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BRIEF REVIEWS

IL-2, Regulatory T Cells, and Tolerance

Brad H. Nelson[1]

IL-2 is a potent T cell growth factor that for many years was assumed to amplify lymphocyte responses in vivo. Accordingly, IL-2 has been used clinically to enhance T cell immunity in patients with AIDS or cancer, and blocking Abs to the IL-2R are used to inhibit T cell responses against transplanted tissues. It was later shown in mice that, unexpectedly, disruption of the IL-2 pathway results in lymphoid hyperplasia and autoimmunity rather than immune deficiency, indicating that the major physiological function of IL-2 is to limit rather than enhance T cell responses. This apparent paradox has recently been resolved with the discovery that IL-2 is critical for the development and peripheral expansion of $CD4^+CD25^+$ regulatory T cells, which promote self-tolerance by suppressing T cell responses in vivo. Our new understanding of IL-2 biology prompts a re-evaluation of how best to clinically manipulate this important immunoregulatory pathway. The Journal of Immunology,* 2004, 172: 3983–3988.

Interleukin-2 was the first T cell growth factor to be molecularly cloned and remains the cytokine of choice for the propagation of T cells in culture (1). Because IL-2 can potently induce T cell expansion in vitro, it was assumed for many years that IL-2 played an analogous role in amplifying T cell responses in vivo. This assumption led to the development of therapeutic strategies aimed at modulating IL-2 signal strength for clinical benefit. On the one hand, IL-2 itself is infused in patients with cancer or AIDS to enhance T cell numbers and function (2, 3). In contrast, Abs to the IL-2R are used to inhibit IL-2 signaling to suppress the rejection of transplanted organs (4). These agents show clinical efficacy in some cases, lending support to the notion that IL-2 serves as an important T cell growth factor in vivo. However, this same notion is strongly challenged by studies from the past decade showing that mice engineered to lack the *IL-2* or *IL-2R* genes are not markedly immunocompromised but instead develop severe T cell-mediated autoimmune disease (5–7). This raises the striking paradox of a cytokine that drives T cell proliferation in vitro being somehow required to limit T cell responses to self-Ags in vivo. Today, while clinicians move forward with human trials in which IL-2 signaling is enhanced to promote immunity or inhibited to promote tolerance, many basic immunologists now view IL-2 as having precisely the opposite properties in vivo. Clearly, we need to better understand the physiological role of IL-2 in immunity and self-tolerance so that clinical manipulation of IL-2 signaling can be rationally tailored to achieve the maximum therapeutic benefit in patients.

IL-2 basics

IL-2 is a typical four α helix cytokine and is produced primarily by activated $CD4^+$ T cells, although expression by naive $CD8^+$ T cells, dendritic cells, and thymic cells has also been reported (8–11). In T cells, IL-2 synthesis is tightly regulated at the mRNA level by signals from the TCR and CD28 (12). IL-2 binds to and signals through a receptor complex consisting of three distinct subunits designated IL-2Rα (CD25), IL-2Rβ (CD122), and common γ-chain (γ_c;[2] CD132) (13). All three subunits are required for high-affinity binding of IL-2. In the absence of IL2Rα expression, IL-2Rβ and γ_c can form an intermediate affinity receptor that is fully competent to signal. However, the high-affinity receptor appears to be the only physiologically relevant form of the IL-2R, as CD25-deficient mice (which express the intermediate affinity IL-2R only) are phenotypically indistinguishable from IL-2-deficient mice (5, 6, 14). One cannot discuss IL-2 without also considering the closely related cytokine IL-15, which signals through the β and γ_c subunits of the IL-2R but utilizes a unique IL-15Rα chain instead of CD25 (15). As a result of the shared usage of IL-2Rβ and γ_c, IL-2 and IL-15 appear to generate identical intracellular signals. However, the cytokines have distinct in vivo properties, presumably due to different expression patterns of the cytokines and their respective α receptor subunits.

Phenotypic consequences of IL-2 and IL-2R deficiency

In young mice lacking a functional *IL-2* or *IL-2R*α gene, mainstream T, B, and NK cell development and seeding of the periphery is largely normal (5, 6), although there is impaired development of TCR$\gamma\delta$ T cells and TCR$\alpha\beta$ T cells of the CD8$\alpha\alpha$ subset (16, 17). *IL-2R*$\beta^{-/-}$ mice also show grossly normal T and B lymphopoiesis, but, owing to lost IL-15 signaling, these mice completely lack NK cells and extrathymically derived T cells (18). When tested in vitro, T cells from *IL-2*- and *IL-2R*α-deficient mice show impaired proliferation and effector functions (5, 6, 19–21). Nevertheless, these mice are

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[2] Abbreviations used in this paper: γ_c, common γ-chain; RAG-2, recombination-activating gene 2; AICD, activation-induced cell death; Treg, T regulatory cell.

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generally immunocompetent, can resolve experimental viral infections, and can reject cardiac and islet cell allografts, albeit with reduced cytolytic activity (19, 20, 22–24).

Despite beginning life with an overtly normal immune system, at 4–6 wk of age IL-2- and IL-2Rα-deficient mice start to show massive enlargement of lymph nodes, spleen, and gut-associated lymphoid tissue due to polyclonal expansion of T and B cells (5, 7). The T cells at these sites have an activated or memory phenotype ($CD69^+CD44^+$) (5, 7, 14) and elevated serum Abs and autoantibodies appear (5, 6). Fatal autoimmune complications ensue. Between 8 and 20 wk, 25–50% of IL-2- or IL-2Rα-deficient mice die from severe hemolytic anemia. Other mice develop fatal colitis that is reminiscent of inflammatory bowel disease in humans and is associated with inflammation, lymphocyte and neutrophil infiltration, circulating anticolon Abs, thickening of the bowel wall, ulceration, diarrhea, and wasting (5, 7). The precise pathology is strain dependent, as *IL-2*$^{-/-}$ BALB/c mice develop fatal anemia and multiorgan autoimmune disease more rapidly than C57BL/6 mice yet fail to develop colitis (14). Like *IL-2*$^{-/-}$ mice, IL-2Rβ-deficient mice develop T and B cell abnormalities, but this occurs as early as 3 wk of age (25). By contrast, IL-15- and IL-15Rα-deficient mice do not develop autoimmune disease (15).

