Regulatory T Cells as Targets for Immunotherapy of Autoimmunity and Inflammation

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Abstract: Regulatory T (Treg) cells are emerging as key players in the regulation of different immune responses, thereby representing potential candidates for therapeutic interventions in a broad variety of immunological disorders. While the reduction or loss in function would be of benefit during the treatment of cancer, induction and/or expansion of Treg cell function might be helpful to interfere with unwanted immune responses in transplantation medicine, during autoimmunity, allergy and inflammation. However, a better understanding of Treg cell biology is a prerequisite to specifically modulate its function during immune responses *in vivo*. In the present review we will discuss current concepts on different cell types, components and some novel surface receptors expressed by Treg cells, namely Neuropilin-1, CD83 and G protein-coupled receptor 83 which might represent promising targets for the modulation of Treg cell function in human disease.

Keywords: Regulatory T cells, surface receptor, Foxp3, Neuropilin-1, G protein-coupled receptor 83, CD83.

INTRODUCTION

The immune system, a highly effective and dynamic cellular network, protects the host from pathogens. Several regulatory mechanisms are used to maintain the immune homeostasis, prevent autoimmunity and moderate inflammation induced by pathogens. Among these mechanisms, regulatory T (Treg) cells play a pivotal role in preventing autoimmune diseases like diabetes type 1 and multiple sclerosis as well as limiting chronic inflammatory diseases such as inflammatory bowel disease [1, 2]. Their importance is emphasized by the fact, that a deficiency in Treg cells results in a fatal autoimmune syndrome affecting multiple organs [1, 3]. Due to their immunosuppressive function, they may also block beneficial responses like immunity against certain pathogens and limiting antitumour immune responses. Therefore, deletion of Treg cells or attenuation of Treg cell function would be a promising approach for cancer immunotherapy. However, for the treatment of autoimmune diseases, allergy or transplant rejection, an increase in Treg cell number or function would be of benefit. Major efforts have been made to expand both polyclonal and antigen-specific naturally occurring Treg cells *in vitro*. Nevertheless, the isolation of antigen-specific Treg cells and the expansion of substantial cell numbers without outgrowth of contaminating and potentially harmful effector T cells remain challenging. A very attractive alternative approach for the therapeutic use of Treg cells is the direct induction of this immunosuppressive T cell population *in vivo*.

Naturally occurring Treg (nTreg) cells can be generated intrathymically upon encounter with T cell receptor (TCR) agonist ligands expressed by thymic epithelial cells [4]. These nTreg cells express high levels of Foxp3, a transcriptional regulator, which is essential for the development and function of nTreg cells [5]. However, the generation of Treg cells is not restricted to the thymus, as formation of these cells can be induced in peripheral lymphoid tissues. This review focuses on different strategies which have been developed over the last few years for the induction of Treg cells as therapeutic targets for the treatment of autoimmunity and inflammation.

INDUCTION OF ANTIGEN-SPECIFIC TREG CELL FUNCTION *IN VIVO*

For the treatment of unwanted immune responses, generation of antigen-specific tolerance in the mature immune system is of great interest as nonspecific immunosuppression dramatically impairs the normal host response against microbes. Such antigen-specific Treg cell generation *in vivo* has been demonstrated in different transgenic mouse models. Expression of specific antigen under control of the immunoglobulin kappa promoter mainly in B cells in TCR-transgenic mice leads to the induction of antigen-specific Treg cells [6-8]. Re-isolation of these antigen-specific Treg cells revealed a regulatory phenotype with suppressive activity during $CD8^+$ T cell-mediated intestinal inflammation [8, 9]. In another approach to induce antigen-specific Treg cells, minute amounts of antigen were administrated by means of an osmotic pump into TCR-transgenic mice harbouring T cells with the same antigen specifity. This prolonged subcutaneous infusion of antigen led to the induction of antigen-specific $Foxp3^+$ Treg cells *in vivo*, which can persist for long periods of time in absence of antigen and confers specific immunologic tolerance upon challenge with antigen [10]. However, this *de novo* induction of antigen-specific Treg cells has been described for artificial transgenic mouse models. For the use of these protocols for the treatment of human diseases, it is important to translate these results to non-transgenic organisms. Most recently, Verginis and colleagues have described the generation of male-specific Treg cells in wildtype (WT) mice [11]. They made use of the Y chromosome-encoded transplantation antigen (HY), which might have life-threatening consequences in clinical transplantation. Female WT mice were infused by osmotic

