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Review Article

The role of ubiquitin ligases in the control of organ specific autoimmunity

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Abstract: Diabetes mellitus is characterized by chronic hyperglycemia caused by a deficiency in insulin action, insulin secretion or both. Type 1 diabetes is classified as the destruction of beta cells leading to a deficiency in insulin production. Type1 diabetes accounts for 5-10% of patients with diabetes and most commonly is caused by the autoimmune destruction of the beta cells in the pancreas. The adaptive immune system is composed of antigen specific T and B lymphocytes which play a central role in protecting the human body from infectious pathogens but occasionally autoreactive T and B cells can escape immune tolerance, become activated and induce autoimmune diseases. Naïve T cells require two distinct signals one delivered via the antigen receptor and the second through the costimulatory receptor CD28 that leads to the induction of IL-2 gene transcription. IL-2 is an important T cell growth factor that can influence both immunity and tolerance. Given its pivotal role it is not surprising that the immune system places strict regulation over *Il2* gene transcription that is controlled by a number of E3 ubiquitin ligases that modulate TCR and CD28 signaling. This review will examine how different E3 ligases function to control T effector cell differentiation and how studies in gene knockout animal models has been crucial in understanding how these proteins function *in vivo* to regulate immune tolerance in the peripheral circulation.

Keywords: T cells, anergy, Foxp 3, TCR signalling, ubiquitin ligases

Introduction

Diabetes mellitus is a significant problem throughout the world that is caused by chronic hyperglycaemia and without treatment can lead to severe life threatening complications caused by dysfunction and/or failure of various organs including eye, kidney, nerves, heart and blood vessels. Genetic and environmental factors contribute to the etiology of diabetes. Type 1 diabetes (T1D) accounts for 5-10% of patients with diabetes and occurs most commonly in childhood and adolescence [1]. The disease is caused by the autoimmune destruction of the beta cells within the islets of Langerhans in the pancreas due to activation of islet reactive T and B cells. Pancreatic beta cells are the sole source of insulin production in the body and following injury the regenerative potential of the beta cell is limited. This is because beta cells are largely senescent due to the expression of cell cycle inhibitors such as $p27^{kip1}$ and $p16^{Ink4a}$ [2-4]. Also recent studies have identified that there is no contribution to the adult beta cell mass by specialized progenitors or stem cells [5]. Instead adult beta cells are the product of self-duplication [5, 6]. Therefore the partial growth ability of the beta cell is insufficient to permit recovery from cell loss as experienced in overt T1D. As a result the loss of beta cell mass leads to a chronic loss of insulin production and dysregulation of blood glucose homeostasis that eventually leads to the clinical symptoms of diabetes.

Patients with T1D can secrete anti-insulin antibodies due to activation of autoreactive B cells, while islet reactive CD4+ and CD8+ T cells have been identified in the peripheral blood of patients. The main target antigens of diabetogenic T cells include islet derived proteins such as insulin, GAD65 and insulinoma associated protein A2 (IA2) [7]. The activation of both T and B lymphocytes in T1D patients indicates that

the disease is caused by a generalized breakdown of immune tolerance whereby activation of islet reactive CD4+ T cells can lead to cognate activation of islet specific B cells and the generation of autoantibodies directed to islet specific proteins. Animal models have been particularly useful in helping to define the cellular and molecular basis of disease pathology associated with T1D and these have contributed to a better understanding of the disease in humans [8].

Immune tolerance to self antigens is established through central or peripheral mechanisms. Central tolerance occurs during T cell development in the thymus through deletion of self reactive T cells that express a TCR with high affinity for self antigens. This process is directed by the presentation of antigen by medullary epithelial cells that express the autoimmune regulator gene (AIRE) that directs ectopic expression of tissue specific antigens in the thymus [9-11]. In the absence of *Aire* central tolerance is severely compromised and autoreactive T cells can escape deletion and enter the periphery. Patients with the rare autoimmune polyendocrinopathy syndrome 1 (APS-1) have mutations in the *Aire* gene that predisposes to organ specific autoimmunity including thyroiditis and diabetes [12, 13]. The efficiency of clonal deletion is not absolute and so even in healthy individuals a small proportion of autoreactive T cells can escape thymic deletion and enter the peripheral circulation. The immune system must control the activation of these cells to prevent autoimmunity and this is achieved through a combination of dominant (extrinsic) and recessive (cell intrinsic) mechanisms.

