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Genetic control of the inflammatory T-cell response in regulatory T-cell deficient scurfy mice☆

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Abstract

IPEX (Immunodysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome is a rare, recessive disorder in patients with mutations in the *foxp3* gene, the normal expression of which is required for the generation of functional regulatory T-cells. Scurfy mice also bear a mutation in the *foxp3*, and like IPEX patients, spontaneously develop multi-organ inflammation. As reviewed herein, breeding immune response genes into Scurfy mice has provided useful insight into how the inflammatory T-cell response is regulated in the absence of regulatory T-cells and post regulatory T-cell checkpoint. Of particular interest are those that preferentially affect the inflammatory T-cell response in an "apparent" organ-specific manner, implying that specific mechanisms of control exist for individual organs during multi-organ inflammation.

Keywords

Multi-organ inflammation; Scurfy mice; Genetic regulation

Introduction

A major control mechanism for self-tolerance is mediated by a $CD4^+$ T-cell subset termed regulatory T-cells (Treg)[2] [1–3]. The expression of Treg is critically dependent on the X-linked gene encoding the transcription factor Foxp3 [4,5]. Patients bearing a mutation in their *foxp3* gene invariably develop IPEX syndrome which is characterized by systemic autoimmunity manifested as diarrhea, eczematous dermatitis, insulin-dependent diabetes mellitus, anemia, thrombocytopenia, neutropenia, and tubular nephropathy [6]. However, clinical variation of these symptoms is observed among families and within families [6]. Factors such as the severity of the mutational effect on Foxp3 expression and/or function, genetic background that influences Foxp3 expression or function, and environment are likely reasons for the heterogeneity of IPEX clinical outcome. For this reason, it is difficult to study how the variations are developed in the manifestation of the systemic autoimmunity in IPEX patients.

Scurfy (Sf) mice bear a mutation in *foxp3* gene such that they cannot produce functional Foxp3 and are totally devoid of Treg [4]. As a result, spontaneous immune response against host antigens (Ag) develops in an uncontrollable and unabated manner, leading to severe multi-organ inflammation (MOI), and death around 3 to 4 weeks of age. Organs such as skin, tail, lungs and liver are affected first [7]. Unlike IPEX patients, diabetes mellitus and

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diarrhea are not observed but the inflammation in skin, lung and liver is reproducibly developed due to genetic homogeneity despite the fact that the $CD4^+$ T-cell expansion is stochastic and widespread across the Vβ and Vα families [8]. Theoretically, Sf mice have the potential to develop inflammation in other organs. A low frequency of organ-specific T-cells, a limited supply of Ag, the pre-weaning condition, and the early death are potential reasons that prevent this process from coming to fruition. Transfer of Sf T-cells into *Rag1*$^{-/-}$ recipients induced inflammation in additional organs [7]. Severe gastrointestinal inflammation was rapidly developed in neonate *Rag1*$^{-/-}$ recipients just a few days after weaning, suggesting mother's milk and the intestinal microbes play a role in the enteropathy [7]. Moreover, inflammation could be demonstrated in accessory reproductive organs in Sf.*fas*$^{lpr/lpr}$ double mutant mice that lived beyond adulthood [9]. Thus, Sf mice offer a unique system to study how Tinf response against multiple organs is regulated by various immune response genes. Importantly, Tinf response in Sf mice occurs beyond Treg checkpoint and without the confounding factor of the feedback regulation by Treg that are present in conventional immune response [10–12]. A straightforward approach is to breed a specific gene, usually in its mutant form, to Sf mice and then to determine the effect of the gene on Tinf response at the organ, cellular and molecular levels. In this communication, we will review studies that bred various immune response genes into Sf mice to study the specific gene effect on Tinf response (summarized in Table 1).

Sf.*cd4*$^{-/-}$ mice

One of the earliest experiments to study immune response gene effect on Scurfy phenotype is by breeding *cd4*$^{-/-}$ and *β2m*$^{-/-}$ genes into Sf mice [13]. These studies demonstrated that *cd4*$^{-/-}$ but not *β2m*$^{-/-}$ gene affected the mortality, organ inflammation, and immunological parameters of Sf mice, thus implicating $CD4^+$ T-cells are critical to the fatal disease. However, the protection by *cd4*$^{-/-}$ gene was only transient. The lifespan of Sf.*cd4*$^{-/-}$ mice was extended from 4 weeks to 6 weeks and the mice eventually died of MOI with immunological manifestation similar to that of Sf mice. Although *cd4*$^{-/-}$ mice lack $CD4^+$ T-cells, class-II-restricted T-cells are still present. The spontaneous T-cell activation in the Sf.*cd4*$^{-/-}$ mice was likely delayed by the lack of the CD4 co-receptor signal, but the activation of Tinf cells eventually developed and induced Sf-like disease.