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minipumps with a single class II MHC-presented HY peptide. This protocol led to the induction of antigen-specific Treg cells *in vivo* and as a consequence animals were tolerant to all HY transplantation antigens [11]. Thus, this approach represents a promising tool to prevent transplant rejection and graft-versus-host-disease. However, these approaches require a detailed knowledge of the immunological antigens involved in the immunopathology. While antigen-specific Treg cells have to be stimulated in an antigen-specific manner to acquire suppressive activity, they cannot only suppress responses of T cells with the same antigen specifity but also responses of neighbouring T cells most likely in an antigen independent manner. Therefore, it is important to study, whether the *in vivo* induction of antigen-specific Treg cells will lead to a more general immune suppression with major side effects like infectious diseases or tumours.

Antigen presentation by immature, so called steady-state dendritic cells (DCs) has been shown to induce T cell tolerance rather than immunity. Targeting of such DCs with foreign antigens thereby represents a promising approach for the induction of antigen-specific Treg cells *in vivo*. Using a monoclonal antibody against the DEC-205 endocytosis receptor, which is highly expressed on DCs in T cell areas of lymphoid organs, allows antigen targeting to DEC-205$^+$ cells. Antigens coupled to the anti-DEC-205 antibody were specifically delivered to DCs, efficiently processed and presented. By this approach, Kretschmer and colleagues could induce antigen-specific Foxp3$^+$ Treg cells *in vivo* [12]. The addition of TGF-beta or the absence of IL-2 further enhanced the rate of antigen-specific Treg cell induction. Thus, administration of antigen coupled to anti-DEC-205 might be useful for the treatment of autoimmune disease with known antigen specificity. This was first demonstrated by Bruder and colleagues for the prevention of type I diabetes [13]. Double transgenic mice harbouring T cells recognizing the model antigen hemagglutinin (HA) concomitant with HA expression in the β-cells of the pancreas develop spontaneous autoimmune diabetes. Repeated injections of newborn mice with anti-DEC-205 conjugated to HA protected almost all mice from disease development [13]. In addition, within the pool of antigen-specific T cells a significant increase in Foxp3 expression was detectable in the protected mice. These results provide a challenging basis for the development of novel strategies for the treatment of unwanted immune responses.

ROLE OF TGF-BETA AND RETINOIC ACID IN THE INDUCTION OF TREG CELLS

The cytokine TGF-beta is known as a critical factor for the regulation of T cell-mediated immune responses and the induction of immune tolerance [14]. When the TGF-beta-mediated inhibitory pathway is abrogated specifically in T cells by restrictive expression of a dominant negative TGF-beta receptor [15], mice develop unchecked T cell proliferation as well as inflammatory and autoimmune-like diseases, illustrating that a TGF-beta-dependent signal is involved in T cell activation and tolerance *in vivo*. Indeed, Chen and colleagues could demonstrate that antigen-specific activation of naïve T cells in the presence of TGF-beta converts them to Treg cells [16]. These TGF-beta induced Treg cells express elevated levels of Foxp3 and are able to suppress pro-allergic immune reactions in a mouse asthma model [16] and experimental colitis [17].

Most recently, two different studies have independently shown that Treg cell conversion *in vivo* takes place more efficiently in gut-associated lymphoid tissues (GALT) than in other lymphoid organs [18]. They identified a population of CD103$^+$ DCs purified from the GALT that induce the development of Foxp3$^+$ Treg cells. The conversion induced by CD103$^+$ cells is dependent on TGF-beta and the dietary metabolite retinoic acid (RA) [18] as RA alone could not induce Treg cell responses but strongly enhanced the TGF-beta dependent conversion of naïve T cells into Foxp3$^+$ Treg cells *in vitro* [18-20]. In summary, results of these studies show that CD103$^+$ DCs in GALT are specifically equipped for converting antigen-specific T cells into Foxp3$^+$ Treg cells in a RA and TGF-beta dependent manner. Thus, RA seems to be a promising additive for the clinical application of Treg cells as it could enhance the antigen-induced and TGF-beta dependent conversion of naïve T cells into Treg cells. However, due to possible side effects of RA other RAR agonists may be better suited for future *in vivo* experiments. Moreover, with respect to the use of RA as potential therapeutic, it has to be clarified, if RA is also suitable for the conversion of previously activated and polarized T cells, and thereby, to interfere with unwanted, ongoing immune responses.

