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IL-2: A Two-Faced Master Regulator of Autoimmunity

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Abstract

CD4⁺ T-cell (Th) cytokines provide important regulatory and effector functions of T-cells. Among them, IL-2 plays a unique role. IL-2 is required for the generation and maintenance of regulatory T-cells (Treg) to provide lifelong protection from autoimmune disease. Whether IL-2 is also required for autoimmune disease development is less clear as *Il2*^{-/-} mice themselves spontaneously develop multi-organ inflammation (MOI). In this communication, we discuss evidence that support the thesis that IL-2 is required for the development of autoimmune response, although some aspects of autoimmune response are not regulated by IL-2. Potential IL-2-dependent mechanisms operating at specific stages of the inflammation process are presented. The interplays among Treg, IL-2, autoimmune response and adaptive immunity are discussed. Overall, available information indicates that IL-2 is a two-faced master regulator of autoimmunity: one to prevent autoimmunity while the other promotes autoimmune response. The latter is an unfortunate consequence of IL-2 function that is used to promote the adaptive immune response against foreign antigens and pathogens.

Keywords

IL-2; Regulatory T-cells; Th cytokines; Trafficking; Inflammation; Autoimmunity; Scurfy

1. Introduction

Immunity is a two-edged sword. It provides the necessary weapon to protect the host from foreign pathogens but when uncontrolled, it can attack the host with severe and even fatal consequences. It appears that the potential anti-self response is the necessary collateral during thymic selection that produces MHC-restricted T-cells for the protective adaptive immunity against foreign Ag. However, this anti-self response is contained by Treg under normal condition [1–3]. IL-2 is a critical component for Treg development and function and as such it serves as a negative regulator of autoimmunity. However, IL-2 is also well known for its role in T-cell activation and expansion, raising the question as to whether it also promotes autoimmune response. Although the T-cell activation function of IL-2 is well appreciated, this activity was demonstrated only in in vitro experiments. Indeed, the in vitro mitogen response of *Il2*^{-/-} T-cells was diminished but not abolished completely and *Il2*^{-/-}

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mice develop autoimmune disease. With the exception of Treg, the distribution of lymphocyte subsets appears normal in *Il2*^{-/-} mice before the severe autoimmune response develops. These observations suggest that the exact functions of IL-2 cannot be easily determined because of the compensatory effects of other cytokines in the *Il2*^{-/-} mice [4,5]. However, new evidence has emerged from our studies that strongly support a crucial role of IL-2 in the development of autoimmune disease [6,7]. The following discussion addresses how IL-2 serves as a two-faced master regulator of autoimmunity.

2. IL-2: a critical component of a genetic program designed to contain autoimmunity

The T-cell immune system originates from the thymocyte development process by which T-cell subsets with distinct functions are generated. This development program is mediated by TCR selection based on their affinity toward the Ag-MHC complex. T-cells expressing the CD4 marker are selected by the peptide Ag-MHC-II complex. The selection process generates two subsets distinguishable by the expression of the Foxp3 transcription factor [8]. The CD4⁺Foxp3⁻ conventional T-cells (Tconv) contain mostly T-cells with a low to moderate affinity for the selecting Ag-MHC-II complex due to thymic negative selection. Many of the CD4⁺Foxp3⁺ T-cells (abbreviated as tTreg) with high affinity for self Ag-MHC-II complex survive the negative selection process. Another important difference is that the selection of Tconv cells does not depend on cytokines, but the development of tTreg involves IL-2. Whether IL-2 significantly affects the size of Treg in the thymus remains unsettled [9–11]. Without IL-2, the Treg level in the thymus of *Il2*^{-/-} mice is significantly reduced to about 55% of B6 control (our unpublished observation). IL-2 also increases the competence of Treg [9,10].

Using Foxp3-GFP knock-in mice, Fontenot et al have convincingly demonstrated that the expression of Foxp3 on CD4⁺ SP T-cells during thymocyte development requires the presence of TCR/MHC components in the thymus [8]. Foxp3 could be detected in cells as early as they are at the DN stage [9]. The observation that TCR⁺CD4⁺CD25⁺Foxp3⁻ thymocytes could be directly induced to express Foxp3 upon stimulation with IL-2 in vitro suggests that the thymocyte acquisition of IL-2 responsiveness for Foxp3 expression also occurs at TCR/Ag-MHC-II selection stage [12,13]. Although the Treg exodus to the periphery is delayed as compared to the Foxp3⁻ CD4⁺ SP cells [14], its presence in the periphery is prior to the establishment of peripheral adaptive immune response, suggesting that the primary and major role of IL-2 is for the maintenance of self-tolerance in the periphery through its control of Treg development.

