Signal transduction in CD4+CD25+ regulatory T cells: CD25 and IL-2

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1. ABSTRACT

IL-2 was originally identified as a growth factor critical for T cell proliferation in vitro. Although the early studies of IL-2 strongly implied an obligate role of IL-2 in T cell growth, it was later shown that mice deficient in IL-2 or in IL-2R developed an unexpected lymphocytic hyperproliferation and subsequent autoimmune disease. In separate studies of autoimmunity, it was observed that a population of CD4+ T cells suppressed the induction of autoimmunity in several in vivo models of autoimmune disease. It was not until the characterization of this subpopulation of CD4+ T cells demonstrated that they co-expressed the IL-2R-alpha chain (CD25) that the puzzling phenotype observed in IL-2 deficient mice began to be truly explained. The constitutive expression of the IL-2R-alpha chain on CD4+CD25+ T cells led to the obvious speculation that IL-2 signaling in CD4+CD25+ T cells was important to these cells. Recent studies have examined the role of IL-2 in the generation, the expansion, the survival and the effector function of CD4+CD25+ T cells. It is now evident that IL-2 is critical for the development of CD4+CD25+ T cells and the phenotype observed in IL-2 and IL-2R deficient mice is most readily explained by the absence of these potent suppressors.

2. INTRODUCTION

IL-2 is a potent growth factor produced by activated T cells that acts to promote the in vitro proliferation of T cells. The receptor for IL-2 (IL-2R) is expressed on activated T cells as well as NK cells and is comprised of three subunits: an alpha chain (CD25), a beta chain (CD122) and the common cytokine receptor gamma chain (CD132) (Figure 1). High affinity binding requires both CD25 and CD122, while signal transduction requires CD122 and CD132. In addition, CD122 is also a subunit of the IL-15R and CD132 is a subunit of the receptors for IL-4, IL-7, IL-9, IL-15 and IL-21 (1). Contrary to expectations, the inactivation of the IL-2 gene did not result in a T cell proliferation defect and the loss of immune responses, but rather, resulted in the uncontrolled expansion of T cells and subsequent autoimmunity (2). IL-2 deficient mice developed splenomegaly and lymphadenopathy by 3 to 4 weeks after birth and death of 50% of the animals occurred by 9 weeks. The surviving animals exhibited autoimmune anemia and inflammatory bowel disease (IBD). Similar results were observed when CD25 and CD122 deficient mice were generated. In CD25 deficient mice, LN and spleen were enlarged by 4 to 6 weeks and death of 25% of the animals occurred within 8
The focus of this review is to examine the role that IL-2 signaling has on CD4+CD25+ T cells. Studies in the last few years have demonstrated that IL-2 signaling is involved not only in the function of CD4+CD25+ cells, but also for their development, expansion and maintenance.

3. CD4+CD25+ T CELLS

After the identification of CD4+CD25+ T cells as the population of cells that prevented the induction of autoimmune gastritis induced by d3Txs, in vitro characterization of these cells demonstrated that they are a unique subset of CD4+ T cells that suppress the proliferation of conventional CD4+ T cells as well as the proliferation and effector function of CD8+ T cells (9). Once activated, CD4+CD25+ T cell-mediated suppression is not MHC restricted. Suppression of in vitro proliferation is a cell contact event that, through an undefined mechanism, leads to the inhibition of IL-2 production in responder T cells. Although CD4+CD25+ T cell derived TGF-beta has been implicated as the mechanism of suppression, the subject remains controversial and a widely accepted mechanism has not been established. An important characteristic of CD4+CD25+ T cells is that they are anergic to TCR stimulation in vitro and require exogenous IL-2, in addition to TCR stimulation, in order to proliferate. The requirement for IL-2 is a result of the inability of CD4+CD25+ T cells to produce IL-2 (9). However, TCR stimulation in the presence of IL-2, IL-4 and to some degree, IL-7, can also cause a proliferative response in CD4+CD25+ T cells, but other common gamma chain cytokines cannot (10). A recent advance in the characterization of CD4+CD25+ T cells was the identification of a transcription factor, FoxP3, that is specific to CD4+CD25+ T cells (11-13). In the absence of FoxP3, mice exhibit a profound lymphoproliferative disorder and die within 3 weeks of birth (14). Moreover, retroviral transduction of FoxP3 into CD4+CD25-T cells conferred both anergy and suppressive activity to these cells and thus recapitulated the CD4+CD25+ T cell phenotype (11, 13). The transfer of CD4+CD25+ T cells has been shown to prevent the induction of disease in many animal models of autoimmunity, including gastritis, diabetes, thyroiditis and (deleted full spelling of IBD) IBD (8, 15-17). Although CD4+CD25+ T cells were originally discovered through their ability to suppress autoreactive cells, it is now evident that these cells can also regulate responses to infectious diseases, tumors and transplants (18-20).