Much less is known about the effects of IL-2 deficiency in humans; however, a male child with a mutant, nonexpressed form of the *IL-2R*α gene has been described (26). This patient was immune compromised and, similar to IL-2Rα-deficient mice, showed signs of T cell autoreactivity evidenced by lymphadenopathy, hepatolosplenomegaly, chronic inflammatory disorders, and dense lymphocytic infiltration of multiple organs including lung, liver, gut, soft tissue, and bone.

Etiology of lymphoid hyperplasia and autoimmunity in IL-2-deficient mice

Several lines of evidence indicate that T cells are necessary and sufficient to initiate the autoimmune syndrome seen in IL-2- or IL-2R-deficient mice. Autoimmunity fails to develop in *IL-2*$^{-/-}$ × *RAG-2*$^{-/-}$ mice, which lack T and B cells (27). Selective ablation of the B cell lineage prevents the development of autoantibodies and hemolytic anemia, but not other aspects of the *IL-2*$^{-/-}$ phenotype (27). *IL-2R*$\alpha^{-/-}$ mice engineered to additionally lack $CD4^+$ T cells still show abnormal activation and expansion of $CD8^+$ T cells, although B cell abnormalities are prevented (25, 28). Conversely, *IL-2*$^{-/-}$ mice that lack $CD8^+$ T cells still develop colitis, indeed with accelerated kinetics (29). Finally, *IL-2*$^{-/-}$ nude mice, which lack a functional thymus, do not develop any autoimmune manifestations, indicating that thymic rather than extrathymic T cells are required for the pathological process. Indeed, transfer of lymphocytes from euthymic *IL-2*$^{-/-}$ mice into athymic *IL-2*$^{+/+}$ mice results in autoimmunity; therefore, IL-2-replete extrathymic T cells are unable to control the dysregulated activity of IL-2-deficient thymically derived lymphocytes (30).

Some form of antigenic stimulation is required to trigger autoimmunity in IL-2- or IL-2R-deficient mice. For example, colitis does not occur in *IL-2*$^{-/-}$ mice that are raised in the absence of intestinal flora (7, 16). Furthermore, colitis and splenomegaly can be induced in such mice by injecting trinitrophenyl-keyhole limpet hemocyanin or trinitrophenyl-OVA, which seems to trigger a general form of T cell-mediated intestinal inflammation that is resolved in wild-type but not *IL-2*$^{-/-}$ mice (31). Even in the absence of intestinal flora or environmental Ags, abnormal activation and expansion of T cells still occurs at other sites. This process appears to be initiated by self-Ags, because IL-2Rα mice engineered to express a transgenic TCR (DO11.10) do not develop an activated T cell phenotype provided the recombination-activating gene 2 (RAG-2) is also deleted so as to preclude the expression of any endogenous TCRs (28). Interestingly, if the RAG-2 gene is left intact such that endogenous TCRs are expressed on some T cells, T cell dysregulation emerges and involves T cells from both the endogenous and TCR-transgenic subsets.

IL-2 and central tolerance

One early hypothesis to explain the autoimmunity seen in *IL-2*$^{-/-}$ mice was impaired negative selection of self-reactive thymocytes. This was a reasonable model given that thymocytes respond to IL-2 in vitro (13, 32), IL-2 is expressed in the thymus (10, 11, 33), and IL-2Rβ is up-regulated on $TCR^{int}CD8^{low/-}CD4^+$ thymocytes, a subset that is undergoing selection and is near maturation to $CD4^+$ or $CD8^+$ thymocytes (34). However, negative selection is consistently reported as being normal in the absence of IL-2 or IL-2Rα (5, 19, 21, 28) or IL-2Rβ (35, 36). One exception is a report that IL-2-deficient thymocytes are less susceptible to apoptosis induced by systemic anti-CD3 or Ag treatment; however, this model does not necessarily reflect physiological negative selection (11). Thus, the idea emerged early that the autoimmune syndrome seen with IL-2 deficiency might instead reflect impaired peripheral tolerance.

IL-2 and the control of activation-induced cell death (AICD)

A second hypothesis to explain the phenotype of IL-2-deficient mice posited an essential role for IL-2 in sensitizing T cells to AICD. AICD is a process that limits the magnitude of T cell expansion through the programmed death of activated T cells, mediated primarily by signals from Fas and TNF (37). IL-2 has been shown to sensitize T cells to AICD in vitro, in part by up-regulating expression of Fas ligand and the TNFR and down-regulating expression of the caspase inhibitor c-FLIP (38–40). Furthermore, IL-2 promotes synthesis of IFN-γ, which is critical for AICD (23, 41). Consistent with these observations, several groups have reported that T cells from IL-2- or IL-2R-deficient mice are resistant to AICD in vitro and superantigen-induced elimination in vivo (5, 21, 33, 42–44). In contrast, other groups claim these processes occur normally in the absence of IL-2 signaling (28, 35). Similarly, it is controversial whether IL-2 is uniquely able to promote AICD (21, 45) or whether IL-4 and IL-7 can have a similar effect (40).