In addition to tTreg, Treg can also be induced in the periphery upon activation of the naïve CD4⁺Foxp3⁻ Tconv cells and with the help of co-stimulation provided by IL-2 and TGF-β1 [15,16]. These cells are termed inducible Treg (iTreg). They also have suppressive functions. However, differences between tTreg and iTreg have been reported in many studies [16]. As CD4 and CD25 were the best markers available for Treg in earlier studies, the so-called CD4⁺CD25⁺ naturally occurring Treg (nTreg) invariably contained both tTreg and iTreg. Many conclusions drawn from studies using nTreg were complicated by this heterogeneity. For example, it is not clear what proportion of the nTreg of adult *Il2*^{-/-} mice are derived from tTreg and iTreg. Recently, the Helios transcription factor of the Ikaros family has been suggested as a marker for tTreg but not for the iTreg elicited by immobilized anti-CD3/anti-CD28 mAb in vitro [17]. We estimated that the nTreg freshly isolated from adult B6 and *Il2*^{-/-} mice contained 55–70% and 85–95% Helios⁺ Treg, respectively (unpublished observation). The data suggest that the nTreg in the periphery of *Il2*^{-/-} mice contains mostly tTreg. Whether the 5–15% Helios⁻ Treg in *Il2*^{-/-} mice is iTreg generated in the absence of IL-2 remains to be determined.

As IL-2 is such a critical cytokine for Treg generation and maintenance, the question as to whether IL-2 can also be a positive regulator of autoimmunity has been raised. If it could, then how it works and how the two opposing forces harmoniously operate in the immune system must be considered. Here, we present our viewpoints on how IL-2 could serve as a two-faced master regulator of the suppressive and stimulatory processes of autoimmunity.

3. The basic scheme of T-cell tolerance

Before the realization and general acceptance of the presence of Treg and its role in the maintenance of peripheral tolerance, it was believed that thymic negative selection could delete high affinity autoreactive T-cells [18,19]. Tconv cells with low affinity against self Ag escaped thymic negative selection but these Tconv cells were quiescent because their engagement with self Ag in the periphery could not result in signal above the threshold for activation. Autoimmune response occurred as these naïve Tconv cells that after subjecting to various immune stimulations had changed their activation threshold. For example, stimulation by foreign Ag that resembled self Ag could “educate” the low-affinity cells and made them more likely to be activated during subsequent encounter of self Ag. The identification of Treg and the demonstration of its critical function in maintaining peripheral tolerance have changed this concept. It was demonstrated that hosts that are deficient or depleted of Treg rapidly develop MOI. These observations strongly suggest that the stimulation threshold is not the limiting factor restricting the autoimmune Tconv cells from being activated in the periphery. However, negative selection is likely to greatly reduce the total number of self-reactive Tconv cells such that they can be contained and actively suppressed by the Treg in the periphery.

If authentic self-reactive T-cells truly developed in *Il2*^{-/-} mice, their autoimmune inflammatory response should not be affected when they are maintained under germ-free condition. Analysis of *Il2*^{-/-} mice under germ-free condition revealed that the intestinal bowel disease was almost completely inhibited. Only mild foci of inflammation were observed [20]. However, these mice still developed undiminished periportal inflammation in the liver [20]. More recently, MOI was examined in adult mice in which the Treg was ablated by diphtheria toxin-induced deletion and the inflammation status of the mice was followed up by maintaining them under germ-free condition [21]. The study revealed strong inflammation in skin, lungs, submandibular gland and stomach whereas inflammation in the small intestine was delayed but the inflammation in the pancreas was significantly enhanced as compared with mice kept under SPF condition [21]. This study provided evidence for the existence of authentic self-reactive T-cells even though immune response to antigens from food, bedding, and air could not be eliminated under such condition. It is also important to point out that the system employs a Treg deletion mechanism that is different from the naturally-derived, Treg-deficient scurfy (Sf) mice in which the “abnormal Treg” is not depleted and could potentially switch to become part of the inflammation-inducing T-cells. Nevertheless, the data suggest that despite lacking such potential source of autoimmune T-cells, the Tconv cells must have a repertoire large enough to elicit autoimmune response against various organs and they are actively inhibited by Treg under normal condition. Thus, the simplest form of Treg-controlled peripheral tolerance would be a homeostatic immune reaction in which self Ag-reactive Tconv cells are stimulated by the antigen-presenting cells (APC) that presents the self Ag. The Treg cells that recognize the presented self Ag react to this response by suppressing it. This check-and-balance process eventually reaches equilibrium that forms a background level of immune homeostasis that does not cause organ damage and yet maintains the constant expression of the autoimmune Tconv and Treg cells (Fig. 1).