TCR transgenic (Tg) Sf mice

Because the MOI in Sf mice is mediated by polyclonal $CD4^+$ T-cells, genetic manipulation that restricts TCR repertoire has a major impact on the fatal disease. Breeding foreign Ag-specific TCR Tg into Sf mice delayed but did not eliminate the fatal MOI [14]. Importantly, TCR Tg Sf mice in *Rag*$^{-/-}$ background did not develop Sf disease [14]. Although such mice lack Treg, their T-cells express only the Tg TCR and are unable to respond to host and foreign Ag not recognized by the Tg TCR. Because Tg expression of TCR genes greatly reduces but does not totally freeze endogenous TCR gene rearrangement (unless in *Rag*$^{-/-}$ background), a substantial fraction of their T-cells, estimated to be around 30%, expresses dual-TCR [15,16]. Although there is a loss of diversity, the TCR repertoire derived from endogenous genes is large enough to generate Tinf response and MOI. Also, the endogenous TCR is usually less abundantly expressed than the Tg TCR on a dual-TCR T-cell. These considerations satisfactorily explain the delayed but still fatal MOI in Sf mice bearing foreign Ag-specific TCR Tg.

The power of Treg control of dual-TCR T-cell expansion is displayed in Sf mice bearing the ovalbumin$(OVA)_{323–339}$-specific Tg TCR (OT-II.*foxp3*^{sf}) [17]. The expansion of the T-cells bearing dual-TCR begins as early as 6 days after birth and reaches to as much as 85% of the lymphocyte population. By contrast, T-cells bearing only Tg TCR are not activated and

remain naïve phenotype even in the total absence of Treg control because they can only be activated by $OVA_{323–339}$ peptide which is absent in the mice. This expansion occurs in the peripheral lymphoid tissues but not in the thymus. Importantly, transfer of T-cells that had been depleted of the Tg TCRβ into *Rag1*$^{-/-}$ mice induced MOI, suggesting MOI could be induced by T-cells bearing endogenously derived TCRβ [17].

It is generally believed that autoimmune T-cells are contained by Treg and in the absence of Treg, they expand and MOI develops. Several examples of breeding Tg TCR that has specificity to “natural self-Ag” into Sf mice have been reported. Several such studies are described below.

K/BxN.*foxp3*^{sf} mice

In an attempt to study thymic T-cell selection, Kouskoff et al. [18] generated a Tg mouse line in B6 background designated KRN mice whose TCRα and TCRβ genes were derived from a B10A.4R T-cell hybridoma specific to RNase and restricted by I-A^k. It was fortuitously discovered that when KRN mice were crossed with NOD mice, the progeny (KRN Tg TCR in [B6xNOD]F1) developed arthritis with severe joint inflammation and swelling [18]. The development of arthritis is critically dependent on the Tg TCR and antibodies with specificity to glucose-6-phosphate isomerase (GPI) [19]. Apparently, a fortuitous cross-reactivity of the Tg TCR with the GPI, processed and presented by the I-A^{g7} antigen-presenting cells, initiates the disease process. Although the cross-reactivity deleted thymic T-cells expressing the Tg TCR, some Tg TCR T-cells (likely expressing dual-TCR) emerged in the periphery and initiated the disease process [18].

The lifespan of K/BxN.*foxp3*^{sf} mice was prolonged similar to that of other TCR Tg Sf mice. A faster and more aggressive arthritis developed. This change was attributed to the lack of Treg in the affected joints and an increased production of antibodies against the GPI [20] but the expansion of GPI-specific T-cells is also a likely contributing factor. Like other TCR Tg Sf mice, the great majority of $CD4^+$ T-cells in the secondary lymphoid tissues are $CD44^+$. In theory, these cells could be expanded both by GPI-specific activation through the Tg TCR or by endogenous TCR of the dual-TCR T-cells as what has been shown for OT-II.*foxp3*^{sf} mice [17].

NOD.*foxp3*^{sf} mice

The NOD.*foxp3*^{sf} mice developed more severe inflammatory pathology in multiple organs as compared with B6.*foxp3*^{sf} mice [21]. However, their survival rate appears to be similar to that of B6.*foxp3*^{sf} mice. In addition, diabetic incidence was not reported, probably because the early death prevented such an analysis [21]. Indeed, the effect of total Treg-deficiency on many chronic autoimmune diseases may not be easily studied because of the dominant and lethal effect of *foxp3*^{sf} mutation.