Dominant tolerance is mediated primarily by the suppressive effects of regulatory T cells in particular the naturally occurring CD4+ Foxp3+ Treg (nTregs) cells that arise during T cell development in the thymus or by inducible Tregs (iTregs) that arise in the peripheral circulation in response to tolerance inducing regimes (e.g. mucosal delivery of antigen) [14, 15]. Recessive tolerance is regulated by cell intrinsic mechanisms that control the fate of autoreactive T cells especially in the periphery. The CD95 (Fas)/ CD95L (FasL) pathway is a member of the TNF receptor family and plays a critical role in regulating programmed cell death of activated T cells [16]. Other inhibitory pathways include PD-1/PD-1L and CTLA-4 which are important for preventing cellular activation and proliferation [17, 18]. Growth factors such as interleukin 2 (IL-2) and transforming growth factor–β (TGF-β) have important roles in regulating T cell proliferation and for maintaining homeostasis of Treg cells in the peripheral circulation [19-21]. Self reactive T cells can undergo functional inactivation through a process referred to as clonal anergy. The development of anergy leads to an abortive activation that makes cells unresponsive to stimulation through the TCR [22, 23]. Several E3 ubiquitin ligases are induced in anergic T cells and they play a central role in ubiquitinating specific signalling molecules located downstream of the TCR to target them for degradation.

In this chapter some of the key E3 ligases that have specific roles in regulating T cell responses will be examined and how defects in the function of these ligases can lead to organ specific autoimmune diseases.

E3 ubiquitin ligases

Ubiquitin is a 76 amino acid polypeptide that is involved in the posttranslational modification of proteins [24]. Ubiquitin is added by the sequential activity of three enzymes; E1 is an activating enzyme, E2 is a conjugating enzyme and E3 is a ligase that attaches the ubiquitin moiety to the target protein. Proteins can either be subject to mono- or poly-ubiquitination [25, 26]. Ubiquitin molecules are generally linked through the lysine (Lys) residue at position 48 or 63 and a protein can be tagged with a single ubiquitin (i.e. monoubiquitinated) or it may be tagged with multiple ubiquitins in an elongated chain which is referred to as (polyubiquitination) [27, 28]. Proteins that become tagged with multiple ubiquitins on Lys48 are destined for degradation in the 26S proteosomal complex. In contrast proteins that are monoubiquitinated or have the addition of multiubiquitins to lysine residues apart from Lys48 can alter protein trafficking between subcellular compartments or protein function [29].

Three families of E3 ligases have been identified including the really interesting new gene (RING) type, homologous to the E6 associated protein carboxy terminus (HECT) type and the U-box type proteins [24]. The E3 ligases are involved in the transfer of the ubiquitin from an