4. Treg repertoire and number

Although still unresolved, it is believed that Treg bearing TCR specific to self Ag is less likely to be deleted as compared to Tconv cells bearing the same TCR during thymic selection [22–24]. Therefore, a sizable fraction of the Treg TCR with high affinity is directed against self Ag and the potential repertoire of Treg is comparable to if not larger than Tconv cells [22,23]. Clearly, the absolute number of Treg is smaller than Tconv cells because nTreg constitutes only 5–10% of the CD4⁺ T-cell compartment. However, the absolute number of self Ag-reactive Tconv cells is likely to be quite small as a result of thymic negative selection. The 5–10% level of nTreg in the peripheral CD4⁺ T-cell compartment appears to be the critical proportion that allows self tolerance to be maintained and immune response against a given foreign Ag to occur under appropriate stimulating conditions.

Conceptually, self-reactive Treg must also be present in the iTreg compartment because iTreg is derived from naïve Tconv that contains self-reactive T-cells as revealed by the Treg ablation experiment described above [21]. As stated earlier, the majority of the Tconv cells bearing self-Ag specificity are deleted during thymic negative selection. According to this thinking, iTreg against self Ag would be small under in vivo condition in which only a mild background adaptive immune response is maintained. However, transfer of a large number of iTreg that were generated poly-clonally in vitro has been shown to inhibit the MOI development in newborn Sf mice for as long as 4 weeks [25]. The large number required as compared to the small number of nTreg under similar condition also supports the model and implies that it is the self-reactive iTreg that maintains the peripheral tolerance in this artificial system [25,26].

5. How foreign Ag-specific response occurs?

We hypothesize that under homeostatic condition, significant suppression of immunity is provided by the Treg that recognizes self-Ag presented on APC. Because Treg suppression is Ag-nonspecific and Treg number for self Ag as a whole is large and because self Ag is abundant and constantly presented by APC, the suppression provided by self Ag-specific Treg is sufficient to maintain a background level of immune homeostasis. The question that can be asked is how a given foreign Ag induces specific immune response. A likely possibility is that when a foreign Ag is picked up and processed by APC, it effectively competes and replaces other Ag (mostly self Ag) naturally present in the peptide/MHC-II pool such that the APC's ability to maintain the Treg presence on the APC is greatly reduced. In addition, the increased foreign Ag peptide/MHC-II complex will attract more Ag-specific Tconv cells. This will reduce the suppressive force imposed by the Treg and allow Ag-specific Tconv cells to be activated (Fig. 2). This interpretation assigns little role to the iTreg in controlling the foreign Ag-specific response at the beginning of the immune stimulation. As the immune response progresses, IL-2 and TGF- β 1 are produced and these cytokines favor the generation of iTreg and the competitiveness of Treg suppressive activity while the foreign Ag level falls (Fig. 2). This model satisfactorily explains how immune response against foreign Ag is dynamically regulated by Treg and why MOI develops in the absence of Treg.