As described above, the early mortality of Sf mice can be significantly reduced if TCR Tg was bred into Sf mice. This strategy was used to study Treg effect on type-1 diabetes [22]. In this case, the NOD mice bearing the BDC2.5 TCR Tg specific to an islet Ag was used to generate BDC2.5/NOD.*foxp3*^{sf} mice. Both MOI and mortality as compared with NOD.*foxp3*^{sf} mice were significantly ameliorated. Interestingly, diabetes developed as early as 14 days after birth and 100% incidence occurred at 18 days of age, indicating that Ag specificity of T-cells is a critical factor for organ-specific inflammation. BDC2.5/NOD.*Rag*$^{-/-}$ mice also lacked Treg and developed diabetes earlier than BDC2.5/NOD mice [22]. Diabetes developed at 20 days after birth and 100% incidence occurred at 30 days of age. This “subtle” difference was attributed to the delay of insulitis in BDC2.5/NOD.*Rag*$^{-/-}$ mice. The T-cells in BDC2.5/NOD.*foxp3*^{sf} mice but not BDC2.5/NOD.*Rag*$^{-/-}$ mice contain

endogenous TCR. Based on the study of *OT*-II.*foxp3*^{sf} mice, dual-TCR T-cells expanded as early as 6 days after birth in an $OVA_{323–339}$-independent, foreign Ag-dependent manner [17]. Conceivably, such expansion turns naïve T-cells into memory/effector cells and collaterally facilitates the pathogenecity of the dual-TCR T-cells on pancreas. Education and expansion of T-cells that exclusively express BDC2.5 TCR may not be as fast because it occurs only in the pancreatic draining lymph nodes. It will be of interest to determine whether the dual-TCR T-cells are preferentially enriched in the islets of BDC2.5/NOD.*foxp3*^{sf} mice.

Sf.*aire*$^{-/-}$ mice

The *aire* gene is expressed in thymic stromal cells. It is responsible for the synthesis of a family of peripheral tissue Ag whose presence is required for the deletion of the Ag-specific autoimmune T-cells during thymic selection [23]. Indeed, mice with mutant *aire* gene develop autoimmune diseases directed mostly against endocrine organs [23]. Although naturally occurring Treg develops in the thymus, this process does not seem to be affected by *aire*. In addition, the effect of Treg occurs in the periphery. Sf.*aire*$^{-/-}$ mice have a gravely shortened lifespan [21]. However, the affected target organs were not extended; particularly those endocrine organs remained free from inflammation. It could be argued that the inflammation in these organs did not have time to develop due to early death. Nevertheless, it is possible that the role of *aire* in central tolerance is not limited to Ag of endocrine organs, and as such *aire* defect may work in synergy with *foxp3*^{sf} to fasten or augment MOI [21].

Sf.*cd28*$^{-/-}$ mice

CD28 on $CD4^+$ T-cells interacts with B7 on antigen-presenting cells. This interaction provides the co-stimulation signal required for optimal T-cell activation of $CD4^+$ T-cells. Breeding *cd28*$^{-/-}$ gene into Sf mice greatly extended the lifespan of the double mutant mice; 50% of which lived more than 200 days [24]. The spontaneous T-cell activation is greatly reduced in Sf.*cd28*$^{-/-}$ mice as reflected in the presence of a lower fraction of $CD44^+$ T-cells and the inability of their T-cells upon activation to produce high levels of IFN-γ, IL-4 and IL-10 as compared with Sf mice. Paradoxically, IL-2 production upon T-cell activation by anti-CD3 and anti-CD28 was comparable among B6, Sf and Sf.*cd28*$^{-/-}$ mice. Serum IgE and IL-4, which were high in Sf mice, were reduced significantly in Sf.*cd28*$^{-/-}$ mice. Consistent with the lack of activation, Sf.*cd28*$^{-/-}$ mice did not show detectable inflammation in liver and lungs [24]. The effect on skin inflammation was not described in this study but one would expect it was also inhibited based on the strong inhibition of general T-cell activation in these mice.

Sf.*Il2*$^{-/-}$ mice

IL-2 knockout *(Il2*$^{-/-}$) mice develop lymph node enlargement and inflammation in colon, liver and salivary glands [9,25]. *Il2*$^{-/-}$ mice are deficient in but not totally devoid of Treg [26]. The Treg-deficiency may explain the lympho-proliferation and MOI. However, the MOI in *Il2*$^{-/-}$ mice is remarkably different from Sf mice that develop severe inflammation in skin and lungs [7]. The phenotype of *Il2*$^{-/-}$ mice is dominant because Sf.*Il2*$^{-/-}$ mice also failed to develop skin and lung inflammation whereas their liver inflammation was not affected [25]. Thus, Sf.*Il2*$^{-/-}$ mice provided the first evidence that the MOI in Sf mice is controlled in an apparent “organ-specific” manner.