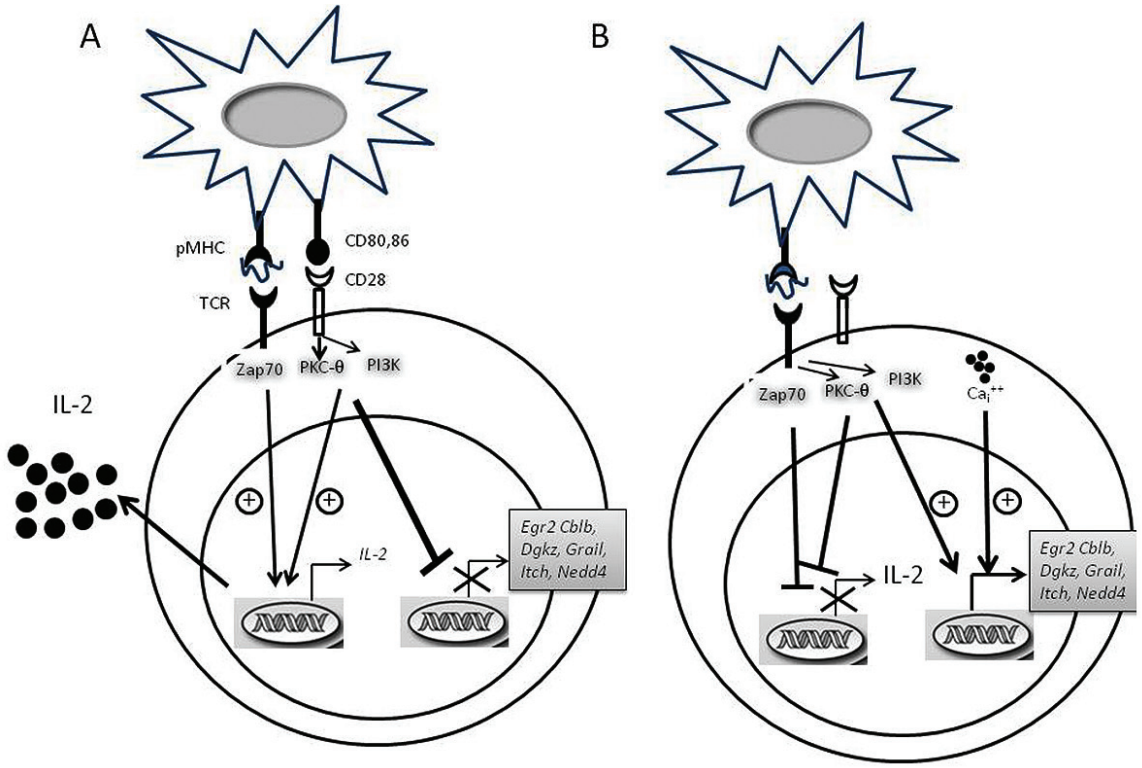

Figure 1. Induction of ubiquitin ligases in response to T cell anergy. A: T cells become fully activated in response to signals through the TCR and CD28 costimulatory receptor. Intracellular signals are transduced from the TCR and CD28 to the nucleus leading to IL-2 gene transcription and the secretion of the cytokine promoting autocrine and paracrine growth of T cells, but ubiquitin ligase gene expression is inhibited. B: T cells that receive a signal only through the TCR or experience sustained elevated levels of Ca_i^{++} leads to abortive activation and the development of T cell anergy. The anergic state is characterized by inhibition of IL-2 gene transcription but increased expression of a range of ubiquitin ligase genes.

appropriate E2 ubiquitin donor to the protein substrate. Several RING and HECT type E3 ligases have been implicated in the regulation of immune function and disrupting their function *in vivo* can have important consequences on the development of autoimmune diseases [30]. This outcome highlights that the immune system relies on the E3 ligases to restrain the autoimmune potential of self-reactive lymphocytes. In the following discussion we will focus on the role of key E3 ligases such as Cbl-b, Itch, Grail and Roquin to illustrate how these molecules control T cell activation.

To achieve complete activation of naïve T cells requires the delivery of two discrete signals, one transmitted through the TCR and the other which is delivered via the costimulatory receptor CD28 in response to binding to its ligands CD80/CD86 on the surface of APCs. Both signals facilitate the recruitment of a number of transcription factors (e.g. NF-kB, AP-1, NFAT) to the promoter of the *Il2* gene locus that lead to the de-repression of the *Il2* gene, *Il2* mRNA transcripts become stabilized and are translated into protein to drive T cell proliferation [31] (**Figure 1A**). IL-2 can act in an autocrine and paracrine fashion to drive T cell proliferation and to enable effector cell differentiation. Therefore it is not surprising that the immune system places the regulation of *Il2* gene transcription under tight transcriptional control to prevent inappropriate activation of naïve T cells in the periphery and the possibility of autoimmunity.

Naive T cells that receive only a TCR signal undergo an abortive activation that leads to a state of immune unresponsiveness termed clonal anergy. Clonal anergy was originally defined using mouse CD4+ Th cell clones stimulated in the absence of CD28 costimulation and human CD4+ T cell clones can also be rendered anergic following stimulation with pep-

tide antigen in the absence of APCs [32-34]. To study the molecular and biochemical basis of T cell anergy Rao and colleagues cultured T cells in the presence of ionomycin to artificially raise intracellular calcium levels and this was sufficient to induce a state of anergy [35, 36]. These studies revealed that the induction of anergy in T cells was an active process that is dependent on new protein synthesis and is associated with the increased expression of a range of E3 ubiquitin ligases such as Cbl-b, Itch, Grail and Nedd4 and other negative regulators of TCR signaling including *diacyglycerol kinase*, *caspase3*, *Traf6*, *Ikaros*, *Egr2*, *Egr3* and *CREM* (cyclic AMP response element modulator) [37-43] (**Figure 1B**).