4. IL-2 AND THE GENERATION OF CD4+CD25+ T CELLS

The autoimmune disease that develops in IL-2 and IL-2R deficient mice substantiates the importance of IL-2 signaling in CD4+CD25+ T cells (1). Papiernik et. al. demonstrated that CD4+CD25+ were present in the thymus and subsequent studies have confirmed that CD4+CD25+ T cells are, in fact, generated in the thymus (9, 21). It is also now evident that the thymic generation of CD4+CD25+ T cells depends upon IL-2 signaling. IL-2 and CD122 deficient mice have greatly reduced numbers of thymic and

![Figure 1](image-url). IL-2 signaling cascades. The IL-2 receptor is comprised of alpha (CD25), beta (CD122) and gamma (CD132) subunits. Upon binding to its receptor, IL-2 initiates the recruitment and phosphorylation of STAT5 and Shc through JAK1 and JAK3. Shc activates both the PI3K and MAPK arms of the cascade.

...to 20 weeks (3). Similar to IL-2 deficient animals, the surviving animals developed anemia and IBD. CD122 deficient mice, however, displayed a more severe phenotype. Splenomegaly and lymphadenopathy developed by 3-4 weeks but death of all animals occurred by 12 weeks (4). While CD25 expression is limited to activated T cells, CD122 is expressed on cells such as NK cells, B cells and monocytes thus, the broader distribution of CD122 may explain the phenotype observed in CD122 deficient mice.

Well before the studies on IL-2 and its role in immunity (or the surprising lack of a role), several in vivo models of autoimmunity had been well established. The most significant of these was developed in the late 1960s and was well characterized by the mid-1970s (5, 6). In this model, mice that were thymectomized on day 3 (d3Tx) of life developed a variety of organ specific autoimmune diseases. The severity and number of organs involved depended upon the strain of mice used in the study. Significantly, disease was not observed in mice that were thymectomized earlier or later than day 3 of life. More importantly, autoimmune disease could be prevented by the transfer of normal splenocytes, specifically by CD4+ T cells (7). It wasn’t until two decades later that Sakaguchi et. al. demonstrated that the population of CD4+ T cells responsible for the inhibition of disease induction was a small subpopulation of cells that co-expressed CD25 (8). With this discovery, the obvious, presumptive role for IL-2 signaling in the function of CD4+CD25+ T cells quickly became a subject of intense focus. These studies merged with those of the IL-2/IL-2R deficient mice and from these studies, an explanation for the contradictory phenotypes observed in IL-2 and IL-2R deficient mice can be explained by a lack of tolerance due to the absence or impaired function of CD4+CD25+ T cells. Thus, IL-2 and CD4+CD25+ T cells play a major role in maintaining tolerance rather than immunity.
peripheral CD4+CD25+ T cells while CD4+CD25- T cells by default, are not observed in CD25 deficient mice (21, 22). It is unclear at this time if CD25 deficient mice are totally devoid of cells with suppressive function, but with the recent identification of FoxP3 as a specific marker for CD4+CD25+ T cells, it is possible to determine if CD25 deficient mice contain FoxP3 positive cells. If such cells do exist, they, as well as the few residual CD4+CD25- T cells in IL-2 and CD122 deficient mice, are of insufficient frequency or function as they are unable to control the development of autoimmunity.