In addition to the role of TGF-beta in the induction of Treg cells, it has also been proposed that TGF-beta produced by Treg cells may mediate suppression of T cells [21]. It has also been described that Treg cell–mediated suppression of CD8$^+$ T cells requires an intact TGF-beta receptor II on CD8$^+$ T cells [22, 23]. Thus, the inhibition of TGF-beta by small molecules that might penetrate the interface between contacting T cells have been suggested to be a valuable tool for the inhibition of Treg cell activity during antitumor immunotherapy. Most recently, Gil-Guerrero and colleagues have shown that a short synthetic peptide called P17 and which inhibits TGF-beta was able to interfere with Treg cell activity *in vitro* and *in vivo* [24]. Administration of this peptide to mice bearing a tumour which expresses a model antigen, leads to enhanced proliferation of antigen-specific T cells, reduced numbers in Foxp3$^+$ antigen-specific Treg cells and to an inhibition of tumour growth [24]. Thus, peptide inhibitors of TGF-beta might be valuable tools to interfere with Treg cell activity and thereby breaking tolerance against pathogens or tumours. However, possible side effects of this therapeutic approach have to be evaluated as TGF-beta participates in other cellular processes.

MOLECULES INVOLVED IN TREG CELL FUNCTION AS POTENTIAL TARGETS FOR IMMUNOTHERAPY

Neuropilin-1

Neuropilin-1 (Nrp-1) has been initially identified as a ligand-binding receptor for class III semaphorins [25] involved in axonal guidance in the nervous system. During the last few years it could be demonstrated that Nrp-1 is also expressed by different cell types of the immune system. We have shown for the first time that Nrp-1 is highly expressed by CD4$^+$CD25$^+$ Treg cells but not in activated T cells. In addition Nrp-1 expression could be induced by ectopic expression of Foxp3 [26, 27] and the administration of the anti-

inflammatory vasoactive intestinal peptide (VIP). Studies on these VIP-induced Nrp-1^+ T cells revealed the induction of Treg cells which are able to inhibit T cell proliferation *in vitro* and are able to interfere with delayed type hypersensitivity and graft-versus-host disease *in vivo* [28]. Thus, induction of Nrp-1^+ Treg cells by VIP might represent a potential therapeutic target for the treatment of autoimmunity. On the other hand, reduction in Nrp-1^+ T cells might represent a promising approach for the treatment of cancer. Recently, Battaglia and colleagues identified a subset of Treg cells in human lymph nodes, which express high levels of Nrp-1. Following preoperative chemoradiotherapy the amount of Nrp-1^+ Treg cells in tumour-draining lymph nodes dropped in a direct relationship with the reduction in tumour mass [29]. This observation suggests that elimination of Nrp-1^+ suppressor T cells may facilitate the generation of T cells mediating the destruction of the neoplastic cells left over after cytotoxic therapy.