Casitas B-lineage lymphoma (Cbl) proteins

The Casitas B-lineage lymphoma (Cbl) family of RING type ubiquitin ligases are key negative regulators of cell surface growth factor receptor signals [44, 45]. Mammals have three Cbl homologues c-Cbl, Cbl-b and Cbl-3 while D-Cbl and Sli-1 are the *Drosophila melanogaster* and *Caenorhabditis elgans* homologous proteins respectively [46]. Cbl-3 does not appear to have any role in the immune system but c-Cbl and Cbl-b are required to modulate TCR signalling. C-cbl is predominantly expressed in the thymus and testes [47] while Cbl-b proteins are expressed primarily in peripheral T cells [48]. In T cells c-Cbl functions to regulate the threshold of TCR signalling by forming a complex with Zap70 and CD3ζ to promotes CD3ζ ubiquitination. This leads to down regulation of the TCR/CD3 complex following ligand binding to peptide/MHC complexes. In the absence of c-Cbl thymocytes display high level expression of the TCR/CD3 complex at the cell surface that appears from the DP stage of development. Negative selection of autoreactive thymocytes proceeds normally in the thymus of *c-Cbl*$^{-/-}$ mice but positive selection of thymocytes is enhanced [49, 50]. There is no evidence of spontaneous autoimmunity in *c-Cbl*$^{-/-}$ mice consistent with the intact negative selection and normal Treg cell differentiation [49, 50] Ho, Hoyne unpublished observations).

The Cbl-b protein is dispensable for T cell development but it does play important roles in TCR modulation and the induction and maintenance of T cell clonal anergy. Studies by Naramura et al showed the combined loss of c-Cbl and Cbl-b leads to constitutive expression of TCR/CD3 at high levels on thymocytes and these cells are resistant to CD3 induced TCR modulation [48]. Cbl-b can interact with CrkL adapter protein and C3G the guanine nucleotide exchange factor for Rap1 [51]. The loss of Cbl-b does not affect the stability of CrkL but its association with C3G that can lead to enhanced expression of LFA-1 and clustering of T cells following TCR signalling [51]. These results imply that Cbl-b is a negative regulator of CrkL-C3G signaling. In addition Cbl-b plays a crucial role as a negative regulator of CD28 signaling in T cells to vav and Rac1 [52] (**Figure 2**). Target proteins of Cbl-b mediated ubiquitination include the p85 subunit of phosphatidyl inositol kinase (PI3K) which is activated in response to CD28 signalling [53, 54] and is also required to inhibit phosphorylation of PLC-γ1 [55]. As *Cblb* deficient mice are unable to restrain TCR signaling they become highly susceptible to develop spontaneous autoimmunity and T cells display hyperproliferation in response to TCR signaling and secrete IL-2 in the absence of a costimulatory signal [48, 52, 56]. However, a recent report by St Rose et al showed that *Cblb* deficient T cells were still resistant to anergy induction when adoptively transferred to recipient mice expressing a neo-self target antigen expressed either in soluble form or on mesenchymal tissues [57].

Additional evidence for the crucial role Cbl-b plays in the regulation of immune tolerance came with the identification of the Komeda diabetes prone (KDP) rat strain that develops spontaneous type 1 diabetes due to a loss of function mutation in *Cblb* [58]. The emergence of autoimmune diabetes in the KDP strain requires additional susceptibility factors including a diabetes-susceptible MHC haplotype that leads to selection islet reactive T cells in the thymus [58]. Studies with inbred strains of mice indicate that Cbl-b on its own is not sufficient to cause spontaneous autoimmunity. However, diabetes can develop if the frequency of islet reactive CD4+ T cells is elevated by transgenic expression of a TCR specific for a neoself antigen expressed on islet beta cells. The *TCR x insHel* model is a highly sensitized mouse strain that has been used extensively to study defects in negative selection and defective nTreg differentiation on the development of islet autoim-