Although IL-2 can clearly sensitize T cells to AICD in vitro, the notion that this is the primary mechanism of IL-2-mediated self-tolerance in vivo is challenged by several observations. First, the IL-2R has been shown to induce AICD by activating the downstream transcription factor STAT5 (42), yet mice engineered to express a mutated IL-2R that fails to activate STAT5 do not develop lymphadenopathy or autoimmunity (46). Second, as discussed further below, mice engineered to express IL-2Rβ in the thymus but not in the periphery (where AICD occurs) do not develop autoimmunity (47). Finally, in vivo tracking of IL-2- and IL-2Rα-deficient T cells has shown reduced T cell expansion relative to wild-type T cells without a major effect on T cell apoptosis or contraction (28, 48). Conversely, T cells engineered to have enhanced IL-2R signaling

show increased expansion in vivo with no evidence of increased apoptosis (8). Indeed, claims that IL-2 limits T cell expansion in vivo have generally been based on system-wide perturbations (33, 49, 50), which may produce unintended secondary effects. Thus, while IL-2 is clearly able to promote AICD in vitro and reduced T cell apoptosis has been observed in IL-2-deficient mice under some conditions, this may not be the primary tolerogenic mechanism for IL-2 in vivo.

IL-2 and regulatory T cells

There is a growing body of evidence that the tolerogenic properties of IL-2 are mediated through regulatory interactions between T cells rather than cell autonomous mechanisms such as impaired negative selection or AICD. Currently, there is evidence for at least three classes of regulatory T cells, including IL-10-producing Tr1 cells, TGF-β-producing Th3 cells, and $CD4^+CD25^+$ (regulatory T cells (Tregs)) (51). $CD4^+CD25^+$ Tregs develop in the thymus and constitute 5–10% of the circulating T cell population in healthy humans and mice. They potently inhibit T cell proliferation in vitro and suppress the activity of autoreactive T cells in vivo (52). Activated $CD4^+$ T cells also express CD25, but this is generally transient and of lower magnitude compared with Tregs (53, 54). Other gene products that appear to distinguish (albeit imperfectly) $CD4^+CD25^+$ Tregs from conventional, activated $CD4^+$ T cells include the cell surface receptor glucocorticoid-induced TNFR and the transcription factor FoxP3 (55–57). The mechanism by which $CD4^+CD25^+$ Tregs suppress other T cell responses is controversial but requires cell-cell contact in vitro and may involve cytokines such as IL-10 and TGF-β in vivo (52). Importantly, there are differing observations and viewpoints regarding the phenotype and effector mechanisms of Tregs, and it seems likely that additional subclasses will be identified with further research (52).

The fact that CD25 (IL-2Rα) distinguishes a major subset of Tregs immediately suggested a role for IL-2 in $CD4^+CD25^+$ Treg activity. Moreover, the systemic autoimmunity seen in IL-2- and IL-2R-deficient mice is reminiscent of that seen when $CD4^+CD25^+$ Tregs are depleted by neonatal thymectomy, FoxP3 deficiency, or other means (52). Indeed, several recent studies provide strong evidence that IL-2 is required for the development, expansion, and/or function of $CD4^+CD25^+$ Tregs. The first suggestion came from studies in which *RAG-2*$^{-/-}$ mice were reconstituted with bone marrow containing a 30%:70% mixture of IL-2-replete and IL-2-deficient cells. Autoimmune pathology failed to develop in these mice, indicating that IL-2-replete T cells can prevent IL-2-deficient T cells from undergoing uncontrolled expansion and initiating autoimmune disease (30), which is consistent with a Treg mechanism. It was later shown that mice deficient for the *IL-2* or *IL-2R*β genes lack $CD4^+CD25^+$ T cells (36, 58, 59). By contrast, mice with chimeric T cell compartments containing mixtures of IL-2R-replete and -deficient cells develop a stable $CD4^+CD25^+$ subset and maintain normal immune homeostasis (36, 59). Finally, adoptive transfer of wild-type $CD4^+CD25^+$ T cells into IL-2Rα- or IL-2Rβ-deficient mice prevents the development of lymphadenopathy and autoimmunity, and these cells can be reisolated and shown to possess classic Treg suppressor activity in vitro (36, 59). Thus, there is correlative and functional evidence supporting an important role for IL-2 in the development and/or function of $CD4^+CD25^+$ Tregs.

Is IL-2 signaling important for Treg development in the thymus? As mentioned above, there is evidence for IL-2 and IL-2R expression in the thymus (10, 11, 32–34, 60), therefore IL-2 could potentially play a role in the thymic development of Tregs. To evaluate this issue, Malek et al. (36, 47) rescued *IL-2R*$\beta^{-/-}$ mice with an IL-2Rβ transgene that was expressed predominantly in the thymus with negligible expression in the periphery. Remarkably, this transgene restored $CD4^+CD25^+$ T cell development and prevented lymphadenopathy and autoimmunity. This suggests that Tregs require IL-2 signaling primarily during thymic development and less so in the periphery. The precise thymic source of IL-2 during Treg development remains obscure. It does not appear to come from the Tregs themselves, since mice reconstituted with chimeric bone marrow containing equal proportions of IL-2-deficient and IL-2Rα-deficient cells develop functional $CD4^+CD25^+$ Tregs, which by definition must be *IL-2*$^{-/-}$ (59). There may be a nonhemopoietic source of IL-2 for Treg development, since lethally irradiated *RAG-2*$^{-/-}$ mice that are reconstituted with *IL-2*$^{-/-}$ bone marrow develop a $CD4^+CD25^+$ T cell subset (59).

Is IL-2 signaling required for peripheral expansion/maintenance of Tregs? Peripheral $CD4^+CD25^+$ T cells from wild-type mice express all three IL-2R subunits, therefore in addition to its role in the thymus, IL-2 could potentially serve as a growth factor for Tregs in the periphery (36). Consistent with this, $CD4^+CD25^+$ T cells engineered to lack IL-2Rβ in the periphery fail to expand upon transfer to wild-type mice (36). Furthermore, $CD4^+CD25^+$ T cells from wild-type mice fail to expand in IL-2$^{-/-}$ mice (36). Thus, IL-2 signaling appears to be required for both the thymic development and peripheral expansion/maintenance of $CD4^+CD25^+$ T cells.