However, the biological function of Nrp-1 expression on Treg cells remains unclear. Ablation of Nrp-1 expression in T cells using transgenic mice revealed no difference in Foxp3 expression in Nrp-1 deficient T cells in comparison to wildtype T cells [30]. This observation suggests that Nrp-1 deficient T cells exhibit similar regulatory function as Nrp-1^+ Treg cells, but no functional data on Nrp-1 deficient Treg cells are available until now. However, Nrp-1 expression seems not to have an intrinsic influence on Treg cells. Most recently, it could be demonstrated that Nrp-1 is part of the immunological synapse [31, 32]. Intravital imaging studies of Treg cell dynamics in autoimmune-disease models have shown that Treg cells make stable contact with antigen-bearing DCs that precedes the contact with antigen-specific naïve T cells with DCs and inhibits T cell activation [33, 34]. Sarris and colleagues could demonstrate that Nrp-1 expressed by Treg cells as well as by DCs is directly involved in the interaction of Treg cells with DCs [32], indicating that Nrp-1 functions as a component of the immunological synapse. They have shown that Nrp-1 promoted prolonged interactions with immature DCs (iDCs), resulting in higher sensitivity to limiting amounts of antigen. In addition, antibody-mediated blockade of Nrp-1 reduces the number of long interactions between Treg cells and iDCs [32], demonstrating a functional role of Nrp-1 expression on Treg cells. The addition of anti-Nrp-1 antibodies to cocultures of antigen-specific naïve T cells with Treg cells in the presence of antigen-loaded DCs significantly reduces the suppressive capacity of Treg cells [32]. These results suggest that modulation of Nrp-1 expression might represent a promising new target for the therapeutic intervention in patient with dysregulated immune responses.

CD83

CD83 is a glycosylated Immunoglobulin-like type 1 transmembrane protein that was initially described as a specific surface marker for activated human dendritic cells (DCs) [35]. The murine CD83 protein shares an overall homology of 63%, suggesting a possible conserved function. CD83 protein expression was not only reported on mature DCs, but also on a broad variety of other immune cells like activated B and T lymphocytes, activated macrophages, a regulatory subset of NK cells and activated neutrophils [36]. In addition, we could demonstrate that CD83 is also expressed by natural occurring $CD4^+CD25^+$ Treg cells [37].

Although its specific role in modulation of immune responses remains poorly understood, manipulation of the CD83 pathway has been proposed to be of therapeutic use for the treatment of inflammatory and autoimmune diseases. Besides the membrane-bound form of CD83, a soluble form of CD83 (sCD83) has been detected in human serum of healthy adults [38], in synovial fluid form rheumatic arthritis patients [39] and in serum from some chronic lymphocytic leukaemia or mantle cell lymphoma patients [40]. This soluble form has been suggested to have a direct immunosuppressive function.

Lechmann and colleagues reported that the extracellular immunoglobulin domain of human CD83 blocked DC-mediated allogenic and peptide-specific T cell proliferation *in vitro* [41]. In addition, in the experimental autoimmune encephalomyelitis (EAE) mouse model injections of sCD83 prevented the disease-associated paralysis under prophylactic as well as therapeutic settings *in vivo* [42]. Scholler and coworkers reported that recombinant expression of a CD83-hIg and CD83-mIg fusion protein inhibited DC-mediated T cell stimulation *in vitro* in a concentration-dependent manner [43]. In addition, they demonstrated that sCD83 downmodulates anti-tumour immune responses *in vivo* using the murine P815 tumour model [43]. Most recently, Xu and colleagues have shown, that a limited course of sCD83 delayed acute cellular rejections of MHC-mismatched mouse skin allografts [44]. It is well known that viruses target functionally important molecules of the immune system in order to overcome and/or escape anti-viral immune responses. In this respect, Senechal *et al.* reported that the membrane bound CD83 surface molecule is a target for Human Cytomegalovirus (HCMV) [45]. Surprisingly, as a consequence of the HCMV infection, a soluble form of CD83 is shed from the cell surface and subsequently blocks T cell stimulation, thereby, resembling the mode of action of recombinantly expressed sCD83. Thus, all these data clearly support an immunoregulatory function of soluble CD83.

Most recently, we could demonstrate that CD83 is also expressed by naturally occurring $CD4^+CD25^+$ Treg cells [37]. To gain further insights into its role in Treg cells we have retrovirally expressed CD83 in freshly isolated naïve T cells. As a consequence, these CD83-transduced T cells exhibit suppressive function *in vitro* and are able to interfere with contact hypersensitivity reactions and Experimental Autoimmune Encephalitis (EAE) *in vivo* [37]. Based on these results one might speculate about secretion of the ectopically expressed CD83. However, the addition of supernatants from cultured CD83-transduced T cells had no significant effect on T cell proliferation *in vitro*. Moreover, CD83-transduced T cells and $CD4^+CD25^+$ Treg cells lost their *in vitro* suppressive capacity in the absence of cell-cell contact and the majority of *in vitro* stimulated $CD4^+CD25^+$ Treg cells expressed membrane-bound CD83 (mCD83) [37]. Thus, secretion of sCD83 by immunoregulatory $CD4^+$ T cells seems to be unlikely. We rather suggest, that Treg cells mediate their suppressive capacity *via* interaction of membrane bound CD83 with its putative ligand.