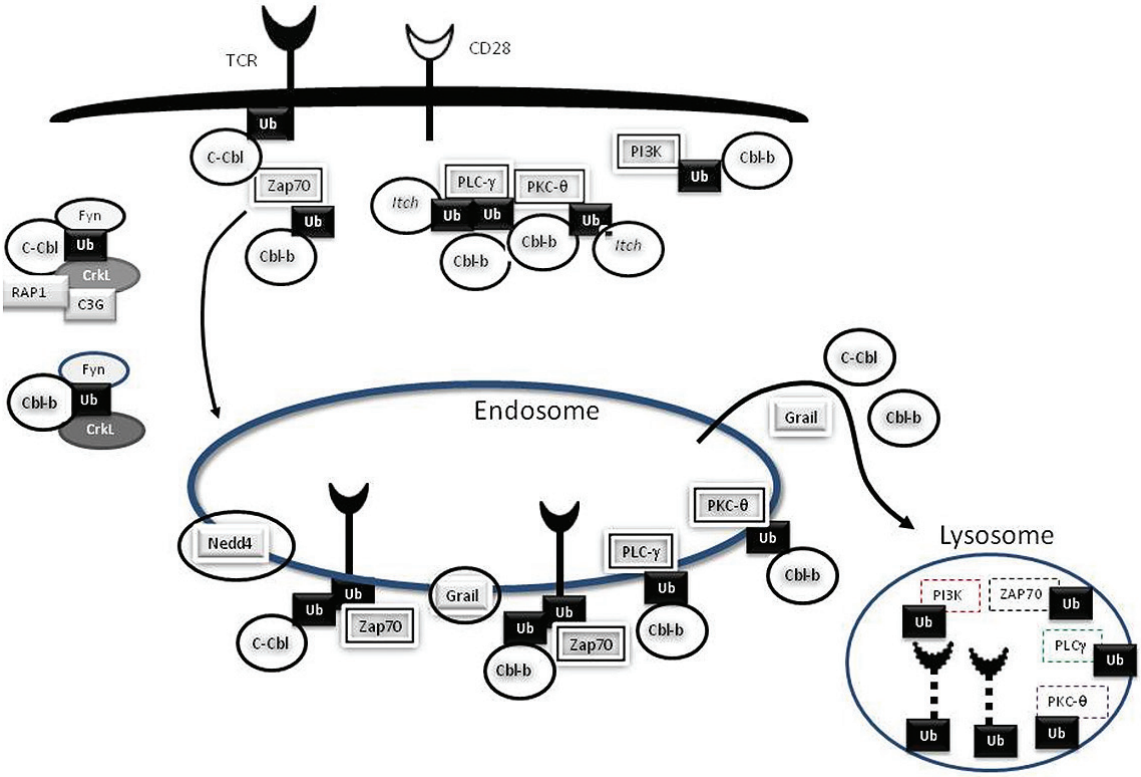

Figure 2. E3 ubiquitin ligases play a crucial role in regulating TCR signalling. The E3 ligases c-Cbl and Cbl-b can ubiquitinate the TCR leading its internalization and targeting to endosome. The Cbl proteins also play an important role in attenuating CrkL/C3G signalling. Cbl-b, Itch and Grail can also ubiquitinate intracellular signalling proteins to dampen signalling into the nucleus. The ubiquitinated proteins are targeted to endosomes and these form lysosomes that result in the destruction of the proteins.

munity [11, 59-61]. The *Cblb* deficiency does not affect negative selection of autoreactive T cells in the *TCR x insHel* model, nor the differentiation of nTregs that develop in the thymus but ~ 80% of *Clbb*$^{-/-}$ *TCR x insHel* double transgenic mice develop type 1 diabetes with extensive lymphocyte infiltration and immune mediated destruction of pancreatic beta cells [62]. However the studies identified a paradox whereby the numbers of islet reactive T cells in the peripheral circulation of *Clbb*$^{-/-}$ *TCR x insHel* double transgenic mice were not dissimilar to that of wild type *TCR x insHel* animals indicating that the immune system was able to control the homeostasis of naïve autoreactive T cells. However the islet reactive T cells from *Clbb*$^{-/-}$ *TCR x insHel* double transgenic mice proliferated extensively and secreted effector cytokines following restimulation with HEL antigen *in vitro* indicating a breakdown in T cell anergy compared to wild type cells from *TCR x insHel* which remained unresponsive to the Hel stimulation.

Grail and Itch and their role in T cell anergy

Grail is a RING type E3 ubiquitin ligase that controls autoimmunity in mice that is separate to the function of Cbl-b described above. Grail is encoded by the *Rfn128* gene was originally identified through a differential display PCR screen of anergic CD4+ T cells. Its expression is strongly induced following the induction of anergy in CD4+ T cells and functions to inhibit IL-2 production following TCR ligation [63]. Grail controls TCR signalling in T cells by selectively inhibiting RhoGTPase activity but it does not affect Ras activation or MAPK signaling [64] (**Figure 2**). *Rfn128* deficient mice are more susceptible to autoimmune diseases and naïve T cells from the gene knockout mice hyperproliferate *in vitro* following TCR stimulation in the absence of costimulation which is similar to the phenotype observed for *Clbb*$^{-/-}$ T cells [65]. Grail also appears to have an important role in Treg cell function as the increased susceptibility to

autoimmunity of *Rfn128* deficient mice is due to a lack of suppressive activity of Tregs [65].