Is IL-2 signaling required by Tregs to exert their suppressor function? When Tregs undergo homeostatic expansion in vivo, they lose expression of CD25 and hence are no longer responsive to IL-2; nevertheless, they retain potent Treg activity when isolated and tested in vitro (61). Moreover, as described above, Tregs engineered to lack expression of IL-2Rβ in the periphery nonetheless prevent autoimmunity and exhibit suppressor activity in vitro (36). Indeed, many groups have shown that addition of IL-2 to Tregs abolishes their suppressive activity in vitro (52). Thus, it appears that IL-2 signaling is not required for the suppressor activity of Tregs and may even disrupt it.

Which Treg subsets require IL-2? As mentioned above, there are other Treg subsets apart from the $CD4^+CD25^+$ class, including $CD4^+CD25^-$ and $CD8^+$ subsets, Tr1 cells, Th3 cells, $\gamma\delta$ T cells, and NKT cells (51, 52, 62, 63). The role of IL-2 in the development and function of these other regulatory subsets is not well understood. It can be inferred that $CD8^+$ Tregs require IL-2 signaling much like $CD4^+$ Tregs, since IL-2Rα-deficient mice develop lymphadenopathy and autoimmunity even when $CD4^+$ T cells are absent (25, 28). Indeed, wild-type $CD8^+$ T cells can correct the abnormal T cell activity in IL-2Rβ-deficient mice, possibly through a cytolytic mechanism (64). Whereas IL-2-dependent Tregs span both the $CD4^+$ and $CD8^+$ subsets, in FoxP3-deficient mice $CD4^+$ T cells are necessary and sufficient for autoimmune pathology (65). Thus, IL-2 appears to be broadly required for both $CD4^+$ and $CD8^+$ Treg function, whereas FoxP3 appears to be essential for only $CD4^+$ Tregs. Notably, some forms of Treg activity are still

present in IL-2-deficient mice. For example, Furtado et al. showed that $CD4^+$ T cells from wild-type or *IL-2*$^{-/-}$ mice are equally able to suppress the development of experimental autoimmune encephalitis in a myelin basic protein TCR transgene model, as well as an Ag-induced hyper-IgE response in a dual T and B cell-transgenic mouse model (66). These and other observations highlight the diversity of Treg subsets and suggest the IL-2 pathway represents a major but not exclusive mechanism for their development and function.

How do IL-2-dependent Tregs maintain lymphoid homeostasis and prevent autoimmunity? The mechanisms by which Tregs suppress the proliferation and activity of other T cells remain poorly defined; however, observations from IL-2- and IL-2R-deficient mice may shed light on this issue. One prominent feature of the IL-2-deficient phenotype is a massive expansion of lymph nodes and spleen due to infiltration by activated T cells, which is consistent with impaired regulation of homeostatic proliferation (5). Homeostatic T cell proliferation is a natural control mechanism that sustains optimal numbers of circulating lymphocytes. It is triggered by low-affinity TCR-mediated recognition of self-peptides, and the subsequent extent of proliferation is determined by the amount of available lymphoid "space," which lymphocytes sense through ill-defined signaling mechanisms (67). IL-2-dependent Tregs could potentially generate or enforce the space signal that normally limits homeostatic proliferation. In the absence of this signal, T cells triggered by low-affinity interactions with self-peptides would be expected to expand uncontrollably, leading to large numbers of autoreactive T cells followed by lymphadenopathy and autoimmunity.

Almeida et al. (59) evaluated the relationship among Tregs, homeostatic proliferation, and IL-2 signaling. They first showed that adoptive transfer of $CD4^+CD25^+$ T cells prevents the severe lymphoid hyperplasia that normally befalls IL-2Rα-deficient mice. Moreover, by adoptively transferring different $CD4^+$ T cell subsets into T cell-deficient hosts (CD3ϵ knockouts), they found that naive $CD4^+$ T cells expanded on average to $\sim$1–2 $\times$ 10^7 cells, whereas $CD25^+CD4^+$ Tregs expanded to a 10-fold lower plateau, indicating a fundamental difference in the expansion limits of naive vs regulatory T cells. The naive T cells eventually induced autoimmunity in these mice, whereas the Treg subset did not. Finally, when the different subsets were coadministered, $CD4^+CD25^+$ Tregs were found to limit the expansion of naive T cells in a dose-dependent manner (59). Thus, $CD4^+CD25^+$ Tregs can regulate the expansion plateau of naive $CD4^+$ T cells under lymphopenic conditions and prevent the uncontrolled expansion of IL-2Rα-deficient T cells, which is consistent with Tregs generating or enforcing a space-determining signal.

Wolf et al. (68) also examined the relationship among IL-2 signaling, Tregs, and T cell expansion, although in this case proliferation was triggered by cognate Ag rather than self-peptides. IL-2-deficient T cells expressing the DO11.10-transgenic TCR were adoptively transferred into nude mice, which lack a functional thymus, thereby creating a situation where neither donor nor host T cells contained a $CD4^+CD25^+$ Treg subset. After activation by Ag, the DO11.10 T cells underwent a dramatic and prolonged expansion phase, which could be suppressed by cotransfer of wild-type $CD4^+$ T cells and, in particular, $CD4^+CD25^+$ T cells. To assess potential mechanisms of suppression, they evaluated the surface phenotype of the responding DO11.10 T cells. Responding T cells that had been suppressed by unfractionated $CD4^+$ T cells were found to express CD69 and CD25, indicating they had received an activation signal but failed to expand. By contrast, responding T cells that had been exposed to $CD4^+CD25^+$ Tregs did not express CD25 or CD69, suggesting they had experienced a more proximal block in activation. The authors concluded there may be two distinct mechanisms of suppression deployed by $CD4^+$ Tregs, and the net effect in each case was to limit the magnitude of T cell expansion. Although other interpretations are possible, these results are again consistent with a model in which IL-2-dependent Tregs set the threshold for T cell expansion in vivo.