Additional studies provide further evidence for the role of IL-2 signaling in the generation of CD4+CD25+ T cells. First, IL-2 treatment of neonatal IL-2 deficient mice prevented the lymphoproliferative syndrome as well as the development of autoimmune disease (23). Second, the transfer of wild-type CD4+CD25+ T cells to CD122 or CD25 deficient mice completely protected the mice from the development of autoimmunity (22, 24). In addition, the expression of an IL-2R-beta (CD122) transgene under control of the proximal lck promoter in CD122 deficient mice led to the thymic expression of CD122 in otherwise CD122 deficient mice (22, 25). The transgene expression resulted in the restoration of thymic and peripheral CD4+CD25+ T cells and protection from autoimmunity. Although peripheral CD4+CD25+ T cells did not respond to TCR and IL-2 stimulation, demonstrating the specificity of the thymic expression of CD122, the cells were able to respond to phorbol myristate acetate (PMA) and IL-4. Finally, in a model to examine the hyperproliferation of CD4 T cells in IL-2 deficient mice, Wolf et. al. transferred TCR Tg IL-2 deficient cells to athymic mice (26). Antigen stimulation resulted in the accumulation of CD4+ T cells and the induction of autoimmune disease, but the co-transfer of normal CD4+CD25+ T cells prevented this accumulation as well as the subsequent autoimmunity.

In further studies, Almeida et. al. demonstrated that the lethality of CD25 deficient mice was due to a lack of regulatory cells that express CD25 and require IL-2 for their generation (24). This was shown by the generation of bone marrow chimeric mice in which lethally irradiated Rag2 deficient mice were reconstituted with bone marrow from CD25 deficient mice mixed with wild type bone marrow. Although mice reconstituted with bone marrow from CD25 deficient mice (deleted cells) died within 6 to 7 weeks after reconstitution, mice reconstituted with CD25 deficient bone marrow cells and as little as 10% wild type bone marrow cells were completely protected from disease. Second, the induction of autoimmunity observed with the transfer of CD25 deficient bone marrow cells was inhibited by bone marrow from IL-2 deficient mice. As all CD4+CD25+ T cells were of IL-2-” origin, the thymic generation of CD4+CD25+ T cells required IL-2. However, the required IL-2 was not produced by CD4+CD25+ cells.

Furtado et. al. however, concluded that IL-2 was not required in the thymus but was required for CD4+CD25+ T cell function in the periphery (27). In this model, mice transgenic for an MBP-specific T cell receptor were bred to TCR alpha and TCR beta deficient mice (T/alpha’beta’). These mice developed spontaneous EAE but the co-transfer of wild type CD4+ T cells protected the animals from disease. When CD4+ T cells from young IL-2 deficient mice were transferred to T/alpha’beta’ recipients they were also protected from the development of EAE but the transfer of CD4+ T cells from CD25 deficient mice was insufficient to protect the mice. As protection was observed with cells generated in the absence of IL-2, Furtado et. al. concluded that IL-2 was not necessary for the thymic generation of CD4+CD25+ T cells. IL-2 was required in the periphery, though, as cells from CD25 deficient mice lacked IL-2 signaling and were unable to function. However, CD4+ T cells from IL-2 deficient mice have a reduced proportion of CD4+CD25+ cells while CD25 deficient mice clearly possess none. (deleted However) An alternate explanation is that the few CD4+CD25+ cells that were generated in the thymus, once transferred, survived and expanded in the IL-2 competent T/alpha’beta’ host. The few, if any, regulatory cells from the CD25 deficient donor would be unable to survive, even in the T/alpha’beta’ host due to the lack of IL-2 signaling.