The HECT (Homologous to the E6 associated protein carboxy terminus) type E3 ubiquitin ligases also play a role in the regulation of T cell anergy [35, 66]. The Itchy mouse strain develops a spontaneous and lethal systemic proinflammatory disease consistent with a failure of peripheral tolerance. The disease is associated with an expansion of Th2 type T cells that trigger a chronic pulmonary interstitial inflammation with elevated levels of IgE antibodies that results in skin irritation and the mice itch incessantly [53, 67]. A mutation in the Itch gene was responsible for the disease manifestation in these mice [67]. As an E3 ligase itch promotes ubiquitination of target proteins including JunB, Cbl-b, PKC-q and Bcl-10 [35, 53, 68]. It was originally shown that Itch targets JunB for degradation which is a transcription factor required for the formation of the AP1 transcription factor that regulates Il-2 gene transcription. In response to activation of calcium/calcineurin signaling in T cells Itch targets the degradation of both PLC-γ1 and PKC-θ to dampen TCR signalling because reduced levels of these two proteins reduces the longevity of the immune synapse formed between the T cell and APC [35] (**Figure 2**).

Additional roles for Cbl-b and Itch in the control of inducible Treg cell function

Thymus derived $CD4^+$ T regulatory cells or natural Tregs (nTreg cells) are essential for controlling autoimmune and inflammatory responses. nTreg cells represent a specialized subpopulation of T cells that arise in the thymus during CD4+ TCRaβ+ cell differentiation through expression of the fork head winged helix transcription factor Foxp3 [15]. The expression of Foxp3 leads to a program of gene expression that directs to Treg differentiation and there is a requirement for sustained Foxp3 expression to maintain their immunoregulatory function in the periphery [69-71]. The importance of CD4+ Foxp3+ Tregs in the maintenance of immune tolerance is supported by the lethal multiorgan autoimmunity that occurs in both humans and mice due to a failure in Treg cell differentiation [72, 73]. The selection of Treg cells in the thymus requires strong TCR: MHC interactions [74] and co-stimulatory signals from CD28 [75-77].

Mature nTreg cells migrate from the thymus and enter the peripheral T cell pool where their homeostasis is controlled by distinct mechanisms compared to naive and memory T cells. IL-2, a member of γ_c cytokine family, is critical for the long term survival of Treg cells in the periphery [78]. A second subset of cells with immunosuppressive functions have been characterized. These are known as inducible Tregs (iTregs) and are derived in the periphery from naïve CD4+ CD25- T cells [79]. iTreg cells can be readily isolated *in vitro* by culturing naïve CD4+ T cells in the presence of TGF-β (and IL-10, IL-2) and they begin to express *Foxp3* and they differentiate as a Treg cells [80-83] . The iTregs function in an equivalent manner in being able to suppress responses of other T cells whether *in vitro* or *in vivo*. The mechanism of suppression by Tregs is mediated by secretion of inhibitory cytokines (e.g. IL10, IL-35 or TGF-β) and/or cell-cell contact [15].

Naïve CD4+ CD25- T cells from *Cblb*$^{-/-}$ and *Itch*$^{-/-}$ mice show poor induction of *Foxp3* expression and the iTregs induced are functionally less suppressive in co-culture experiments with wild type naïve T cells [62, 66, 84, 85]. The mechanism by which Cbl-b and Itch regulate iTreg cell differentiation in response to TGF-b stimulation is quite distinct. An important target of Itch in naïve CD4+T cells is the TGF-b induced early gene (TIEG1, Klf10) product [85]. Studies by Liu and colleagues have revealed that Itch and TIEG1 can bind to the promoter of Foxp3 to allow transactivation [85]. When wild type naïve CD4+ T cells are cultured in the presence of TGF-b T cell proliferation is inhibited and Itch and TIEG1 lead to the induction of Foxp3 directing iTreg cell differentiation. In the absence of Itch, naïve CD4+ T cells are resistant to the inhibitory effects of TGF-β, and TIEG1 is unable to promote efficient expression of Foxp3 and preventing iTreg cell differentiation [85]. Similarly, CD4+ *TIEG1*$^{-/-}$ T cells are resistant to the effects of TGF-b *in vitro* and are unable to induce *Foxp3* expression [85]. Taken together these studies have identified an important role between the E3 ubiquitin ligase Itch and TIEG1 in the differentiation of iTreg cells *in vivo*.