Despite a lot of efforts in several labs a specific ligand for CD83 (CD83L) has not yet been identified. Studies on

CD83L expression are controversial, but have suggested that a putative CD83L is expressed on DCs [41], monocytes [46], activated $CD8^+$ as well as $CD4^+$ lymphocytes [47]. CD83L was expressed on activated T cells only upon stimulation through both CD3 and CD28 but not CD3 alone suggesting that CD83L only functions when T cells are activated in the presence of costimulatory signals provided by $CD83^+$ APCs [40]. This observation might explain the function of CD83 on Treg cells and the immunosuppressive function of CD83-transduced T cells, respectively. Binding of Treg cells *via* CD83/CD83L interaction to activated T cells (A) might establish the cell-cell contact described to be important for the immunosuppressive function of Treg cells or/and (B) might interfere with T cell/DC interactions and thereby compete with further T cell stimulation by professional APCs providing costimulatory signals (Fig. **1**). However, the identification of the CD83L and its intracellular signalling pathway would be helpful to better understand the role of CD83/CD83L interactions in the regulation of different immune responses.

In summary, available data imply that blocking interactions between membrane CD83 and its ligand or the direct binding of soluble CD83 with its ligand may modulate T cell activation during *in vitro* and *in vivo* assays. However, studies using anti-CD83 antibodies could not demonstrate a ligand-blocking or function-blocking activity until now. But one could not exclude a possible antibody engagement of CD83, which might generate transmembrane signals complicating blocking studies. Thus, the identification and characterization of CD83 antibodies which block ligand binding is critical for a better understanding of the role of CD83 in immunoregulation.

G Protein-Coupled Receptor 83

Over the last few years extensive studies have been carried out with the aim of a detailed understanding of the developmental and functional aspects of Treg cells. By using microarray analyses to study genes that are differentially expressed in $CD4^+CD25^+Foxp3^+$ Treg cells the G protein-coupled receptor 83 (GPR83) was identified to be specifically up-regulated in Treg cells [3, 48]. GPR83 (also termed GIR, hGPR72) is an orphan GPR homologous to the tachykinin and neuropeptide Y receptors [49, 50]. It was originally identified among genes induced by glucocorticoids and cAMP in the T cell line WEHI-7TG [51], and later it was shown to be induced by dexamethasone in murine thymocytes [52]. It encodes a putative seven-transmembrane-domain protein [49] that is highly expressed in mouse brain and thymus [51, 53]. Whereas most of the genes highly expressed by Treg cells were also up-regulated upon activation of naïve T cells, GPR83 is specifically expressed in the $CD4^+CD25^+Foxp3^+$ Treg cell subset only [54, 55]. In addition, overexpression of Foxp3 by retroviral gene transfer induces GPR83 expression [54]. These observations suggest a close link between GPR83 and Foxp3 expression. Thus, GPR83 might be an interesting target for the modulation of