In addition to the requirement for IL-2 signaling in the thymus for the generation of CD25 cells, there is evidence for the requirement of CD28 as well. CD28 deficient NOD mice have greatly reduced numbers of CD4+CD25+ cells and exhibit an increased incidence of diabetes (28). Given that CD28 co-stimulation induces IL-2 production in conventional peripheral cells (29), one explanation for the CD28 dependency is that CD28 co-stimulation in the thymus is required for the induction of IL-2 production. This is unlikely though, as CD4+CD25+ T cells do not produce IL-2 and CD28 dependent co-stimulation in the thymus leads to negative selection in conventional CD4+ T cells (30). Tai et. al. recently demonstrated that in fact, CD28 costimulation is required for thymic selection of CD4+CD25+ T cells but that it is required independently of its ability to induce IL-2 (31). CD28 molecules with mutations in the cytosolic tail were introduced as transgenes into CD25 deficient mice. Although CD28 signaling was required to generate thymic and peripheral CD4+CD25+ T cells, they did not require the phosphatidylinositol 3-kinase (PI3K) domain or the Itk kinase binding domain of CD28, but did require the lck binding domain. Moreover, it was determined that IL-2 production also required an intact lck binding domain, but not a PI3K or Itk kinase binding domain. However, mixed bone marrow chimeras of wild type bone marrow and bone marrow from IL-2 deficient mice transferred into lethally irradiated wild type mice generated animals with equal frequencies of both phenotypes. The CD4+CD25+ cells of IL-2-” (no underline) origin isolated from the periphery possessed the phenotype of wild type CD4+CD25+ T cells. Cells of wild type origin provided IL-2 for the IL-2 deficient cells therefore, the IL-2 requirement was not cell intrinsic. Next, bone marrow from CD28 deficient mice mixed with bone marrow from IL-2 deficient mice transferred into lethally irradiated CD28 deficient mice did not generate CD4+CD25+ T cells of IL-2-” origin. Thus, T cells of CD28-” origin did not produce sufficient IL-2 to generate CD4+CD25+ T cells of IL-2-” origin and CD28 costimulation is required for this IL-2 production. Finally,
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lethally irradiated wild type mice reconstituted with bone marrow cells from wild type mice mixed with bone marrow cells from CD28 deficient mice only gave rise to CD4+CD25+ T cells of wild type origin. Therefore, even the presence of extrinsic IL-2 was not sufficient to generate CD4+CD25+ T cells in the absence of intrinsic CD28 signaling.

5. IL-2 AND THE EXPANSION AND MAINTENANCE OF CD4+CD25+ T CELLS

In addition to the requirement for IL-2 in the generation of CD4+CD25+ T cells, IL-2 signaling is also required for the expansion and survival of the CD4+CD25+ T cells upon export from the thymus. A recent study by Bayer et al. demonstrated that when carboxyfluorescein diacetate, succinimidyl ester (CFSE) labeled CD4+CD25+ T cells were transferred to neonatal CD122 deficient mice, the cells migrated preferentially to the lymph nodes and rapidly divided (32). When transferred in the presence of anti-IL-2, CD4+CD25+ T cells did not expand and could not be detected in significant numbers. Furthermore, wild type neonates treated with anti-IL-2 exhibited reduced numbers of CD4+CD25+ T cells.