Interestingly, lymph nodes of Sf.*Il2*$^{-/-}$ mice are significantly larger than Sf mice and have a higher number of lymphocytes. Apparently, lacking IL-2 did not inhibit T-cell activation and proliferation *in vivo*. Other lympho-proliferative lymphokines such as IL-4, IL-7, and

IL-15 were not obviously higher in Sf.*Il2*$^{-/-}$ mice as compared with Sf mice (Sharma and Ju, unpublished observation). Reduced FasL (CD178) expression has been implicated in the lymphadenopathy in *Il2*$^{-/-}$ mice but FasL expression was somewhat higher in Sf.*Il2*$^{-/-}$ mice than in Sf mice [25].

Inhibition of lymphocyte trafficking, either out of lymph nodes or into peripheral tissues, or both, may cause accumulation of lymphocytes in the lymph nodes. Our unpublished observation suggests that the CD4^{+} T-cells in Sf.*Il2*$^{-/-}$ mice fail to up-regulate trafficking/chemotactic/retention receptors necessary for entering into skin and lungs to induce inflammation [25]. Considering the large areas of skin and lungs and the large number of lymphocytes accumulated in these areas in Sf mice, this mechanism cannot be overlooked. This interpretation further implies that the infiltration of the inflammation-inducing T-cells into liver is not affected by the absence of IL-2. Importantly, lymphocyte trafficking to and retention in specific target organs involves many receptors and how IL-2 regulates altogether their expression and controls skin and lung inflammation in such a dominant manner is a challenging issue to address.

Sf.*Igtαε*$^{-/-}$

Integrinαε(CD103)β7 is a cell surface receptor that binds to E-cadherin expressed mainly by epithelial cells. As a result, cells expressing CD103 are retained more in tissues like skin and lungs. In Sf mice, the frequency of CD4^{+}CD103^{+} T-cells in the lymph nodes is significantly higher than their B6 counterpart [27]. An even higher frequency is observed in the skin and lungs. The high frequency of CD4^{+}CD103^{+} T-cells is dramatically reduced in Sf.*Il2*$^{-/-}$ mice, demonstrating the requirement of IL-2 for CD103 expression on CD4^{+} T-cells. *In vitro* culture experiments demonstrated that optimal expression of CD103 also required TGF-β1 which is not limiting in Sf mice. IL-2 requirement for CD103 expression is specific for CD4^{+} T-cells including CD4^{+}Foxp3^{+} Treg. Interestingly, the Treg deficiency in *Il2*$^{-/-}$ mice is observed mainly in the CD103^{-} Treg [27], suggesting a subtle difference in the regulation of CD103 and Foxp3 expression in *Il2*$^{-/-}$ mice, perhaps the IL-15- or IL-7-induced Treg does not efficiently express CD103 [26]. CD103 expression on CD8^{+} T-cells and dendritic cells appears comparable between Sf and Sf.*Il2*$^{-/-}$ mice [27].

Lacking CD103 expression in Sf.*Igtαε*$^{-/-}$ mice delayed the skin and lung inflammation for a few weeks, indicating that the IL-2-controlled CD103 expression on CD4^{+} T-cells contributed to the “organ-specific” development of skin and lung inflammation. However, the lack of CD103 cannot fully explain the lifetime protection from skin and lung inflammation in Sf.*Il2*$^{-/-}$ mice because the MOI in Sf.*Igtαε*$^{-/-}$ mice eventually came back with a severity comparable to that observed in Sf mice. Thus, IL-2 must controls additional components of the skin and lung inflammatory process [27].

Sf.*stat6*$^{-/-}$ mice

IL-4, IL-5, IL-13, and IgE are important components for allergic skin and lung inflammation. The spontaneous development of skin and lung inflammation in Sf mice is associated with a high level of expression of serum IgE and Th2 lymphokines IL-4, IL-5, and IL-13, although Th1 lymphokines such as IL-2 and IFN-γ are also strongly expressed [28]. As STAT6 is the transcription factor critical to Th2 response, breeding *stat6*$^{-/-}$ gene into Sf mice of Balb/c background was conducted to determine how much the Th2 lymphokines and IgE contributed to the skin and lung inflammation. Serum IgE was greatly reduced and so was the ability of activated Sf.*stat6*$^{-/-}$ T-cells to produce IL-4, IL-5 and IL-13. Blood eosinophils reached to near normal level. With respect to the immunopathology of the MOI, the expression of mucus in Goblet cells as determined by Periodic Acid-Schiff-staining was inhibited, but severe inflammation in the lungs remained,

suggesting a Th2-independent lung inflammation in these mice. Skin inflammation was not described, perhaps due to early death of these mice (T. A. Chatila, personal communication). Sf.*stat6*$^{-/-}$ mice lived 1 week more than Sf mice, suggesting Th2 regulated allergic inflammatory response in the lungs and skin was not necessary for mortality to occur [28].