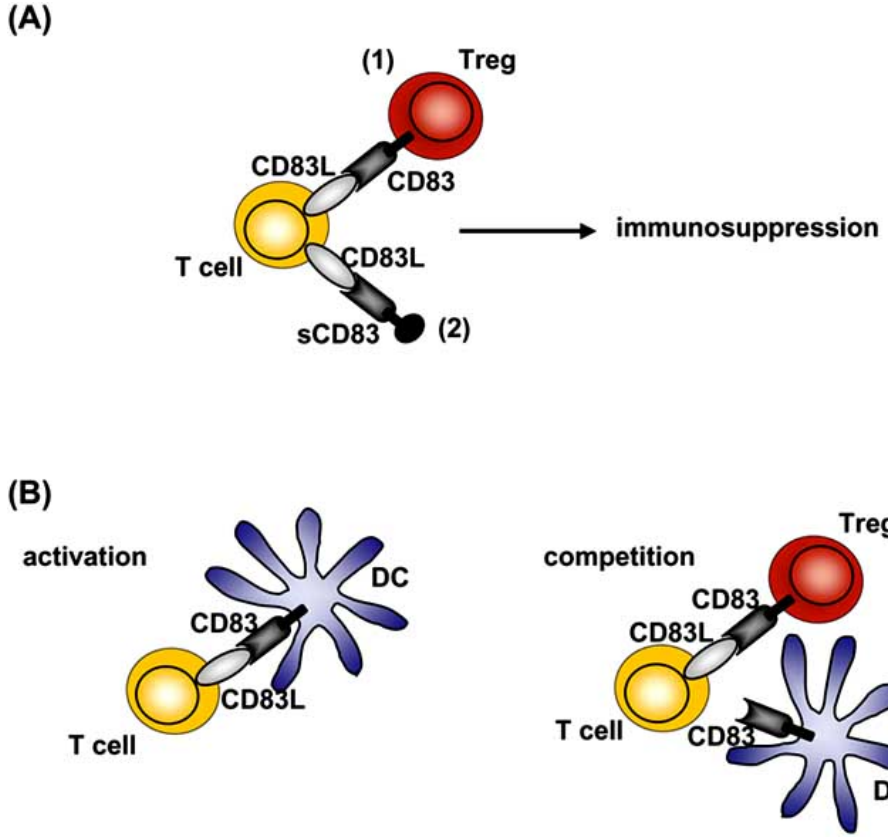


Fig. (1). The picture illustrates the potential function of CD83/ CD83 ligand interactions. (**A**) Expression of a putative CD83 ligand (CD83L) has been shown for T cells, whereas CD83 is expressed by regulatory T (Treg) cells. Engagement of CD83L by membrane bound CD83 (1), or soluble CD83 (2) might contribute to the suppression of T cells. (**B**) Mature dendritic cells (DC) also express CD83 on their surface. It was proposed that interactions of CD83 with its ligand (CD83L) play an important role in the course of T cell activation. Competition for DC/T cell interactions *via* CD83/CD83L by CD83 expressing Treg cells might inhibit an appropriate activation of T cells by DCs.

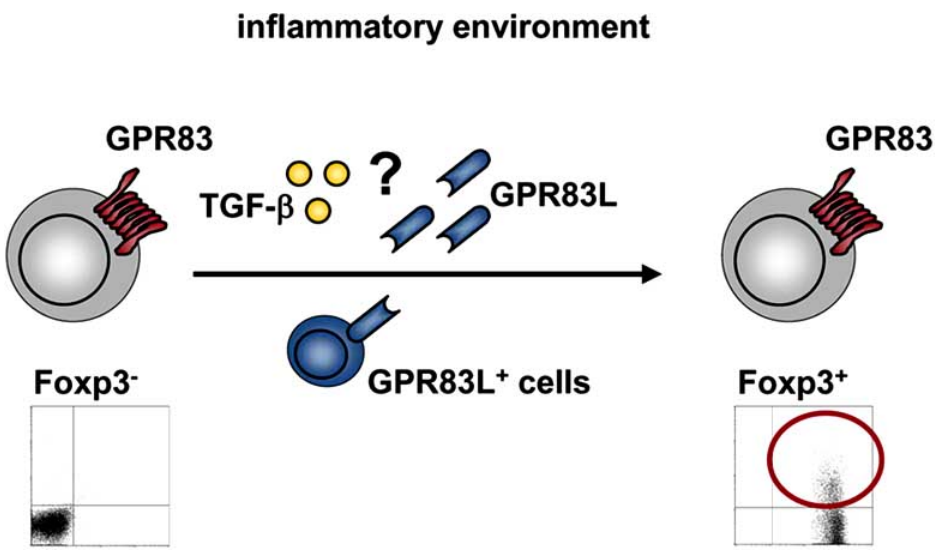


Fig. (2). GPR83 is involved in the induction of regulatory T (Treg) cells *in vivo*. GPR83$^+$Foxp3$^-$ T cells convert to Foxp3$^+$ Treg cells in an inflammatory environment *in vivo*. It has been suggested that TGF-beta and/or a specific ligands (soluble or in a membrane bound form) are involved in GPR83-dependent Treg cell induction *in vivo.*

Treg cell numbers and/or Treg function by receptor agonists and antagonists.