6. IL-2 as a positive stimulator of autoimmunity

Until recently, evidence that IL-2 is required for the development of autoimmune disease is lacking [27]. When an lck promoter-controlled *foxp3* transgene was introduced into *Il2r β ^{-/-}* mice, peripheral tolerance was restored despite the fact that the Treg was derived exclusively from the thymus [27]. Based on this study, it was proposed that the main purpose of IL-2/IL-2R signaling system is for the generation and maintenance of Treg that

serves to maintain peripheral tolerance [28–30]. It was further argued that this signaling system is not required for the peripheral adaptive immunity [30]. This contention was based on many reports that showed that the adaptive immunity was not impaired in the *Il2*^{−/−} and *Il2ra*^{−/−} mice as compared to wild-type controls [30]. This assessment is not accurate because these mutant mice lack Treg and that any reduction of adaptive immunity could have been compensated and masked by the absence of Treg, resulting in the apparent lack of a positive influence by IL-2/IL-2R signaling system. For example, the susceptibility to Herpes Simplex Virus type 2 as determined by survivability showed no difference between *Il2ra*^{−/−} mice and wild-type control [31]. This protection is mediated by CD8⁺ T-cells. However, the total number and the proportion of CD8⁺ T-cells in the lymphocytes of *Il2ra*^{−/−} mice is significantly higher than wild-type control as a result of the spontaneous lympho-proliferation in the Treg-deficient *Il2ra*^{−/−} mice. In addition, their serum level of IL-2 was extremely high [31,32]. If these factors were taken into account, it would appear that the IL-2/IL-2R signal is required for optimal adaptive response. Thus, the adaptive immunity of the *Il2ra*^{−/−} CD8⁺ T-cells against the virus cannot be accurately assessed. In this regard, perhaps an objective assessment of the effect of IL-2/IL-2R signal on adaptive immunity could be made by comparing the adaptive immune response of the *Il2rβ*^{−/−} mice bearing the lck-promoter-controlled *foxp3* gene with normal B6 mice. Still, such study cannot address the effect of IL-2/IL-2R signal on autoimmunity because the mice do not develop MOI.

To fairly assess the role of IL-2 as a positive regulator of autoimmunity, we introduced *Il2*^{−/−} gene into Sf mice (*Sf. Il2*^{−/−}) so that comparison is made on a leveled plane of autoimmune response initiated by the total lack of Treg [6,7,33]. That IL-2 had an impact on the spontaneous autoimmune disease became clear immediately because the *Sf. Il2*^{−/−} mice lived significantly longer than Sf mice. In addition, they lacked skin inflammation and their lung inflammation was greatly reduced [6,33]. Conversely, the lymphocyte number in the lymph nodes of *Sf. Il2*^{−/−} mice was significantly higher than that in Sf mice [33], perhaps reflecting the inability of T-cell emigration to skin and lungs. It was also found that IL-2 up-regulated the expression of CD103 on CD4⁺ T-cells. CD103 is a component of the integrin $\alpha_E\beta_7$ that binds to E-cadherin on the epithelial cells to allow long retention of T-cells in the skin and lungs [6].

7. Genome-wide analysis revealed that IL-2 positively targets several specific stages of the autoimmune response

Effect of IL-2 on trafficking receptors and chemokines

That IL-2 is a positive regulator for autoimmunity is revealed by the genome-wide analysis of the differentially expressed genes among the CD4⁺ T-cell samples from B6, Sf, and *Sf. Il2*^{−/−} lymph nodes [7]. Hundreds of genes involved in T-cell proliferation, activation, and trafficking are highly expressed in the Sf samples. Of particular significance is the differential regulation of a large array of genes for trafficking/chemotaxis/retention and chemokines. Four patterns of regulation are observed (Table 1). Group A are those that are highly expressed only in Sf samples. Many genes in this group are known to associate with a strong Th2 response [7]. The data demonstrate that IL-2 is critically required for the induction of trafficking genes associated with Th2 response. Group B are those that are highly expressed in *Sf. Il2*^{−/−} samples when compared to Sf sample. The number of genes belonging to this group is small. The same genes are slightly up-regulated in Sf samples as compared with B6 control. Group C are those that are up-regulated in both samples as compared with B6 control. Most of them in this group are up-regulated modestly. Group D are those that are mildly down-regulated in both samples as compared with B6 control but there is little difference between them. This group contains genes encoding integrins and a

few chemokines. Only *Ccr7* gene that encodes the receptor for lymph node homing was down-regulated. Apparently, the mild change in gene expression in this group did not impact the inflammation observed in Sf and *Sf.II2^{-/-}* mice [7].