Sf.*fas*$^{lpr/lpr}$ mice

Fas (CD95)/FasL interaction has been considered as a major mechanism that regulates T-cell homeostasis but its role in organ damage is less appreciated. As compared with Sf mice, the number of lymphocyte in the lymph nodes of Sf.*fas*$^{lpr/lpr}$ mice is only mildly increased by about 20% whereas 100% increase was observed for Sf.*Il2*$^{-/-}$ mice [25]. By contrast, the average lifespan of Sf.*fas*$^{lpr/lpr}$ mice (12–14 weeks old) is comparable to Sf.*Il2*$^{-/-}$ mice and both lived significantly longer than Sf mice. In addition to the fully developed inflammation in the skin and lungs, the spontaneous inflammation in Sf.*fas*$^{lpr/lpr}$ mice was extended to other organs not observed in Sf mice. Unlike Sf mice, Sf.*fas*$^{lpr/lpr}$ and Sf.*Il*2$^{-/-}$ mice developed inflammation in colon and accessory reproductive organs (9, unpublished observation). These observations strongly suggest that Fas/FasL system has little to do with “organ-specific” inflammation and that FasL-dependent organ damage is an important factor for mortality induced by inflammation. FasL-induced organ damage could be due to its direct cytotoxicity or due to its ability to recruit inflammatory cells through Fas/FasL interaction [25,29].

Discussion/Summary

Participation of Th1 and Th2 responses in MOI

Most allergic inflammation conditions including IPEX are attributed to a preferential Th2 response and IgE over-expression. IL-2 is critically important in IL-4 induction and Th2 development under Th2 induction condition *in vitro* [30,31]. The Sf.*Il2*$^{-/-}$ mice represents the first animal model for which the role of IL-2 in Th2-mediated allergic inflammation can be explored. Sf mice displayed highly up-regulated Th1 and Th2 responses. Interestingly, expression of IL-4, IL-5, and IL-13 in CD4^{+} T-cells and serum IgE level were up-regulated both in Sf and Sf.*Il2*$^{-/-}$ mice as compared with B6 control (Sharma and Ju, unpublished observation), yet skin and lung inflammation in the latter group was strongly inhibited. This is interesting in light of the fact that the hyper-production of Th2 lymphokines (IL-4, IL-5, and IL-13) and IgE in Sf mice was reduced to normal levels in Sf.*stat6*$^{-/-}$ mice and yet, lung (and perhaps skin) inflammation persists in the latter group. This comparison suggests that the skin and lung inflammation in Sf mice also involves Th1 response and that a critical step shared by both Th1 and Th2 responses for skin and lung inflammation must have been inhibited in Sf.*Il2*$^{-/-}$ mice.

How IL-2 controls skin and lung inflammation in Sf mice?

To address this issue, genes differentially expressed between lymph node CD4^{+} T-cells of Sf and Sf.*Il2*$^{-/-}$ mice were determined (Sharma and Ju, unpublished observation). Genes encoding receptors for trafficking/chemotaxis/retention were highly differentially-expressed in Sf lymph node CD4^{+} T-cells as compared with Sf.*Il2*$^{-/-}$ samples. Among them, many skin-homing receptors such as Cysteinyl Leukotriene Receptor 1, Leukotriene β4 Receptor 1, CD103, CCR8, and others are the most differentially expressed (Fig. 1, Also see review in reference 32 for T-cell trafficking). These observations suggest that T-cell entering skin and lungs is a critical step preceding the T-cell activation in these organs and the subsequent inflammatory response. Consequently, even a strong expansion of potential inflammation-inducing T-cells in the lymph nodes cannot induce skin inflammation when the expression of these trafficking receptors is inhibited. The chemotactic factors for T-cell entrance to skin

and lungs are likely produced by mast cells, basophils (for leukotrienes) and dermal microvessels, melanocytes, and Langerhans cells (for CCL1) [32,33]. IL-2, by regulating the receptors for these ligands, enables T-cell infiltration into skin and lungs to induce clinical symptoms (Fig. 1). How IL-2 controls the expression of these receptors is critical to the understanding how "organ-specific" control of MOI in Sf mice is developed.