As described in the upper part of this review, Foxp3 expression could be induced in antigen-activated T cells by the addition of TGF-beta. Toms and colleagues have demonstrated that GPR83 expression is also highly dependent on TGF-beta [55], as GPR83 was only expressed in Foxp3$^+$ T cells but not Foxp3$^-$ T cells upon stimulation in the presence of TGF-beta. In addition, in T cells isolated from transgenic mice expressing a dominant negative form of the TGF-beta receptor II subunit (dnTGF-betaRII) and as such displaying impaired responsiveness to TGF-beta, no GPR83 expression could be detected upon activation [55]. Interestingly, in these mice, CD4$^+$CD25$^+$ Treg cells express normal levels of Foxp3, but showed a significant decrease in GPR83 expression [55]. These observations suggest that GPR83 is closely associated with TGF-beta signalling. However, this association seems not to be a general effect. Analyses of T cells from genetically modified mice with ablation in GPR83 revealed that these T cells indeed exhibit a reduced efficiency in Foxp3 induction upon activation in the presence of TGF-beta, but the generation of Th17 cells, another TGF-beta-dependent cell line was not affected under these *in vitro* conditions [56]. Thus, GPR83 seems to be involved in the induction of Foxp3$^+$ Treg cells but dispensable in Th17 generation.

We could recently demonstrate that GPR83 transduced naïve T cells convert into Foxp3$^+$ Treg cells under inflammatory conditions *in vivo* [54]. However, GPR83 overexpression alone did not induced a regulatory phenotype, i.e., suppressive activity and Foxp3 expression suggesting that a specific ligand might only be present in the inflammatory environment *in vivo* (Fig. **2**). In addition, the presence of antigen-presenting cells (APC) seems to play a role in GPR83 dependent Treg cell induction. Differences in Foxp3 expression in GPR83 deficient T cells *vs* WT T cells upon stimulation in the presence of TGF-beta were not observed in the absence of APCs *in vitro* [56]. Despite the strong link between GPR83 expression and Foxp3 induction, studies in GPR83 deficient mice revealed that GPR83 is dispensable for the development and function of naturally occurring Treg cells [55, 56]. Nevertheless, the finding that GPR83 is a TGF-beta-responsive gene and results from studies with GPR83 overexpressing cells highlight its potential role in the peripheral development of induced Foxp3$^+$ Treg cells. By this, the identification of the specific ligand which might trigger GPR83 signalling and thereby inducing Treg cell function would represent a promising therapeutic target for the treatment of different inflammatory diseases, whereas blocking of GPR83 seems not to have a major influence on Treg cell function as GPR83 deficient Treg cells have a normal phenotype *in vitro* and *in vivo* [55, 56].

Sequence analysis revealed that GPR83 shares high sequence homology with members of the neuropeptide Y family. Indeed, recent studies on rat GPR83 could demonstrate NPY-Y2 receptor activity of GPR83 *in vitro* [57]. This possible link between the neurological and immunological systems has also been described for other molecules such as Neuropilin-1 [26]. In addition a recent study by Sah and colleagues proposed a role of GPR83 in the control of feeding and emotional behaviour [58]. Well in line with this, a tendency to become obese was observed in GPR83-deficient mice [56].

CONCLUSIONS

Tregs cells are essential components of immune homeostasis and thereby provide a potential therapeutic opportunity for active immune regulation and long-term tolerance induction. The identification of molecules specifically expressed by Treg cells may have implications in understanding the regulation of unwanted immune responses e.g. during autoimmunity, inflammation or persistent immune activation caused by chronic infections. Moreover, molecules which have the ability to induce immunosuppressive Treg cell function like GPR83 or CD83 represent promising targets for the modulation of immunity. Induction of Treg cells by triggering these surface receptors would be of benefit for the treat-

ment of dysregulated or over-whelming immune responses, whereas blocking the function of these molecules might contribute to amplify immune responses to antigens in the context of vaccine development or boost the immune response to infections and tumours.

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