nodes of control and Tg−/− mice (Fig. 5, A and C). Primary responses in Tg−/− mice were comparable to those seen in control littermate mice. Frequencies of virus-specific CD8+ T cells during secondary responses were somewhat lower in Tg−/− mice (Fig. 5, B and C); however, this difference was not statistically significant (p = 0.21).

In vitro stimulation of spleen cells from the virus-infected control mice in the presence of PMA plus exogenous IL-2 generated strong proliferative responses (Fig. 6). IL-2-dependent responses were not generated by Tg−/− T cells in the presence of PMA, confirming the absence of signaling through the IL-2R by Tg−/− peripheral T cells. By contrast, T cells from Tg−/− mice generated substantial, but somewhat reduced, responses to both PMA/IL-4 and anti-CD3 when compared with T cells from littermate controls, confirming that T cells from Tg−/− mice were not anergic. Furthermore, nearly comparable CD8+ T cell responses to the S510 peptide as well as CD4+ T cell responses to UV-inactivated
vJS510 indicated that virus-specific T cells from Tg\(^{-/-}\) mice and control mice display similar capacities for expansion in direct response to specific Ags in vitro. However, exogenous IL-2 enhanced these proliferative responses to S510 peptide and UV-inactivated vJS510 by control, but not Tg\(^{-/-}\) T cells, when assayed days 5 and 7 post primary infection and day 5 post secondary infection (data not shown). These data provide additional evidence for the IL-2 nonresponsiveness of Tg\(^{-/-}\) T cells when assayed directly ex vivo.

To determine functional capabilities of anti-viral T cells in the absence of IL-2/IL-15 signaling, splenocytes from either control or Tg\(^{-/-}\) mice during either primary or secondary responses to vJS510 (data not shown). By contrast, cytolytic activity was readily detected from both Tg\(^{-/-}\) and control splenocytes after culture of unfractionated spleen cells for 6 days in the presence of the S510 peptide (Fig. 7A). Although 60% fewer viable cells were recovered from the cultures of Tg\(^{-/-}\) splenocytes, cytolytic activity from both groups of cells was similar based on comparable numbers of live effector cells in the CTL assay.

Determination of the frequency of S510-specific CD8\(^{+}\) T cells via IFN-\(\gamma\) ELISPOT 7 days post primary infection with vJS510 revealed a striking difference in the number of IFN-\(\gamma\) secreting cells between Tg\(^{-/-}\) mice and littermate controls, i.e., 36 vs 241 per 10\(^{6}\) splenocytes, respectively. As the frequencies of D\(^{b}\)S510\(^{+}\)CD8\(^{+}\) T cells were almost identical during primary responses, this result suggested that Tg\(^{-/-}\) T cells are impaired in generating IFN-\(\gamma\)-secreting CD8\(^{+}\) effector cells. Similar results were obtained 5 days post secondary vJS510 infection. A total of 3- to 4-fold fewer IFN-\(\gamma\)^+CD8\(^{+}\) T cells were detected via intracellular IFN-\(\gamma\) staining in splenocytes from Tg\(^{-/-}\) mice compared with control mice (Fig. 7B). Even though spleens of Tg\(^{-/-}\) mice contain 20% fewer CD8\(^{+}\) T cells during the secondary response compared with control mice (Fig. 7A), the differences in the frequency of IFN-\(\gamma\)-producing cells was not accounted for by the slightly lower fraction of D\(^{b}\)S510 tetramer\(^{+}\)/CD8\(^{+}\) T cells in the spleen. Collectively, these data indicate that Tg\(^{-/-}\) mice developed primary and secondary T cell responses to vaccinia virus, but with an impaired capacity of these T cells to secrete IFN-\(\gamma\).

**Discussion**

Numerous in vitro studies have established the importance of IL-2 as a T cell growth factor and a cytokine that renders Ag-activated T cells susceptible to activation-induced cell death. The immune response pattern of T cells from Tg\(^{-/-}\) mice in vitro further confirmed the essential role of IL-2/IL-15 signaling for extended T proliferation and differentiation into effector T cells (23, 24). Therefore, we were somewhat surprised at the extent of immunocompetence of Tg\(^{-/-}\) mice when challenged in vivo to develop T cell-dependent immune responses. This included induction of Ab responses to OVA that depend on CD4 T cell help, responses to vaccinia virus that depend on CD8 T cells, and rejection of allogeneic skin that uses both CD4 and CD8 T effector cells. The magnitude of both the primary and secondary responses to each of these in vivo immune responses by Tg\(^{-/-}\) mice was largely comparable to that generated by control mice. Although the responses by the Tg\(^{-/-}\) mice were often slightly lower, these differences were usually not statistically significant. Therefore, these findings demonstrate that IL-2/IL-15 signaling, and hence IL-2 and IL-15, are largely dispensable to mount primary and secondary T cell-dependent immune responses in vivo.