Another example that organ-specific control of inflammation exists in the Sf mice is supported by the rstudy of submandibular gland (SMG) [9]. Sf mice do not develop inflammation in SMG but contain $CD4^+$ T-cells capable of inducing SMG inflammation in *Rag1*$^{-/-}$ recipients upon adoptive transfer. Interestingly, oral treatment of Sf mice with LPS or poly-I:C induces SMG inflammation [9]. The SMG in Sf mice is developmentally arrested at the stage equivalent to <2 weeks old normal B6 male mice [9]. This developmental arrest is in part caused by the lack of hormones such as testosterone and thyroxine. Even in 10–16 weeks old Sf.*fas*$^{lpr/lpr}$ mice, the SMG remained undeveloped due to the severe inflammation damage of the accessory reproductive organs that produce testosterone. These observations suggest that the production of chemokines/chemotactic factors in the undeveloped SMG is limited, thus, preventing entrance of the inflammation-inducing T-cells and rendering the SMG resistant to the attack of inflammation-inducing T-cells (Fig. 2).

Implication

Sf defect can be cured by transfer of Treg in newborns and by partial bone marrow transplantation [34,35]. When performed before organ damage, bone marrow transplantation using non-myeloablative conditioning regimens can inhibit clinical symptoms in IPEX patients [36]. The genetic breeding experiments using Sf mice do not address how to cure Treg defect but rather aim at understanding how Tinf response and MOI are developed and controlled. These studies revealed that MOI of Sf mice is controlled by many critical components of the inflammation process. As summarized in Table 1, regulation occurs at many levels, from as early as those involved in T-cell development to those inflammation-inducing molecules involved in the latter stage of the inflammation process. Several studies such as those using BDC2.5 TCR Tg, *stat6*$^{-/-}$, *Il2*$^{-/-}$, and *Igtαε* genes, demonstrated that MOI in Sf mice can be controlled in an "organ-specific" manner, through Ag-specific TCR, cytokines, trafficking receptors, and integrin receptors. These checkpoints may be useful targets for the treatment of organ inflammation. Among them, the IL-2 regulated "organ-specific" inflammation process is highly significant considering the fact that numerous immune diseases occur in the skin and lungs. It seems that inhibiting Ag-specific production of IL-2 or blocking the major signaling component of IL-2 (i.e., STAT5) can be a useful approach to treat these diseases.

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Abbreviations

Ag	antigen
foxp3^{sf}	*foxp3* gene mutation in Sf mice
GPI	Glucose-6-phosphate Isomerase
MOI	Multi-organ Inflammation
$OVA_{323–339}$	peptide #323 to 339 of ovalbumin
Sf	Scurfy mice
SMG	submandibular gland
Tg	transgenic
Tinf	inflammation-inducing T-cells
Treg	regulatory T-cells