Conclusions and future directions

The last few years have brought much insight into the enigmatic properties of IL-2. It remains the preferred cytokine for in vitro propagation of $CD4^+$ and $CD8^+$ T cells. However, at least in mice, the contribution of IL-2 to in vivo T cell expansion is subtle and may be restricted to anatomical niches such as nonlymphoid peripheral tissues (48). Instead, it appears that the major nonredundant role of IL-2 in vivo is to promote the thymic development and peripheral expansion of $CD4^+CD25^+$ Tregs and, though less well characterized, $CD8^+$ Tregs. Loss of Treg activity in IL-2- or IL-2Rβ-deficient mice leads to severe, Ag-triggered lymphadenopathy followed by fatal autoimmunity. The evidence to date is consistent with a model in which IL-2-dependent Tregs establish and/or enforce the size of the peripheral T cell compartment and thereby prevent homeostatic and Ag-induced proliferative responses from continuing unchecked toward a state of pathological autoreactivity.

The existence of IL-2-dependent Tregs is supported by numerous genetic and adoptive transfer experiments, yet still lacking is a direct demonstration that developing Tregs express a functional IL-2R and receive an IL-2 signal in the thymus. Furthermore, since the biochemical signals generated by the IL-2R are similar if not identical to those from the IL-7R and IL-15R, it is unclear why Tregs are uniquely dependent on IL-2 for their development and peripheral expansion compared with conventional T cells, which are well supported by these other cytokines. On this note, it was recently reported that STAT5 mediates a necessary and sufficient signal for Treg development (69, 70), yet this transcription factor is activated by many cytokines in addition to IL-2, including IL-7, IL-9, and IL-15. An additional unresolved issue concerns the sources of IL-2 used by Tregs in the thymus and periphery. Tregs themselves have been ruled out, which implies that these cells themselves are under the control of other thymic and peripheral cells that provide paracrine IL-2. One intriguing possibility is that, in the periphery, the IL-2 produced by conventional T cells upon exposure to Ag serves to attract or expand the Treg subset, which in turn limits the T cell proliferative response, thereby forming a classic negative feedback loop. We also do not yet understand the extent to which the IL-2-dependent Treg subset overlaps with Treg subsets identified in other experimental contexts, such as neonatal thymectomy, FoxP3 deficiency, or tolerance induction. Genetic crosses or adoptive T cell transfers between these different mouse models should help resolve this issue. Finally, it remains unclear to what extent the immunoregulatory properties of IL-2 are attributable to AICD vs Treg activity under different physiological conditions. Conditional deletion of the

IL-2 or *IL-2Rα* genes at different stages of T cell development and function would help resolve this important issue.

As for the clinical modulation of IL-2 signaling, the key lesson from mice is that the physiological functions of IL-2 are complex and currently difficult to predict. Use of anti-CD25 Abs (anti-Tac) during organ transplantation could potentially deplete $CD4^+CD25^+$ Tregs, which may lead to increased alloreactivity. In contrast, since Tregs are capable of homeostatic expansion, anti-CD25 treatment could theoretically create lymphoid space that becomes repopulated with allospecific, tolerogenic Tregs. Furthermore, since IL-2 can reverse the suppressive activity of mature Tregs (at least in vitro), anti-CD25 Abs could potentially sustain the Treg phenotype in vivo by protecting $CD4^+CD25^+$ T cells from IL-2 signals. Similarly, it is difficult to predict how systemic infusion of IL-2 might affect the proliferation and suppressive phenotype of Tregs. Indeed, in murine studies, the timing of IL-2 infusion with respect to the T cell proliferative program dictates whether IL-2 promotes or inhibits T cell expansion (50, 71). Finally, it is important to consider the retention and bioavailability of IL-2 in different tissues, which may have a profound influence on its biological properties (33, 72).

Despite the potential issues raised by genetic studies in mice, the fact remains that, at least in some patients, anti-CD25 Abs can promote tolerance to allografts and systemic IL-2 infusion can boost antitumor immunity, enhance T cell counts in HIV patients, and prolong the survival of infused therapeutic T cells (2–4, 73). This does not reflect a species difference, as similar responses are seen in mice after anti-CD25 treatment or systemic IL-2 infusion (50, 71, 74). Instead, this likely reflects important differences between what IL-2 does under normal conditions vs what IL-2 can do when therapeutically manipulated. Despite some success, there is clearly much room for improvement with respect to the clinical use of IL-2 or anti-CD25 Abs. It seems the best way forward is to carefully monitor the responses of different T cell subsets in patients undergoing IL-2-based immunomodulation so as to test the new hypotheses arising from murine studies, as some groups are beginning to do (75). One can measure increases or decreases in Treg activity using the molecular markers and in vitro suppression assays that are now available, and the specificity and precision of these assays will improve as we further elucidate the different Treg subsets in humans, their physiological target Ags, and their contribution to health and disease. Clearly, IL-2 represents an important control point for manipulating the balance between regulatory and effector T cell function in vivo, and our enlightened understanding of this cytokine offers new hope for the rational control of T cell responses in patients with malignant and infectious diseases.