It is extremely unlikely that the immune responses developed by the Tg\(^{-/-}\) mice were due to some residual IL-2/IL-15 activity by peripheral T cells. We have extensively documented that responses by Tg\(^{-/-}\) T cells in the presence of IL-2 and IL-15 are exceptionally impaired in vitro (23, 24). Confirming these results, T cells from Tg\(^{-/-}\) mice that were first stimulated in vivo remained essentially nonresponsive to IL-2 when assayed directly ex vivo. Furthermore, as discussed more fully below, some T effector cell functions that were impaired in vitro were also diminished in vivo following immunological challenge of Tg\(^{-/-}\) mice, directly demonstrating impaired IL-2/IL-15 function in vivo.
Past studies have shown that immune responses were generated in IL-2-deficient mice (4, 6, 15–19, 35). Ag-specific T cell proliferation by TCR Tg IL-2−/−/CD8+ T cells was induced in vivo by influenza nucleoprotein peptide (19). IL-2−/− mice were shown to be competent to reject allogeneic islet allografts (16). Furthermore, primary and secondary CTL were generated to lymphocytic choriomeningitis (LCMV) and vaccinia virus infections as well as Ab responses to vesicular stomatitis virus (VSV) infection in IL-2−/− mice (15). However, other studies have reported impaired immune responses in IL-2−/− and IL-2R−/− mice (4, 6, 15, 35, 36). For example, the magnitude of the primary response to LCMV was dramatically reduced in IL-2−/− mice although these T cells developed cytolytic activity (17). IL-2Rβ−/− mice failed to mount immune responses to VSV and LCMV (7). More recently, IL-2−/− mice were shown to be resistant to infection with avirulent Salmonella serovar Choleraesuis while IL-2Rβ−/− mice were susceptible to infection (35). The immunocompetence of Tg−/− mice strongly suggests that failed responses in IL-2 or IL-2R nonresponsive animals results from complications of autoimmunity, which become superimposed on evaluations of specific immune responses. Nevertheless, the development of primary and secondary immune responses to a variety of stimuli in autoimmune-free Tg−/− mice rules out the possibility that immune responses were induced in IL-2−/− mice solely as a consequence of autoimmunity. In any case, the severe autoimmunity associated with IL-2/IL-2R deficiency represents a serious limitation in most studies that use these mice to evaluate T cell immunity.

Two recent studies have investigated antiviral CD8-dependent T cell responses in IL-15−/− and IL-15R-deficient mice (21, 22). Both primary and secondary responses to VSV and LCMV were readily generated, although the responses to VSV were suboptimal especially after 5–6 days when compared with control mice. Thus, IL-15 is also not mandatory for an immune response to virus. However, these and other studies revealed that the key role for IL-15 resides in the long-term maintenance of the memory pool (21, 22, 37–41). Although we have not investigated the Ag-specific memory pool, our findings are largely in agreement with these reports. In contrast to the studies described above, an obvious difference in studying T cell immunity in Tg−/− mice is that the contribution of IL-2 and IL-15 to a particular response is assessed simultaneously. Our study indicates that neither cytokine is essential for diverse T cell responses in vivo in the time frame of 1–6 wk for primary and recall responses. This conclusion seems at odds with recent work that suggests that IL-15 may be a key cytokine that promotes the initial growth of Ag-activated T cells in vivo, especially CD8+ T cells (20). In these experiments, T cell proliferation was assessed by adoptively transferred T cells into lethally irradiated allogeneic recipients early after transfer. Lethal irradiation induces host cytokine production and a lymphopenic environment, two conditions that favor IL-15-induced CD8 T cell growth in vivo. This setting is much different from both our and other studies (21, 22) where immune responses were investigated in unirradiated nonlymphopenic mice. We contend that these distinct experimental conditions represent a plausible explanation for markedly different requirements for IL-15 in the initial response to Ag.

FIGURE 5. Antiviral immunity by Tg−/− mice. Tg−/− and control mice were challenged directly with vJS510 (A) or immunized with SINJS510 and challenged 4 wk later with vJS510 (B) to assess CD8+ T cell responses to primary and secondary viral challenges, respectively. Representative analysis of the fraction of S510-specific CD8+ T cells from pooled spleen and lymph nodes. Primary (A) and secondary (B) infections were enumerated 7 and 5 days postinfection, respectively, by flow cytometry after staining with CyChrome anti-CD8 and PE-D b/S510 tetramer. C, Summary of the fraction of CD8+ T cells in the spleen from all mice which are S510-specific following a primary (day 7 postchallenge) and secondary infection (day 5 postchallenge) enumerated by D'S510 tetramer staining. Data from primary and secondary responses are from a total of four to five mice and were derived from two separate experiments.

