Review Article

Cellular Mediators of Inflammation: Tregs and T_H17 Cells in Gastrointestinal Diseases

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Human lymphocyte subpopulations were originally classified as T- and B-cells in the 70s. Later, with the development of monoclonal antibodies, it became possible to recognize, within the T-cells, functional populations: $CD4^+$ and $CD8^+$. These populations were usually referred to as "helper" and "suppressor" cells, respectively. However several investigations within the CD8 cells failed to detect a true suppressor activity. Therefore the term suppressor was neglected because it generated confusion. Much later, true suppressor activity was recognized in a subpopulation of CD4 cells characterized by high levels of CD25. The novel population is usually referred to as T regulatory cells (Tregs) and it is characterized by the expression of FoxP3. The heterogeneity of CD4 cells was further expanded by the recent description of a novel subpopulation characterized by production of IL-17. These cells are generally referred to as T_H17. They contribute to regulate the overall immune response together with other cytokine-producing populations. Treg and T_H17 cells are related because they could derive from a common progenitor, depending on the presence of certain cytokines. The purpose of this review is to summarize recent findings of the role of these novel populations in the field of human gastroenterological disease.

1. Introduction

In 1970 Gershon and Kondo described the role of T-lymphocytes in the induction of tolerance [1]. Much later true regulatory activity was recognized in a subpopulation of $CD4^+$ cells characterized by high levels of CD25, the alpha-chain of IL-2 receptor [2]. This novel population is usually referred to as T regulatory cells (Tregs). IL-2 is essential for the generation of Tregs in the thymus and their survival, expansion, and suppressive function in the periphery [3].

The presence of alternative patterns of cytokine production has been well established in several pathological conditions and is usually referred to as T_H1 cells that produce IL-2 and INF-gamma but not IL-4 and T_H2 cells that produce IL-4 but not IL-2/IFN-gamma. Yet, the concept that the cells producing these alternative patterns of cytokines represent in humans irreversibly differentiated endpoints has been challenged [4, 5]. However, for the sake of simplicity, we will, as it is usually done, refer to these cells as T_H1 and T_H2 indicating not differentiated population but their pattern of cytokine production.

In this review we discuss experimental evidences derived from both human and mouse studies. For the sake of clarity, the reader should assume that we are reviewing human data unless differently specified.

2. Regulatory T-Cells (Tregs)

Regulatory T-cells constitute a minor subpopulation of $CD4^+$ T-cell but their role is crucial for the control of autoreactive T-cells [6]. Naturally occurring $CD4^+CD25^+$ T-cells represent 5%–10% of peripheral $CD4^+$ cells [7, 8]. They develop in the thymus from nonregulatory thymocytes during ontogeny [9]. Nevertheless not all the $CD4^+CD25^+$ T-cells can be considered Tregs. Most of the regulatory T-cells are $CD4^+CD25^{high}$ cells, which represent 2%-3% of the $CD4^+$ T-cells [10]. However, recent data supports

the idea that Tregs can also be CD25 negative [11, 12]. Regulatory T-cells are involved in the regulation of immune response, maintaining immunological self-tolerance and immune homeostasis [13], and the control of autoimmunity and cancer surveillance [14]. Therefore, Tregs play a key role in autoimmunity, allergy, cancer, infectious disease, and the induction of transplantation tolerance. As a consequence, abnormalities in the number and functions of Tregs have been implicated in the pathogenesis of the abovementioned clinical conditions [15].

Tregs are characterized by the expression of FoxP3 (Forkhead box P3), a transcriptional repression factor of the forkhead-winged helix family of transcription factors [16]. FoxP3 is physically associated with the Rel family transcription factors, nuclear factor of activated T-cells (NFATs) and NF-kappaB (NF-κB), and blocks their ability to induce the endogenous expression of their target genes, including key cytokine genes [17]. Mutations in FoxP3 are associated with the inherited autoimmune disease, Scurfy in mice, and IPEX (immune dysregulation, polyendocrinopathy, enteropathy, and X-linked syndrome) in human [18]. IPEX is characterized by the presence of autoimmune disease in multiple endocrines organs, inflammatory bowel disease, allergies, and severe infections [16]. Beyond IPEX, mutations of FoxP3 have been seen associated also with an absence of Tregs [19].

A further evidence of the importance of FoxP3 derives from the observation by Hori et al. that retroviral gene transfer of FoxP3 converts naive T-cells into a regulatory T cell phenotype similar to that of naturally occurring CD4$^+$ regulatory T-cells [20]. FoxP3 can also interact with the promoter of IL-127 receptor (IL-127R) and might contribute to reduce expression of CD127 in Tregs. Thus, CD127 expression inversely correlates with FoxP3 expression [21]. In 2008 Sakaguchi proposed FoxP3 as the crucial marker for Tregs development and function [14], and FoxP3 is currently one of the most accepted markers for Tregs. So Tregs are usually referred to phenotypically as CD3/CD4/CD25^{high}/FoxP3$^+$ cells. Recently, after the description of an inverse relationship between the expression of FoxP3 and CD127 [21], it has become possible to define an alternative phenotype of Tregs as CD3/CD4/CD25^{high}/CD127$^-$ cells.