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References

1. Smith, K. A. 1988. Interleukin-2: inception, impact, and implications. *Science 240:1169.*
2. Dutcher, J. 2002. Current status of interleukin-2 therapy for metastatic renal cell carcinoma and metastatic melanoma. *Oncology (Huntingt.) 16:4.*
3. Pahwa, S., and M. Morales. 1998. Interleukin-2 therapy in HIV infection. *AIDS Patient Care STDS 12:187.*
4. Morris, J. C., and T. A. Waldmann. 2000. Advances in interleukin 2 receptor targeted treatment. *Ann. Rheum. Dis. 59:i109.*
5. Willerford, D. M., J. Chen, J. A. Ferry, L. Davidson, A. Ma, and F. W. Alt. 1995. Interleukin-2 receptor α chain regulates the size and content of the peripheral lymphoid compartment. *Immunity 3:521.*
6. Schorle, H., T. Holtschke, T. Hunig, A. Schimpl, and I. Horak. 1991. Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. *Nature 352:621.*
7. Sadlack, B., H. Merz, H. Schorle, A. Schimpl, A. C. Feller, and I. Horak. 1993. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell 75:253.*
8. Cheng, L. E., C. Ohlen, B. H. Nelson, and P. D. Greenberg. 2002. Enhanced signaling through the IL-2 receptor in $CD8^+$ T cells regulated by antigen recognition results in preferential proliferation and expansion of responding $CD8^+$ T cells rather than promotion of cell death. *Proc. Natl. Acad. Sci. USA 99:3001.*
9. Granucci, F., C. Vizzardelli, N. Pavelka, S. Feau, M. Persico, E. Virzi, M. Rescigno, G. Moro, and P. Ricciardi-Castagnoli. 2001. Inducible IL-2 production by dendritic cells revealed by global gene expression analysis. *Nat. Immunol. 2:882.*
10. Yang-Snyder, J. A., and E. V. Rothenberg. 1998. Spontaneous expression of interleukin-2 in vivo in specific tissues of young mice. *Dev. Immunol. 5:223.*
11. Bassiri, H., and S. R. Carding. 2001. A requirement for IL-2/IL-2 receptor signaling in intrathymic negative selection. *J. Immunol. 166:5945.*
12. Powell, J. D., J. A. Ragheb, S. Kitagawa-Sakakida, and R. H. Schwartz. 1998. Molecular regulation of interleukin-2 expression by CD28 co-stimulation and anergy. *Immunol. Rev. 165:287.*
13. Nelson, B. H., and D. M. Willerford. 1998. Biology of the interleukin-2 receptor. *Adv. Immunol. 70:1.*
14. Sadlack, B., J. Lohler, H. Schorle, G. Klebb, H. Haber, E. Sickel, R. J. Noelle, and I. Horak. 1995. Generalized autoimmune disease in interleukin-2-deficient mice is triggered by an uncontrolled activation and proliferation of $CD4^+$ T cells. *Eur J. Immunol. 25:3053.*
15. Ma, A., D. L. Boone, and J. P. Lodolce. 2000. The pleiotropic functions of interleukin 15: not so interleukin 2-like after all. *J. Exp. Med. 191:753.*
16. Contractor, N. V., H. Bassiri, T. Reya, A. Y. Park, D. C. Baumgart, M. A. Wasik, S. G. Emerson, and S. R. Carding. 1998. Lymphoid hyperplasia, autoimmunity, and compromised intestinal intraepithelial lymphocyte development in colitis-free gnotobiotic IL-2- deficient mice. *J. Immunol. 160:385.*
17. Porter, B. O., and T. R. Malek. 1999. IL-2Rβ/IL-7Rα doubly deficient mice recapitulate the thymic and intraepithelial lymphocyte (IEL) developmental defects of $\gamma_c^{-/-}$ mice: roles for both IL-2 and IL-15 in CD8αα IEL development. *J. Immunol. 163:5906.*
18. Suzuki, H., G. S. Duncan, H. Takimoto, and T. W. Mak. 1997. Abnormal development of intestinal intraepithelial lymphocytes and peripheral natural killer cells in mice lacking the IL-2 receptor β chain. *J. Exp. Med. 185:499.*
19. Kramer, S., C. Mamalaki, I. Horak, A. Schimpl, D. Kioussis, and T. Hung. 1994. Thymic selection and peptide-induced activation of T cell receptor- transgenic CD8 T cells in interleukin-2-deficient mice. *Eur. J. Immunol. 24:2317.*
20. Steiger, J., P. W. Nickerson, W. Steurer, M. Moscovitch-Lopatin, and T. B. Strom. 1995. IL-2 knockout recipient mice reject islet cell allografts. *J. Immunol. 155:489.*
21. Van Parijs, L., A. Biuckians, A. Ibragimov, F. W. Alt, D. M. Willerford, and A. K. Abbas. 1997. Functional responses and apoptosis of CD25 (IL-2Rα)-deficient T cells expressing a transgenic antigen receptor. *J. Immunol. 158:3738.*
22. Kundig, T. M., H. Schorle, M. F. Bachmann, H. Hengartner, R. M. Zinkernagel, and I. Horak. 1993. Immune responses in interleukin-2-deficient mice. *Science 262:1059.*
23. Cousens, L. P., J. S. Orange, and C. B. Biron. 1995. Endogenous IL-2 contributes to T cell expansion and IFN-γ production during lymphocytic choriomeningitis virus infection. *J. Immunol. 155:5690.*
24. Dai, Z., B. T. Konieczny, F. K. Baddoura, and F. G. Lakkis. 1998. Impaired alloantigen-mediated T cell apoptosis and failure to induce long-term allograft survival in IL-2-deficient mice. *J. Immunol. 161:1659.*
25. Suzuki, H., T. M. Kundig, C. Furlonger, A. Wakeham, E. Timms, T. Matsuyama, R. Schmits, J. J. Simard, P. S. Ohashi, H. Griesser, et al. 1995. Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor β. *Science 268: 1472.*
26. Sharfe, N., H. K. Dadi, M. Shahar, and C. M. Roifman. 1997. Human immune disorder arising from mutation of the α chain of the interleukin-2 receptor. *Proc. Natl. Acad. Sci. USA 94:3168.*
27. Ma, A., M. Datta, E. Margosian, J. Chen, and I. Horak. 1995. T cells, but not B cells, are required for bowel inflammation in interleukin 2-deficient mice. *J. Exp. Med. 182:1567.*
28. Leung, D. T., S. Morefield, and D. M. Willerford. 2000. Regulation of lymphoid homeostasis by IL-2 receptor signals in vivo. *J. Immunol. 164:3527.*
29. Simpson, S. J., E. Mizoguchi, D. Allen, A. K. Bhan, and C. Terhorst. 1995. Evidence that $CD4^+$, but not $CD8^+$ T cells are responsible for murine interleukin-2-deficient colitis. *Eur. J. Immunol. 25:2618.*
30. Kramer, S., A. Schimpl, and T. Hunig. 1995. Immunopathology of interleukin (IL) 2-deficient mice: thymus dependence and suppression by thymus-dependent cells with an intact *IL-2* gene. *J Exp. Med. 182:1769.*
31. Ehrhardt, R. O., B. R. Ludviksson, B. Gray, M. Neurath, and W. Strober. 1997. Induction and prevention of colonic inflammation in IL-2-deficient mice. *J. Immunol. 158:566.*
32. Carding, S. R., A. C. Hayday, and K. Bottomly. 1991. Cytokines in T-cell development. *Immunol. Today 12:239.*
33. Wrenshall, L. E., and J. L. Platt. 1999. Regulation of T cell homeostasis by heparan sulfate-bound IL-2. *J. Immunol. 163:3793.*
34. Hanke, T., R. Mitnacht, R. Boyd, and T. Hunig. 1994. Induction of interleukin 2 receptor β chain expression by self-recognition in the thymus. *J. Exp. Med. 180:1629.*
35. Suzuki, H., A. Hayakawa, D. Bouchard, I. Nakashima, and T. W. Mak. 1997. Normal thymic selection, superantigen-induced deletion and Fas-mediated apoptosis of T cells in IL-2 receptor β chain-deficient mice. *Int. Immunol. 9:1367.*