Another study by Setoguchi et al. also demonstrated the important role of IL-2 in the expansion and survival of CD4+CD25+ T cells (33). Anti-IL-2 administration to adult BALB/c mice preferentially reduced the absolute number of CD4+CD25+ T cells. As IL-2 regulates the expression of CD25, they also demonstrated that FoxP3, a marker of CD4+CD25+ T cells, was reduced to the same degree. CD4+CD25+ T cells exhibited a significant degree of BrdU incorporation when compared to conventional CD4+CD25- T cells and in agreement with the premise that IL-2 is important for the expansion of CD4+CD25+ T cells, anti-IL-2 treatment preferentially reduced BrdU incorporation in CD4+CD25+ T cells. The reduced numbers of CD4+CD25+ T cells induced by anti-IL-2 treatment ultimately resulted in autoimmune gastritis that was evident at 3 months post-treatment. However, the number of CD4+CD25+ T cell had returned to normal at 3 months. Moreover, these CD4+CD25+ T cells were functional, suppressive CD4+CD25+ T cells. In addition, anti-IL-2 treatment also reduced CD4+CD25+ T cells in NOD mice and led to an earlier onset of diabetes, an increased incidence of diabetes and the involvement of other organ specific disease not seen in controls. Thus, a transient deficiency in CD4+CD25+ T cells was sufficient to induce disease in normal mice.

Malek et al. offer further support for the requirement of IL-2 signaling in the expansion and maintenance of CD4+CD25+ T cells as wild type CD4+CD25+ T cells do not expand or engraft in neonatal IL-2 deficient mice and do not prevent the induction of autoimmune disease (22). In addition, CD4+CD25+ T cells from IL-2R-beta transgenic CD122 deficient mice do not expand or engraft when transferred to CD122 deficient mice nor do they prevent the induction of disease. However, the CD4+CD25+ T cells isolated from the periphery of IL-2R-beta transgenic CD122 deficient mice are anergic and suppressive when assayed in vitro but are still unable to engraft and function when transferred.

Although the expression of an IL-2R-beta transgene in CD122 deficient mice restores CD4+CD25+ T cells in the thymus and in the periphery, the peripheral CD4+CD25+ T cells are CD122 deficient and are unable to signal through the IL-2R (22). It is therefore possible that IL-2 signaling is not needed in the periphery and other cytokines, such as IL-4 (insert space) or IL-7, may maintain CD4+CD25+ T cell homeostasis. An alternate and more likely explanation is that IL-2 is required in the periphery for the homeostatic maintenance of CD4+CD25+ T cells and that there is considerable death of the CD4+CD25+ T cells. The expression of the IL-2R-beta transgene, however, may compensate for the lack of IL-2 signaling and lead to a constant, increased generation of CD4+CD25+ T cells. In support of this idea, the percentage of CD4+CD25+ T cells is higher in both the thymus and lymph nodes of IL-2R-beta transgenic CD122 deficient mice when compared to non-transgenic CD122 heterozygous animals. BrdU uptake assays to examine the turnover rate of CD4+CD25+ T cells from IL-2R-beta transgenic CD122 deficient mice and/or thymectomy of these mice would easily address this issue.

As discussed above, CD28 costimulation is required for thymic generation of CD4+CD25+ T cells independently of its ability to stimulate IL-2 production. CD28 also appears to be required for peripheral survival but unlike the thymic requirement, the peripheral requirement is IL-2 dependent (34). Although the number of CD4+CD25+ T cells in the thymus and the periphery are greatly reduced in CD28 deficient mice, these mice do not develop a lymphoproliferative syndrome or autoimmunity. This is most likely due to the inability of the effector cells to be activated due to the defect in CD28 costimulation. Wild type CD4+CD25+ T cells transferred to CD28 deficient mice did not engraft and it was concluded that the host CD28 deficient T cells were unable to support the persistence of CD4+CD25+ T cells, most likely due to the absence of IL-2. Surprisingly, an overnight pretreatment of CD4+CD25+ T cells with IL-2 was sufficient to permit the survival of wild type CD4+CD25+ T cells transferred to CD28 deficient mice. Costimulation through CD28 did not appear to function by inducing survival factors, as CD4+CD25+ T cells from mice expressing a transgene for Bcl-xL were still depleted upon treatment with CTLA-4 Ig. Rather, costimulation through CD28 appeared to be important for the maintenance of high CD25 expression on CD4+CD25+ T cells.