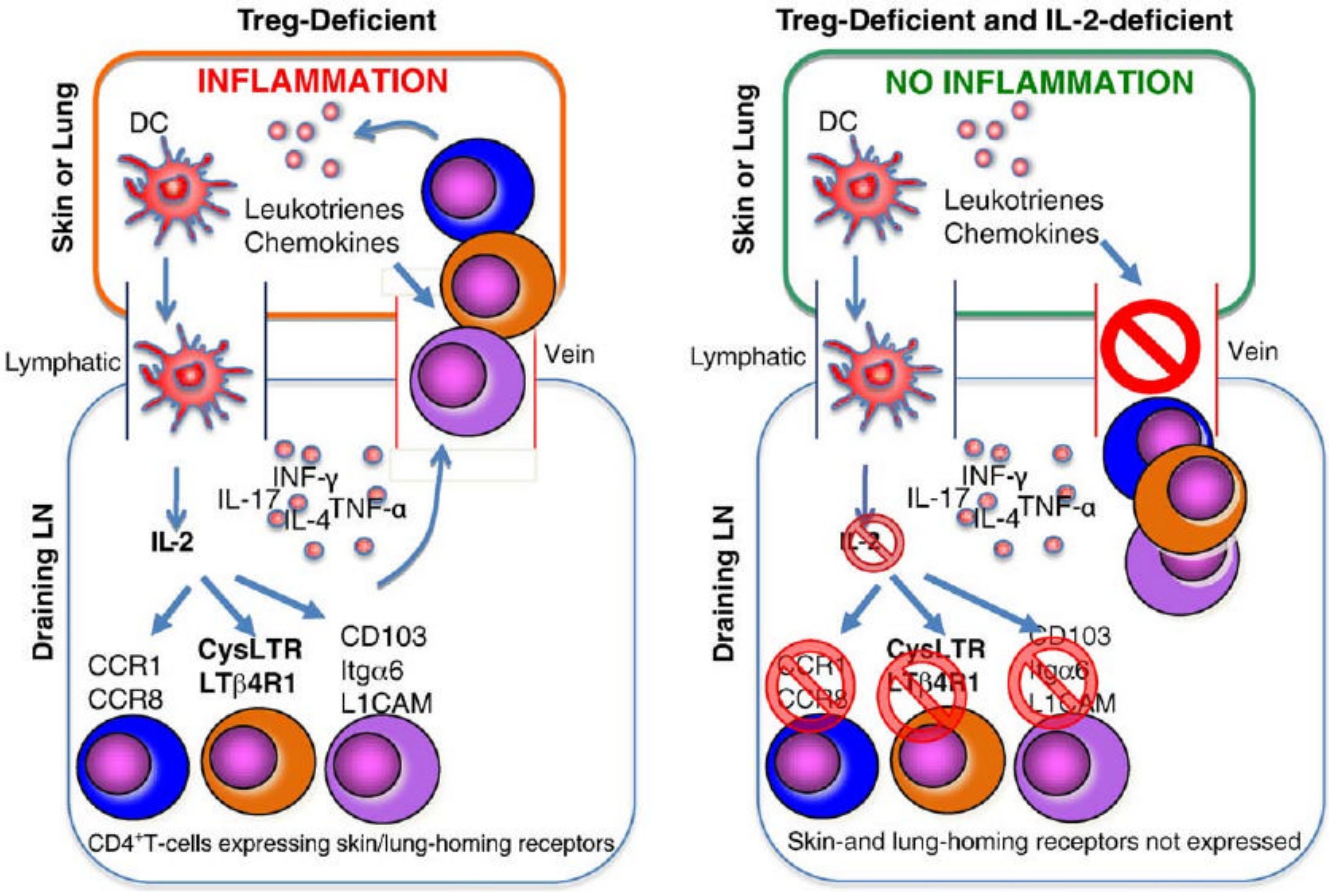

Figure 1.

IL-2 controls trafficking receptor expression for skin and lung inflammation. In the Treg-deficient Sf mice (left panel), both Th1 and Th2 responses are strongly developed in the draining lymph nodes (LN). In the presence of IL-2, receptors for trafficking, chemotaxis, and retention for skin and lungs are induced on $CD4^+$ T-cells, allowing them to enter target organs and induce inflammation. In the absence of IL-2 as in Sf.*Il2*$^{-/-}$ mice (right panel), these receptors are not expressed and despite having an up-regulated Tinf response, the $CD4^+$ T-cells cannot enter the target organs to induce inflammation.