36. Malek, T. R., A. Yu, V. Vincek, P. Scibelli, and L. Kong. 2002. CD4 regulatory T cells prevent lethal autoimmunity in IL-2Rβ-deficient mice: implications for the nonredundant function of IL-2. *Immunity 17:167*.
37. Green, D. R., N. Droin, and M. Pinkoski. 2003. Activation-induced cell death in T cells. *Immunol. Rev. 193:70*.
38. Lenardo, M. J. 1991. Interleukin-2 programs mouse αβ T lymphocytes for apoptosis. *Nature 353:858*.
39. Refaeli, Y., L. Van Parijs, C. A. London, J. Tschopp, and A. K. Abbas. 1998. Biochemical mechanisms of IL-2-regulated Fas-mediated T cell apoptosis. *Immunity 8:615*.
40. Zheng, C. L. Trageser, D. M. Willerford, and M. J. Lenardo. 1998. T cell growth cytokines cause the superinduction of molecules mediating antigen-induced T lymphocyte death. *J. Immunol. 160:763*.
41. Refaeli, Y., L. Van Parijs, S. I. Alexander, and A. K. Abbas. 2002. Interferon γ is required for activation-induced death of T lymphocytes. *J. Exp. Med. 196:999*.
42. Van Parijs, L., Y. Refaeli, J. D. Lord, B. H. Nelson, A. K. Abbas, and D. Baltimore. 1999. Uncoupling IL-2 signals that regulate T cell proliferation, survival, and Fas-mediated activation-induced cell death. *Immunity 11:281*.
43. Khoruts, A., A. Mondino, K. A. Pape, S. L. Reiner, and M. K. Jenkins. 1998. A natural immunological adjuvant enhances T cell clonal expansion through a CD28-dependent, interleukin (IL)-2-independent mechanism. *J. Exp. Med. 187:225*.
44. Kneitz, B., T. Herrmann, S. Yonehara, and A. Schimpl. 1995. Normal clonal expansion but impaired Fas-mediated cell death and anergy induction in interleukin-2-deficient mice. *Eur J. Immunol. 25:2572*.
45. Dai, Z., A. Arakelov, M. Wagener, B. T. Konieczny, and F. G. Lakkis. 1999. The role of the common cytokine receptor γ-chain in regulating IL-2- dependent, activation-induced $CD8^+$ T cell death. *J. Immunol. 163:3131*.
46. Fujii, H., K. Ogasawara, H. Otsuka, M. Suzuki, K. Yamamura, T. Yokochi, T. Miyazaki, H. Suzuki, T. W. Mak, S. Taki, and T. Taniguchi. 1998. Functional dissection of the cytoplasmic subregions of the IL-2 receptor β_c chain in primary lymphocyte populations. *EMBO J. 17:6551*.
47. Malek, T. R., B. O. Porter, E. K. Codias, P. Scibelli, and A. Yu. 2000. Normal lymphoid homeostasis and lack of lethal autoimmunity in mice containing mature T cells with severely impaired IL-2 receptors. *J. Immunol. 164:2905*.
48. D'Souza, W. N., K. S. Schluns, D. Masopust, and L. Lefrancois. 2002. Essential role for IL-2 in the regulation of antiviral extralymphoid CD8 T cell responses. *J. Immunol. 168:5566*.
49. Ku, C. C., M. Murakami, A. Sakamoto, J. Kappler, and P. Marrack. 2000. Control of homeostasis of $CD8^+$ memory T cells by opposing cytokines. *Science 288:675*.
50. Shrikant, P., and M. F. Mescher. 2002. Opposing effects of IL-2 in tumor immunotherapy: promoting CD8 T cell growth and inducing apoptosis. *J. Immunol. 169:1753*.
51. Jonuleit, H., and E. Schmitt. 2003. The regulatory T cell family: distinct subsets and their interrelations. *J. Immunol. 171:6323*.
52. Bach, J. F., and J. Francois Bach. 2003. Regulatory T cells under scrutiny. *Nat. Rev. Immunol. 3:189*.
53. Baecher-Allan, C., J. A. Brown, G. J. Freeman, and D. A. Hafler. 2001. $CD4^+CD25^{high}$ regulatory cells in human peripheral blood. *J. Immunol. 167:1245*.
54. Kuniyasu, Y., T. Takahashi, M. Itoh, J. Shimizu, G. Toda, and S. Sakaguchi. 2000. Naturally anergic and suppressive $CD25^+CD4^+$ T cells as a functionally and phenotypically distinct immunoregulatory T cell subpopulation. *Int. Immunol. 12:1145*.
55. Shimizu, J., S. Yamazaki, T. Takahashi, Y. Ishida, and S. Sakaguchi. 2002. Stimulation of $CD25^+CD4^+$ regulatory T cells through GITR breaks immunological self-tolerance. *Nat. Immunol. 3:135*.
56. Khattri, R., T. Cox, S. A. Yasayko, and F. Ramsdell. 2003. An essential role for Scurfin in $CD4^+CD25^+$ T regulatory cells. *Nat. Immunol. 4:337*.
57. Fontenot, J. D., M. A. Gavin, and A. Y. Rudensky. 2003. Foxp3 programs the development and function of $CD4^+CD25^+$ regulatory T cells. *Nat. Immunol. 4:330*.