The microarray analysis also revealed that IL-2 regulates Th chemokine gene expression [7]. Many of the up-regulated chemokine genes encode ligands for the up-regulated chemokine receptor genes. The co-induction of chemokine/chemokine receptor pairs was also noted in *Sf.II2^{-/-}* CD4⁺ T-cells when compared with wild-type sample, although the number of such pairs was smaller than Sf CD4⁺ T-cells. In addition, the co-induction of chemokine genes was not observed for certain other chemokine receptors. This novel strategy of co-induction of chemokine/chemokine receptor pairs suggests a self-perpetuating mechanism for T-cell recruitment during tissue inflammation. Genes encoding several chemokine/chemokine receptor pairs that have been shown to selectively express in Th1 cells and Th2 cells were observed [7,34].

Effect of IL-2 on Th2 response

Surprisingly, all Th cytokine genes examined were not differentially expressed between Sf and *Sf.II2^{-/-}* samples [7]. A comparison made with B6 control indicated that these Th cytokine genes were highly up-regulated in both samples. As Th cytokines provide various and critical effector functions of autoimmune reaction, the latter finding seems to support the idea that IL-2 may not be required for this limb of the autoimmune response in Sf mice. However, serum levels of IL-4, IL-5, and IL-13 were significantly lower in *Sf.II2^{-/-}* mice as compared with Sf samples. Upon ex vivo activation with PMA plus Ionomycin, the frequency of the IFN- γ -producing Th1 cells was not significantly different between Sf and *Sf.II2^{-/-}* samples but the IL-4, -5, -13-producing Th2 cells were significantly reduced. Collectively, these analyses demonstrate that IL-2 is a positive regulator for many aspects of the autoimmune T-cell response process in Sf mice. Its influence on Th2 response is particularly evident during T-cell activation. However, some specific aspects of the response such as the production of IFN- γ and TNF- α are apparently not influenced by the presence or absence of IL-2 [7].

The observation that the autoimmune response of Sf but not *Sf.II2^{-/-}* mice displayed a heightened Th2 responses and remarkable up-regulation of a large array of genes for T-cell trafficking not only provides direct evidence that IL-2 is a positive regulator for autoimmunity but also reveals the specific targets and stages of its effect. Our data suggest that the optimal induction of Th2 response and its cytokine effector functions by IL-2 are important in the target organs in which the activation of the infiltrated T-cells takes place to elicit tissue inflammation. Our study also suggests that the induction of genes for T-cell trafficking occurs in the draining lymph nodes and the inhibition of skin and lung inflammation in *Sf.II2^{-/-}* mice correlates with the absence of activation of these genes. It is important to indicate that certain trafficking receptor genes and Th cytokines were up-regulated in *Sf.II2^{-/-}* samples as compared with wild-type control. Thus, certain aspects of the autoimmunity are clearly independent from IL-2 in Sf mice. Indeed, inflammation in the liver, pancreas, and colon are not inhibited in *Sf.II2^{-/-}* mice.

8. A general scheme of IL-2 controlled MOI in Sf mice

Based on the above observations, we propose the following scheme for the development of MOI and the specific stages that are controlled by IL-2 in Sf mice. It starts by the dendritic cells that carry the organ Ag to the draining lymph nodes and present them to naïve Tconv cells. A robust, un-regulated and progressive polyclonal response is induced. This result results in CD4⁺ T-cell priming marked by heightened Th2 reaction along with increased Th1 response that provides IL-2. Importantly, this activation induces the hyper-expression on

CD4⁺ T-cells of a large panel of receptors that are critical to the trafficking/chemotaxis/retention of the T-cells to target organs where the corresponding ligands such as chemokines, leukotrienes and E-cadherin are expressed. Due to lack of IL-2, the expression of some of these receptors is inhibited in *Sf.II2^{-/-}* mice and cannot infiltrate into the skin and lungs. Additionally, Th2 cytokine production is also inhibited, particularly during the activation of CD4⁺ T-cells of *Sf.II2^{-/-}* mice. In the target organs of *Sf* mice, the induction of Th cytokines and chemokines could exacerbate the inflammation process through their ability to recruit additional inflammatory cells including neutrophils, macrophages and the same CD4⁺ T-cells that express the receptors for the chemokines. IL-2 activated T-cells in the target organs also produce more IL-3 and M-CSF that could increase the number of myeloid cells, which degranulate and release leukotrienes and other chemo-attractants to fuel the unabated cycles of inflammation (Fig. 3).