As discussed above, Cbl-b plays an important role in modulating the strength of TCR signaling in T cells. Signaling downstream of the TCR and

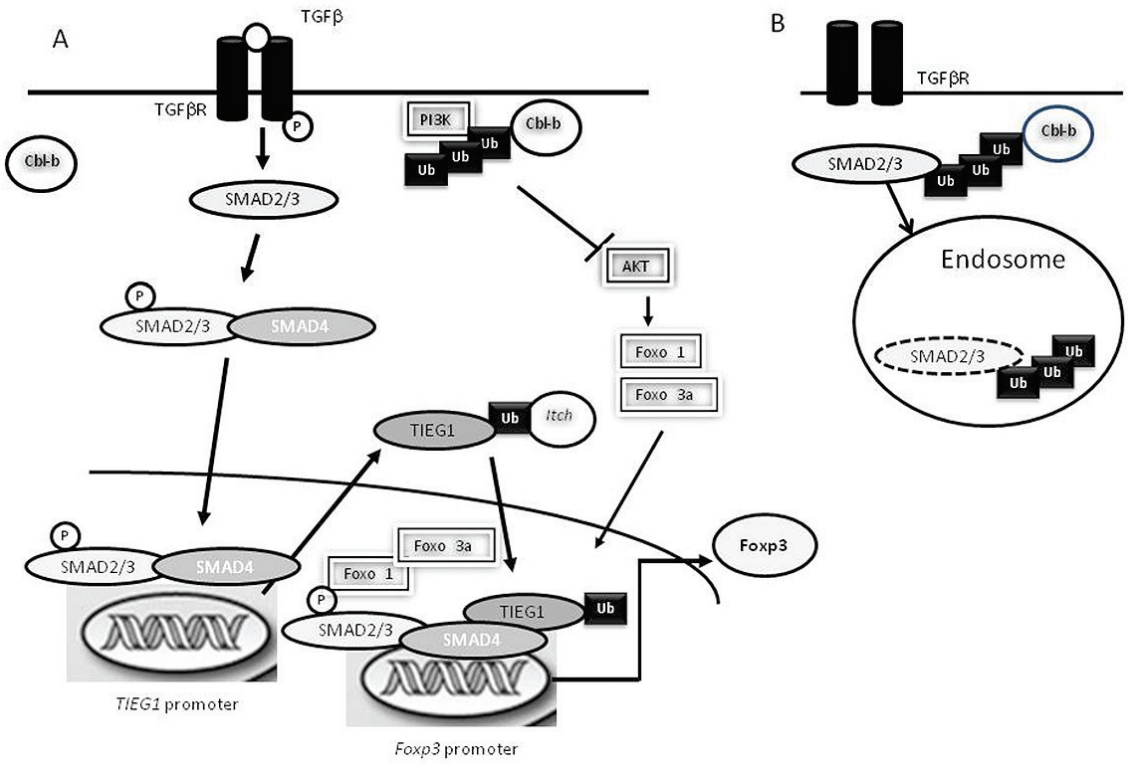

Figure 3. Role of ubiquitin ligases in the induction of *Foxp3* gene expression in iTreg cells. Naïve CD4+ T cells can be induced to differentiate into iTreg cells through induction of the foxp3 gene expression. TGF-b signalling leads to phosphorylation of SMAD2/3 which associates with SMAD4 and the complex translocates to the nucleus where it leads to induction of the TIEG1 gene. The TIEG1 protein can be mono-ubiquitinated by the HECT ligase Itch which promotes its migration to the nucleus where it can bind to the *Foxp3* promoter in association with SMAD complex leading to induction of *Foxp3* expression. The expression of *Foxp3* triggers the Treg differentiation program and leads to control of immune responses. Cbl-b ubiquitinates PI3K targeting it for degradation and this prevents activation of the serine threonine kinase AKT which is required for phosphorylation of the Foxo transcription factors. In the absence of phosphorylation the Foxo transcription factors translocate to the nucleus where they bind to the Foxp3 promoter to induce gene expression. B: In the absence of TGFbR signalling the Cbl-b ubiquitin ligase ubiquitinates the SMAD2/3 complex which targets it for degradation and thus prevents Foxp3 expression.