6. IL-2 AND THE FUNCTION OF CD4+CD25+ T CELLS

In vitro, it appears that IL-2 signaling is also critical for CD4+CD25+ T cell effector function. Studies from our laboratory have demonstrated that IL-2 is required for effector function (10). Although the proliferation of responder CD4+CD25+ T cells is inhibited by anti-IL-2, the induction of IL-2 mRNA in these cells is unaffected by anti-IL-2 treatment. As CD4+CD25+ T cells inhibit
CD4+CD25- responder T cells in vitro through the inhibition of IL-2 mRNA transcription, we were able to examine the effect of anti-IL-2 on CD4+CD25+ T cell function. In the presence of anti-IL-2, inhibition of IL-2 mRNA transcription in CD4+CD25-T cells by CD4+CD25+ T cells was not observed. Thus, we concluded that anti-IL-2 acted upon the CD4+CD25+ T cells and prevented their effector function. A study by de la Rosa et al. also demonstrated the importance of IL-2 signaling for CD4+CD25+ T cell effector function (35). Using a mouse/human chimeric suppression assay to selectively block CD25 and CD122 on mouse CD4+CD25+ T cells they demonstrated that the blockade of IL-2 signaling reversed suppression. As the blockade was selective for mouse molecules, IL-2 signaling was clearly required for CD4+CD25+ T cell function.

Although the in vitro studies support a critical role for IL-2 signaling in CD4+CD25+ T cell effector function, the in vivo data are not as clear. Transgenic IL-2R-beta expression in CD122 deficient mice restores the generation of CD4+CD25+ cells as well as the appearance of these cells in the periphery (22). However, peripheral CD4+CD25+ T cells are deficient for CD122 and are unable to transduce an IL-2 signal, yet they are functional when assayed for suppressive activity in vitro. This argues against the requirement for IL-2 signaling not only for maintenance as discussed above, but also for effector function. Alternate explanations are that other cytokines such as IL-4 or IL-7 can substitute for IL-2 or that the thymic IL-2R-beta transgene expression results in the export of activated CD4+CD25+ T cells into the periphery.

7. IL-2 IN THE SIGNALING OF CD4+CD25+ T CELLS

Considerable effort has gone into the study of the IL-2 signaling requirements for CD4+CD25+ T cells, but very little is known regarding the molecular events triggered by IL-2R engagement. A hallmark of CD4+CD25+ T cells is their inability to respond to TCR stimulation and to produce IL-2. Several recent studies have begun to elucidate the molecular mechanisms responsible for this unique phenotype. In an analysis of the signaling cascade required for IL-2 transcription, Su et al. demonstrated that stimulation of freshly isolated cells with PMA and ionomycin led to comparable phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) in both Ras-mitogen-activated protein kinase (MAPK) and PMA and ionomycin led to comparable phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) in both Ras-mitogen-activated protein kinase (MAPK) and PI3K, was not observed (42). The inability to activate PI3K arm of the IL-2 signal cascade has been shown to be critical for the antiapoptotic effects of IL-2 (43). However, freshly isolated CD4+CD25+ T cells, when cultured in vitro with IL-2 alone, did not proliferate but exhibited a marked increase in survival when compared to cells cultured in media alone, despite the absence of Akt activation. The survival advantage conferred by IL-2 treatment appeared to be dependent on Bcl-XL, in contrast to the results of Tang et al. (34). PI3K activation appeared to be normal, however PI3K is negatively regulated by the lipid phosphatase, phosphatase and tensin homologue deleted on chromosome 10 (PTEN) (44). In