FIGURE 6. In vitro proliferation by Tg−/− T cells after viral infections. Proliferative responses by splenocytes from mice of the indicated IL-2Rβ genotype after primary and secondary virus challenge (7 and 5 days post-challenge, respectively). Splenocytes (2 × 10⁶/well) were cultured with the indicted stimuli for 48 h and [3H]thymidine was added during the last 6 h of culture. Data are representative of two experiments in which each group consisted of a pool of spleen cells from two to three mice.
T cell responses in vitro are highly dependent upon IL-2Rβ signaling, including the induction of IFN-γ secretion (24). Although IL-2Rβ was largely dispensable for T cell immunity in vivo, optimal IFN-γ secretion still required IL-2Rβ. Slightly diminished class switching to IgG2a, which in part is dependent upon IFN-γ, and a substantially lower frequency of IFN-γ-producing virus-specific CD8+ T cells was observed for immunized Tg−/− mice. The impairment in IFN-γ production to vaccinia virus was not accounted for by a lower precursor frequency of S510-specific CD8+ T cells. Other studies have observed impaired IFN-γ production in the absence of IL-2Rβ signaling in vitro and in vivo (6, 17, 42). Thus, IL-2 appears to be an important cytokine in vivo for efficient production of IFN-γ by T effector cells.

Primary stimulation of Tg−/− T cells in vitro resulted in markedly impaired production of CTL due to reduced induction of perforin and nearly absent induction of granzyme B (24). By contrast, induction of CTL activity in vivo was only sometimes impaired in Tg−/− mice when compared with control mice, notably to alloantigens after priming with skin grafts. In contrast, when Tg−/− mice were challenged with anti-CD3, they readily produced CTL when assayed ex vivo. Other studies point to a variable requirement for IL-2 in vivo for induction of CTL (15, 17, 19). These findings suggest that other cytokines or cell interactions may bypass the requirement for IL-2Rβ signaling, and such a signal may be limiting in some immune responses in vivo. In this regard, it is pertinent that exogenous IL-4 in vitro effectively overcame the impairment of CTL development by Tg−/− T cells (24). However, in very limited experiments to date, we have been unsuccessful in implicating IL-4 as a redundant cytokine for production of CTL by Tg−/− mice in vivo (data not shown). Furthermore, LCMV-specific CTL and protective viral responses were induced in vivo in IL-2/IL-4 double knockout mice (18). Thus, it is likely that IL-4-independent signals may substitute for the absence of IL-2Rβ for production of CTL. It is also interesting to note that although no direct ex vivo cytolytic activity was detected after primary or secondary challenge with S510-modified vaccinia virus from either control or Tg−/− mice, upon in vitro culture, cytolytic activity was readily induced from both type of T cells, although many fewer cells were recovered from the cultures containing Tg−/− T cells. We believe that the signals necessary for S510-specific CTL were received during the in vivo priming, perhaps due to an innate immune response, because direct in vitro stimulation of Tg−/− T cells by anti-CD3 or alloantigen resulted in negligible induction of CTL (24).

The immunocompetence of Tg−/− mice demonstrates that there must be compensatory pathways that permit relatively efficient T cell immunity in the absence of IL-2 and IL-15. However, the magnitude of some of the responses in Tg−/− mice was sometimes slightly diminished and effector activity, such as IFN-γ production, was impaired, indicating that such compensatory mechanisms do not fully substitute for failed IL-2 and IL-15 signaling in vivo. Therefore, there are likely some situations where effective immunity may still depend on IL-2 and/or IL-15. In this regard, it is important to point out that when IL-15−/− mice were infected with the highly virulent neurotropic WR vaccinia virus strain, all mice died 4–9 days after infection (14). This result markedly contrasts with the nonlethal infection by attenuated recombinant vJS510 vaccinia virus that nevertheless stimulated strong T cell responses in both control and Tg−/− mice.

The main unanswered question and the subject for future investigation from this work is what accounts for the capacity to induce T cell responses in vivo in the absence of IL-2Rβ-dependent IL-2 and IL-15 signaling. With respect to γc-dependent cytokines, it is unlikely, as discussed above, that IL-4 is solely responsible for these responses. Studies of alloantigen-induced T cell proliferation in adoptively transferred lethally irradiated mice demonstrated a role for IL-15 in driving the initial proliferative response (20). The one very attractive feature of this result is that IL-15 is produced by nonlymphoid cells and is readily available to stimulate T cells when present at a low frequency, as expected at the initiation of the response. Perhaps nonlymphoid-derivied IL-7, whose signaling is very similar to that induced by IL-2 and IL-15, might substitute for lack of IL-2Rβ in a nonmanipulated host in a manner analogous to that recently reported for IL-7 and IL-15 in the regulation of CD8+ memory T cells (40, 41, 43). Another candidate might be IL-21, which is secreted by activated CD4+ T cells and uses a receptor related to IL-2Rβ and IL-4Rα (44). Alternatively, there are many surface molecules on T lymphocytes whose function in T cell immunobiology are very poorly understood and may serve to promote immune responses in an IL-2/IL-15-independent fashion. Thus, although the IL-2R is essential for in vitro T cell responses, there are undoubtedly alternative pathways to induce T cell-dependent immune responses in vivo.

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