The microarray analysis indicates that IL-2 controls autoimmune response at the stage of T-cell activation/expansion, trafficking, Th2 development, and the production of cytokines for inflammatory effector functions in target organs. The data provide convincing evidence that IL-2 is critically required for the development and progression of the autoimmune inflammation in the skin and lungs, but not for the inflammation in liver, pancreas, and colon. Although inflamed, the Th2 response may be deficient in the latter organs in *Sf.II2^{-/-}* mice. This apparent “organ-specific” control of autoimmune inflammation by IL-2 in *Sf* mice is likely mediated by its regulation of trafficking receptor expression in the draining lymph nodes and Th2 expansion in the target organs. Although IL-2 can promote Treg control of autoimmunity, the dilemma of IL-2 acting as a positive regulator of autoimmunity should be considered regardless when IL-2 is used to treat autoimmune diseases.

9. The case of IL-2 regulation of type-I diabetes

The NOD (Non-Obese Diabetic) mice develop MOI but it is the autoimmune Insulin-Dependent Diabetes Mellitus (IDDM) that is most extensively studied. NOD mice express a variant form of IL-2, a CTLA-4 splice variant lacking B7 binding activity, and a somewhat reduced level of nTreg [35–37]. Each of these characters could have contributed to diabetes progression by affecting Treg development and function. Treatment of NOD mice with a low dose of IL-2 has been shown to be protective and therapeutic [38,39]. The effect is attributed to the increased presence of Treg in the pancreas [39]. In *NOD.foxp3^{sf/Y}* mice, MOI appeared worsened as a result of Treg absence but the analysis of diabetes was seriously limited by the *Sf* mutation-induced early death [40]. We were also unable to assess the effect of IL-2 on diabetes in *NOD.II2^{-/-}* mice due to early death (unpublished observation). When a diabetogenic TCR transgene (BDC2.5) was introduced into *NOD.foxp3^{sf/Y}* mice, the early death was delayed due to the reduction of the autoimmune repertoire and density of the non-transgenic TCR imposed by the transgene [41,42]. Diabetes developed faster and was more severe in these mice as compared to the Treg-sufficient, TCR transgenic NOD mice. These observations indicate that the total absence of Treg further impinges on autoimmune diabetes progression.

In an artificial mouse model of diabetes, mice whose pancreatic β -cells were genetically modified to express hen egg lysozyme (HEL) were bred with mice bearing the HEL-specific TCR transgene [43]. Breeding *II2^{-/-}* gene into these mice induced diabetes. The development of diabetes was prevented by treatment with a low dose IL-2. The low dose IL-2 appeared to preferentially induce Treg in the thymus [43]. The lack of IL-2 effect on Tconv trafficking suggests that trafficking receptors regulated by IL-2 are not critical to Tconv cell infiltration to islets. In *Sf.II2^{-/-}* mice, inflammation in pancreas also remains undiminished as compared to *Sf* mice [7].

The dual effect of IL-2 on tolerance and immunity can be avoided during therapy by using Treg expanded in vitro rather than using IL-2 for treatment. This is best exemplified by the studies of NOD mice [44]. The nTreg (CD4⁺CD25⁺CD62L^{low}) in NOD mice decrease with age and diabetes progression. Transfer of a very large number of the Treg from young NOD mice was necessary to achieve therapeutic efficacy. This was attributed to the low frequency of the Ag-specific Treg in the population. Using CD4⁺CD25⁺CD62L^{low} T-cells from BDC2.5 NOD mice, Ag-specific Treg was expanded by anti-CD3/anti-CD28 beads in the presence of high rIL-2 (2000 IU/ml). This population was highly effective in suppressing diabetes development as well as reversing the diabetes in the new-onset mice [44]. Although a combined treatment with IL-2 and sirolimus is currently being evaluated in a clinical trial [45], the data obtained with Ag-specific Treg in NOD system seems highly promising for the treatment of IDDM without the potential complication associated with IL-2. Thus, it seems plausible that human Treg specific to glutamic acid decarboxylase and proinsulin could be developed and used as a therapeutic agent for IDDM in the future [46].