growth factor receptors (e.g. IL-2R) leads to activation of PI3 kinase (PI3K), the serine threonine kinase AKT and mammalian target of rapamycin (mTOR) to stimulate proliferation and to help activated T cells to avoid the induction of anergy [86, 87]. Activation of AKT increases the activity of mTOR which in turn phosphorylates the Foxo transcription factors (e.g. Foxo 1 and Foxo 3a) which leads to their nuclear export and subsequent degradation [88]. The immunosuppressive drug Rapamycin is an inhibitor of mTOR, and treatment of naïve T cells with rapamycin can induce T cell anergy in vitro [89]. The induction of anergy requires the complete inhibition of mTOR activity and there is heightened activation of the calcineurin/NFAT pathway [23]. It is now apparent that the induction of *Foxp3* expression in naïve T cells to induce iTreg differentiation in response to TGF-b treatment requires proteins that block PI3K activity such as Cbl-b and PTEN [90, 91]. mTOR deficient CD4+ T cells are hypersensitive to TGF-β treatment and switch on *Foxp3*. Two recent studies have identified that regulation of the Foxo1 and Foxo3a transcription factors is essential for TGF-β mediated iTreg cell differentiation [84, 92, 93]. Cblb is required in anergic CD4+T cells to inhibit PI3K signaling by targeting the p85 subunit for degradation [35, 55]. By disrupting PI3K signaling, this prevents AKT activation and the Foxo1 and Foxo3a transcription factors remain de-phosphorylated and can induce expression of *Foxp3* in response to TGF-b signaling. However in the absence of Cbl-b, PI3K/AKT signaling in naïve T cells is not inhibited, and Foxo1 and Foxo3a become phos-

phorylated and are degraded and are not available to induce Foxp3 expression resulting in impaired iTreg differentiation [84, 94] (**Figure 3**).

Roquin constrains autoimmunity by regulating Tfh cell functions in vivo

Roquin is a relatively new member of the RING finger protein family with E3 ligase activity that is encoded by the *Rc3h1* gene [61]. The Roquin protein has emerged to be a key regulator of T follicular helper (Tfh) cell differentiation *in vivo*. Tfh cells are a relatively new Th cell subset that plays a critical role in regulation of germinal centre (GC) responses in the spleen and for promoting B cell responses to antigen. The induction of Tfh cells is dependent on Bcl6 and the cytokine IL-21 [95-97]. Originally discovered through an ENU mutagenesis screen in mice for autoimmune prone phenotypes, the Sanroque mutation is a hypomorphic allele caused by a point mutation in the conserved Roq domain leading to loss of function of the Roquin protein [61]. The *Rch31*$^{san/san}$ mice on a C57BL/6 genetic background develop a systemic autoimmune Lupus-like disease that results in spontaneous GC formation in the spleen, increased numbers of Tfh cells, elevated levels of double stranded DNA antibodies and serum immunoglobulins that leads to the premature death of most *Rch31*$^{san/san}$ mice [61]. CD4+ Tfh cells in *Rch31*$^{san/san}$ mice constitutively express high levels of inducible costimulator (ICOS) at the cell surface of T cells and this phenotype is responsible for driving the spontaneous GC formation in the spleen of Sanroque mice [61]. Roquin plays a key role in regulating the expression of *Icos* mRNA in T cells by binding to the 3' untranslated region targeting it for degradation [98]. The loss of function of *Rc3h1* leads to increased stability of *Icos* mRNA CD4+ Tfh cells express constitutively high levels of ICOS at the cell surface that allows them to interact with autoreactive B cells to promote autoimmune responses.

Conclusion

The RING finger E3 ligases Cbl-b, Itch and Grail play multiple roles in attenuating the activation and differentiation of naïve T cells. The immune system places an important emphasis on restricting the activation of the IL-2 gene in naïve T cells to limit the inappropriate activation and proliferation of autoreactive T cell clones. Thus the E3 ligases are not only play key roles in attenuating TCR signaling, but they appear to have evolved important roles in directing iTreg cell differentiation as well. Thus the breakdown in anergy can not only unleashes TCR signaling in autoreactive T cell clones, but disables an immunoregulatory checkpoint that is normally controlled by iTreg cells.

The development of autoimmune diabetes is controlled by genetic and environmental factors that can lead to a breakdown in the immunoregulatory checkpoints that normally prevent activation of autoreactive T cells. As highlighted in this review there are multiple E3 ubiquitin ligases that are used to control the fate of naïve T cells. Some of these E3 ligases are crucial for dampening TCR signaling, but as highlighted by the function of Roquin, new mechanisms of controlling the activation and/or differentiation of autoreactive T cells could still emerge. Roquin is one of the first E3 ligases to be involved in controlling mRNA degradation of a key protein involved in costimulation of naïve T cells.

The diversity of E3 ligases involved in controlling autoimmunity to organ specific antigens such as in type 1 diabetes makes these proteins potential targets for immune therapy. The link between Cbl-b, Itch and iTreg cell differentiation is an exciting development. There is great excitement as to the role of iTregs in immune cell therapy for the treatment of autoimmune diseases. As we come to a clear understanding of how to induce Foxp3 expression to direct iTreg cell differentiation, the use of Treg therapy may continue to flourish and provide an exciting opportunity to hopefully treat and cure type 1 diabetes in the future.

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