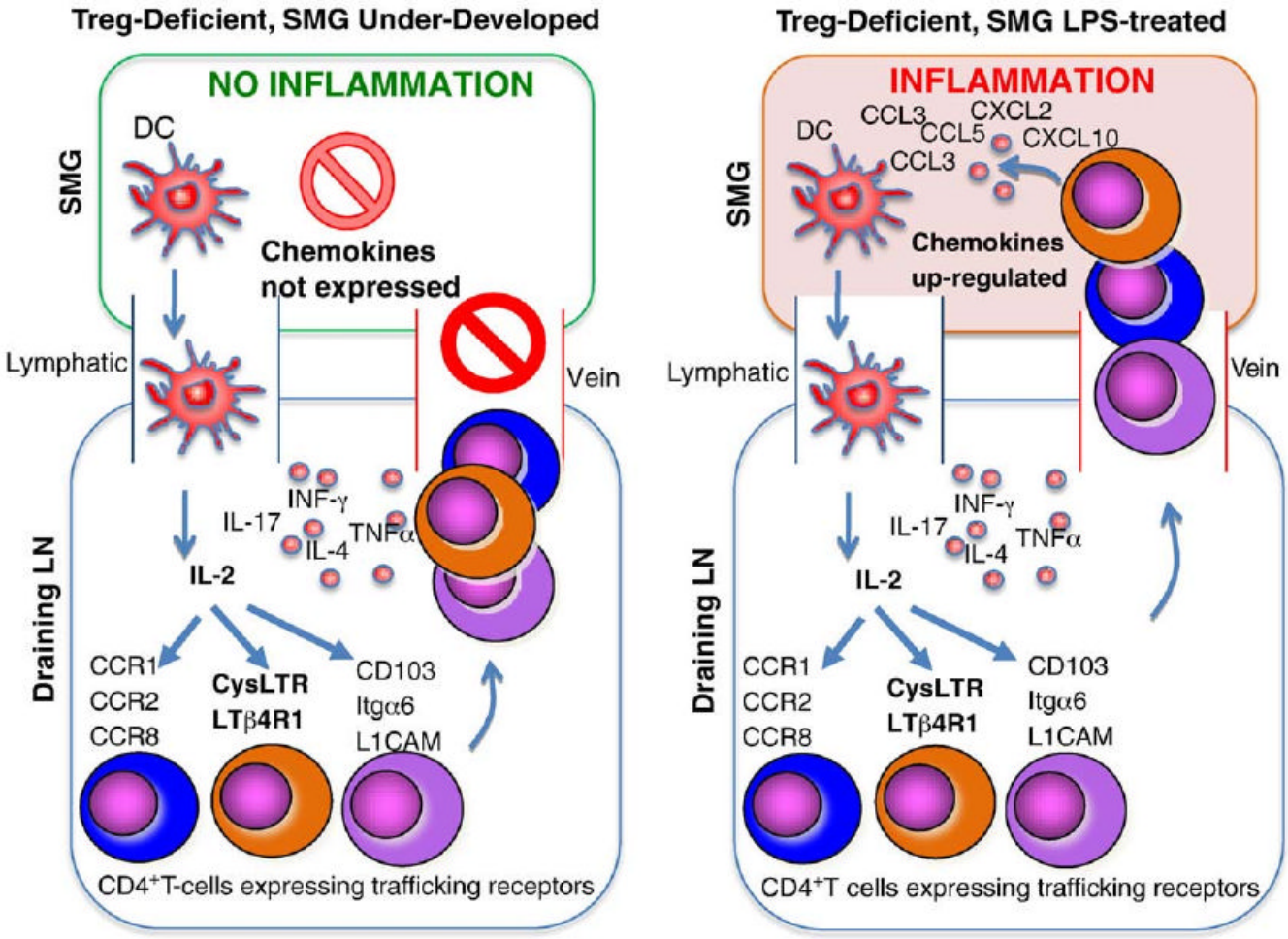

Figure 2.

Chemokine expression controls SMG inflammation. The SMG in Sf mice is free of inflammation despite the fact that the Sf lymph nodes (LN) contain $CD4^+$ T-cells capable of inducing SMG inflammation upon transferring into *Rag1*$^{-/-}$ recipients. The SMG of Sf is severely under-developed and may not produce sufficient chemokines to attract $CD4^+$ T-cells to induce inflammation (left panel). Oral application of LPS or poly-I:C up-regulates chemokine expression in SMG, allowing entrance of the $CD4^+$ T-cells to induce inflammation (right panel).

Table 1

Summary of breeding experiments that address the genetic control of MOI in Sf mice.

Gene examined in Sf mice	Cellular change compared with Sf mice	Change in MOI	Lifespan (weeks)
TCR Tg.rag$^{-/-}$	TCR Tg T-cells only	No MOI	>20
cd4$^{-/-}$	No CD4^{+} T-cells	Delayed 1 week	6
β2m$^{-/-}$	No CD8^{+} T-cells	Not delayed	4
TCR Tg	T-cell number reduced, dual-TCR T-cells expanded	Delayed 2–3 weeks	7
NOD background	N.D.*	More severe than B6.*foxp3*^{sf} mice	N.D.
BDC2.5 TCR Tg in NOD background	Lymphoproliferation was ameliorated	Rapid development of insulitis and diabetes, MOI was not addressed.	N.D.**
aire$^{-/-}$	N.D.	MOI fastened but did not extend to endocrine organs	2–3
cd28$^{-/-}$	Inhibit T-cell activation and lymphokine production	Inhibited	50% lived >30
stat6$^{-/-}$	Inhibited IgE and Th2 lymphokine production	Inhibited eosinophilia and lung Goblet cell metaplasia	5
Igtαε$^{-/-}$	lymphocyte number decreased by ~40%	Delayed 2–3 weeks, developed colitis	6–7
Il2$^{-/-}$	Lymphocyte number in lymph nodes increased 100%, CD103 and trafficking/chemotaxis receptors inhibited	Delayed 3–5 weeks, no skin inflammation, greatly reduced lung inflammation, liver inflammation remained, developed colitis	6–10
fas$^{lpr/lpr}$	Slight increase in lymphocyte number in lymph nodes	Not delayed but lifespan prolonged, adult mice developed severe inflammation in accessory reproductive organs and colitis.	6–18

* Not described.

** TCR Tg should have prolonged the lifespan.