In a closer examination of the IL-2 mediated signaling cascade in CD4+CD25+ T cells, Antov et al. examined CD4+CD25+ T cells in Janus kinase 3 (Jak3) deficient mice (37). IL-2 signaling, as well as that of all cytokine receptors that use the common cytokine-receptor gamma-chain, depends upon the activation of Jak3 kinase. CD4+CD25+ T cells were reduced in Jak3 deficient mice and were comparable to the percentages of cells observed in IL-2 and IL-2R-beta deficient mice. In Bcl-2 expressing mice, the cells were greatly reduced in the spleen but not in the thymus. The transfer of bone marrow cells derived from IL-2 deficient mice that were transduced with an inducible form of STAT5 into irradiated hosts, in fact, led to an increase in functional CD4+CD25+ T cells. The importance of IL-2 signaling through STAT5 is also demonstrated by the findings of Burchill et al. (41). In this study, mice expressing a transgene with a constitutively active form of STAT5b, exhibited a striking increase in CD4+CD25+ T cells that were indeed functional CD4+CD25+FoxP3+ T cells with suppressive activity. Despite the fact CD4+CD25+ T cells express TCR and IL-2R, they are anergic to stimuli through either receptor alone. A combination of TCR and cytokine stimulation is required to elicit a proliferative response. In conventional activated CD4+ T cells, signaling through IL-2R activates Jak1 and Jak3 followed by the recruitment of STAT5 and (deleted spelling of PI3K) PI3K and (deleted spelling of MAPK-moved higher up) MAPK (Figure 1). Bensinger et al. have recently demonstrated that upon IL2R signaling in CD4+CD25+ T cells, Jak/STAT activation is normal, but the activation of Akt and p70s6kinase, downstream targets of PI3K, was not observed (42). The inability to activate the PI3K arm of the IL-2 signal cascade has been shown to be critical for the antiapoptotic effects of IL-2 (43). However, freshly isolated CD4+CD25+ T cells, when cultured in vitro with IL-2 alone, did not proliferate but exhibited a marked increase in survival when compared to cells cultured in media alone, despite the absence of Akt activation. The survival advantage conferred by IL-2 treatment appeared to be dependent on Bcl-XL, in contrast to the results of Tang et al. (34). PI3K activation appeared to be normal, however PI3K is negatively regulated by the lipid phosphatase, phosphatase and tensin homologue deleted on chromosome 10 (PTEN) (44). In
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contrasts to the low levels of PTEN in control primed CD4+ T cells, naïve CD4+ T cells and CD4+CD25+ T cells displayed high levels of PTEN in the presence of IL-2. Furthermore, TCR signaling resulted in the downregulation of PTEN and the phosphorylation of Akt in CD4+CD25+ T cells. Therefore, IL-2 signaling in CD4+CD25+ T cells results in the appropriate activation of STAT5 and PI3K, but PI3K is inhibited by PTEN. In the presence of TCR stimulation, the inhibition of PI3K by PTEN is removed resulting in the proliferation of CD4+CD25+ T cells.

8. CONCLUDING REMARKS

The unexpected phenotype displayed by IL-2- and IL-2R deficient mice puzzled scientists for some time as they tried to fit the contradictory in vivo findings to the premise that IL-2 was the major T cell growth factor. The pieces of the puzzle began to fall into place with the discovery that CD4+CD25+ T cells were key regulators of tolerance. It is now evident that the lymphoproliferation and lethal autoimmunity in IL-2 and IL-2R deficient mice can be explained primarily, if not solely, by the absence of CD4+CD25+ T cells. It is also clear that expression of CD25 on these cells is not merely a marker of activation, but is a critical molecule responsible for the generation, the expansion, the maintenance and the function of CD4+CD25+ T cells. The critical importance of IL-2 signaling in CD4+CD25+ T cells makes it an attractive candidate for therapeutic approaches to autoimmune disorders as well as for tumor vaccines. The manipulation of IL-2 signaling to expand or activate CD4+CD25+ T cells may be possible to treat or prevent autoimmunity, while the manipulation of IL-2 signaling to block CD4+CD25+ T cell function could enhance immune responses to tumor vaccines. Although we have a much better understanding of the role that IL-2 and CD4+CD25+ T cells play in tolerance, there remain numerous questions to be addressed.

9. REFERENCES

20. P. Taylor, R.J. Noelle and B.R. Blazar: CD4+CD25+ immune regulatory cells are required for induction of

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