58. Papiernik, M., M. L. de Moraes, C. Pontoux, F. Vasseur, and C. Penit. 1998. Regulatory CD4 T cells: expression of IL-2R α chain, resistance to clonal deletion and IL-2 dependency. *Int. Immunol. 10:371*.
59. Almeida, A. R., N. Legrand, M. Papiernik, and A. A. Freitas. 2002. Homeostasis of peripheral $CD4^+$ T cells: IL-2Rα and IL-2 shape a population of regulatory cells that controls $CD4^+$ T cell numbers. *J. Immunol. 169:4850*.
60. Zuniga-Pflucker, J. C., and A. M. Kruisbeek. 1990. Intrathymic radioresistant stem cells follow an IL-2/IL-2R pathway during thymic regeneration after sublethal irradiation. *J. Immunol. 144:3736*.
61. Gavin, M. A., S. R. Clarke, E. Negrou, A. Gallegos, and A. Rudensky. 2002. Homeostasis and anergy of $CD4^+CD25^+$ suppressor T cells in vivo. *Nat. Immunol. 3:33*.
62. Stephens, L. A., and D. Mason. 2000. CD25 is a marker for $CD4^+$ thymocytes that prevent autoimmune diabetes in rats, but peripheral T cells with this function are found in both $CD25^+$ and $CD25^-$ subpopulations. *J. Immunol. 165:3105*.
63. Graca, L., S. Thompson, C. Y. Lin, E. Adams, S. P. Cobbold, and H. Waldmann. 2002. Both $CD4^+CD25^+$ and $CD4^+CD25^-$ regulatory cells mediate dominant transplantation tolerance. *J. Immunol. 168:5558*.
64. Suzuki, H., Y. W. Zhou, M. Kato, T. W. Mak, and I. Nakashima. 1999. Normal regulatory α/β T cells effectively eliminate abnormally activated T cells lacking the interleukin 2 receptor β in vivo. *J Exp. Med. 190:1561*.
65. Blair, P. J., S. J. Bultman, J. C. Haas, B. T. Rouse, J. E. Wilkinson, and V. L. Godfrey. 1994. $CD4^+CD8^-$ T cells are the effector cells in disease pathogenesis in the scurfy (sf) mouse. *J. Immunol. 153:3764*.
66. Furtado, G. C., M. A. Curotto de Lafaille, N. Kutchukhidze, and J. J. Lafaille. 2002. Interleukin 2 signaling is required for $CD4^+$ regulatory T cell function. *J. Exp. Med. 196:851*.
67. Surh, C. D., and J. Sprent. 2002. Homeostatic T cell proliferation: how far can T cells be activated to self-ligands?. *J. Exp. Med. 192:F9*.
68. Wolf, M., A. Schimpl, and T. Hunig. 2001. Control of T cell hyperactivation in IL-2-deficient mice by $CD4^+CD25^-$ and $CD4^+CD25^+$ T cells: evidence for two distinct regulatory mechanisms. *Eur. J. Immunol. 31:1637*.
69. Snow, J. W., N. Abraham, M. C. Ma, B. G. Herndier, A. W. Pastuszak, and M. A. Goldsmith. 2003. Loss of tolerance and autoimmunity affecting multiple organs in STAT5A/5B-deficient mice. *J. Immunol. 171:5042*.
70. Antov, A., L. Yang, M. Vig, D. Baltimore, and L. Van Parijs. 2003. Essential role for STAT5 signaling in $CD25^+CD4^+$ regulatory T cell homeostasis and the maintenance of self-tolerance. *J. Immunol. 171:3435*.
71. Blattman, J. N., J. M. Grayson, E. J. Wherry, S. M. Kaech, K. A. Smith, and R. Ahmed. 2003. Therapeutic use of IL-2 to enhance antiviral T-cell responses in vivo. *Nat. Med. 9:540*.
72. Wrenshall, L. E., J. L. Platt, E. T. Stevens, T. N. Wight, and J. D. Miller. 2003. Propagation and control of T cell responses by heparan sulfate-bound IL-2. *J. Immunol. 170:5470*.
73. Yee, C., J. A. Thompson, D. Byrd, S. R. Riddell, P. Roche, E. Celis, and P. D. Greenberg. 2002. Adoptive T cell therapy using antigen-specific $CD8^+$ T cell clones for the treatment of patients with metastatic melanoma: in vivo persistence, migration, and antitumor effect of transferred T cells. *Proc. Natl. Acad. Sci. USA 99:16168*.
74. Shimizu, K., R. C. Fields, M. Giedlin, and J. J. Mule. 1999. Systemic administration of interleukin 2 enhances the therapeutic efficacy of dendritic cell-based tumor vaccines. *Proc. Natl. Acad. Sci. USA 96:2268*.
75. Sereti, I., H. Martinez-Wilson, J. A. Metcalf, M. W. Baseler, C. W. Hallahan, B. Hahn, R. L. Hengel, R. T. Davey, J. A. Kovacs, and H. C. Lane. 2002. Long-term effects of intermittent interleukin 2 therapy in patients with HIV infection: characterization of a novel subset of $CD4^+/CD25^+$ T cells. *Blood 100:2159*.