10. Concluding remark

In this review, evidence that IL-2 is both a negative and a positive regulator of autoimmunity is presented. In addition, specific aspects of the mechanisms of IL-2-mediated regulation are discussed. The finding that IL-2 has a pro-inflammatory function through regulating a large panel of genes involved in the inflammation process should open up a whole new area for inflammation research and should move the field forward. Possible use of Ag-specific Treg in lieu of IL-2 to avoid the potential pro-inflammatory effect of IL-2 is considered. The interesting question is why IL-2 is the major cytokine that is endowed with this unique property. Although the reason is not entirely clear, the fact of the matter is that this unique manifestation of IL-2 as a two-faced master regulator of both immune stimulator and immune suppressor provides one of the most efficient, rapid, and buffered control system to deal with autoimmunity and the adaptive immune response (Figs. 1–3). In this manner, the protection against pathogens and autoimmunity can be most effectively addressed with minimal autoimmune injury to the host.

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Fig. 1.

Treg control of peripheral tolerance: Self Ag are abundant and are always presented by APC. The number of nTreg as a whole is large. In contrast, the number of self Ag-reactive Tconv cells is small as a result of deletion by thymic negative selection. The check-and-balance between the two opposing reactions eventually reaches equilibrium such that a background but non-harmful co-existence of self Ag-reactive Tconv and nTreg is maintained.

Fig. 2.

Induction of foreign Ag-specific response: When sufficient foreign Ag is picked up and processed by APC, it competes with self Ag for the MHC and replaces the self Ag to form foreign Ag-MHC complex. This results in loss of Treg and increase in foreign Ag-specific Tconv cells on the APC. The loss of Treg allows the activation of the foreign Ag-specific Tconv cells. As the reaction progresses and proceeds to its later stage, IL-2 and TGF-β1 are produced and the foreign Ag level becomes low. The condition favors the production of foreign Ag-specific iTreg and the return of the nTreg binding to the APC and its competitive suppressing activity.

Fig. 3.

A general scheme of IL-2 regulated autoimmune response in Sf mice: Self Ag in organs are picked up by APC and enter the draining lymph nodes through lymphatic. In the lymph nodes, they prime the self-reactive Tconv cells. As a result of total absence of Treg, the activation cannot be contained. In the presence of IL-2, both Th1 and Th2 responses are activated and a large array of trafficking receptors (TrR) is induced. These T-cells travel through blood, and depending on their receptor expression, enter specific target organs. In the target organs, their activation by local APC induces the production of effector cytokines and inflammation. In the *Sf.II2*^{-/-} mice, priming of both Th1 and Th2 cells occurs in the lymph nodes but the critical trafficking receptors for infiltrating skin and lungs are not induced. This effect coincides with the absence of skin and lung inflammation in *Sf.II2*^{-/-} mice. However, *Sf.II2*^{-/-} mice still display inflammation in the liver, pancreas, submandibular gland and colon. Due to the lack of IL-2, the inflammation in these organs is likely to be dominated by Th1 response.

Table 1

Differentially displayed genes among Sf, Sf.*Il2*^{-/-}, and B6 CD4⁺ T-cells can be classified.*

Group	Pattern of regulation	Genes that are selectively regulated
A	↑ in Sf vs Sf. <i>Il2</i> ^{-/-}	<i>Cysltr1</i> , <i>Ltb4r1</i> , <i>Il1rl1</i> , <i>Itgae</i> , <i>Ccr1</i> , 2, 8, <i>Cxcr6</i> ^{**} , <i>Ccl1</i> ^{**} , 8, <i>Cxcl2</i>
B	↑ in Sf. <i>Il2</i> ^{-/-} vs Sf	<i>Cxcr5</i> , <i>Ccr5</i> , <i>Ccl4</i> , 5,
C	↑ in Sf. <i>Il2</i> ^{-/-} & Sf vs B6	<i>Ccr3</i> , 4, 6, <i>Cxcr2</i> , 3, 6, 7, <i>Ccl1</i> , <i>Cxcl3</i> , 8, 9, 10, 11
D	↓ in Sf. <i>Il2</i> ^{-/-} & Sf vs B6	<i>Ccr7</i> , <i>Ccl9</i> , <i>Ccl20</i> , <i>Itga4</i> , <i>Itga6</i> , <i>Itga9</i> , <i>Itgb3</i> , <i>Sell</i> , <i>Selp1g</i>

* The table is compiled from Table 1 in [7].

** Both genes are up-regulated Sf and Sf.*Il2*^{-/-} samples when compared with B6, but much more so in the Sf samples.