In summary, FoxP3, IL-2, and CD25 are essential molecules for the development, function, and survival of Tregs. It is worth noting that induction of FoxP3 is also under the control of TCR and CD28 signaling. Moreover IL-2 and TGF-beta are important for the survival and proliferation of Treg cell precursors [22, 23].

A subset of human Tregs the FoxP3$^+$ regulatory effector/memory-like T- (T$_{REM}$) cells expresses CD39, nucleoside triphosphate diphosphohydrolase-1 [NTPDase 1], an ectoenzyme that degrades ATP to AMP. Different from mice, in which CD39 is expressed in all Tregs, in humans CD39 is a marker of a Treg subset likely involved in the control of the inflammatory autoimmune disease [24]. So, the expression of CD39 in concert with CD73, another ectonucleotidase present on the surface of lymphocytes, can further distinguish CD4$^+$/CD25$^+$/FoxP3$^+$ Treg cells from other T-cells [25].

Alpha(E)beta(7)-integrin (CD103) is an integrin that enable the interaction with epithelial cadherin responsible for tissue-specific retention of lymphocytes. Thus, the integrin alpha(E)beta(7) can be regarded as a novel marker for subsets of functionally distinct Tregs specialized for crosstalk with epithelial environments [26]. CD103 has been seen also connected to the retention and homing of Tregs in inflamed tissues [27].

3. Tregs Subpopulations

Tregs are divided into two subpopulations, which are primarily defined by where they develop:

(1) the natural Tregs (nTregs), which originate directly from the thymus, expressing at once high levels of CD25 and the transcriptional factor FoxP3,

(2) the induced Tregs (iTregs), develop in the periphery from conventional CD4$^+$ T-cells and whose expression can be modulated through antigenic stimulation under a variety of conditions.

A functionally important proportion of Tregs (the nTregs) originates from the thymus; nevertheless, in 2006, Chatenoud and Bach reported that Tregs are not present as such in the thymus; they derive from peripheral precursors that are CD4$^+$CD25$^-$ and differentiate into functional Tregs following adequate stimulation in the presence of the cognate antigen and specialized immunoregulatory cytokines, such as TGF-beta, IL-10, and IL-4; they are generally termed "adaptive" Tregs [28].

In addition several pathological and tolerogenic conditions, such as oral tolerance and the presence of retinoic acid, induce conversion of conventional T-cells into Treg cells. In particular, it has been shown that, in the presence of TGF-beta, all-*trans*-retinoic acid (ATRA) efficiently converts adult human peripheral blood naive CD4$^+$ T-cells into Treg cells with stable and potent suppressive ability [29].

Moreover it has been demonstrated that a substantial percentage of Tregs had transient or unstable expression of the transcription factor FoxP3. These cells have been defined as "exFoxP3" T-cells, and they have an activated-memory T-cell phenotype and produce inflammatory cytokines. exFoxP3 cell numbers increase in inflamed tissues in autoimmune conditions. Analysis of the T-cell receptor repertoire suggested that exFoxP3 cells developed from both natural and adaptive Tregs [30].

4. Mechanisms of Action of Tregs

Several mechanisms have been proposed to explain the function of Tregs, but the effective mechanism of action of Tregs is not yet completely understood. Despite the numerous studies performed about Tregs functions, a number of fundamental questions concerning the origin, phenotypic nature, and mechanism of action of Tregs remain still elusive [15].

Currently it is believed that Tregs rely on several mechanisms as follows.

(1) The first mechanism is metabolic disruption.

(2) Tregs may regulate their target by modulating DC maturation via CD80/86 and CTLA-4 interactions.

(3) Another mechanism is the cytolysis mediated by granzyme A or B in human and mice, respectively.

(4) Moreover Tregs can produce inhibitory cytokines, such as IL-10, TGB-beta, and IL-35.

(5) Finally Tregs are involved also in the regulation of peripheral tolerance to self-antigens.

Tregs were described initially as antigen nonspecific cells that act through an APC (Antigen Presenting Cell) independent mechanism. Tregs require activation via their TCR to become suppressive; but once activated, their suppressor effector function is completely nonspecific [31]. Nevertheless, subsequently it has been verified that may exist populations of Tregs that are antigen specific. More recently, a much larger analysis of hundreds of TCRs found little evidence that the Tregs population preferably recognized self-antigen; besides it was concluded that non-self-antigens are the cognate specificities of Tregs [32].

Other studies concluded that Tregs, like conventional T-cells, have a polyclonal TCR repertoire and are selected on self-peptides. Specificity for self-peptides directs the selection of $CD4^+CD25^+$ regulatory thymocytes by a process that is distinct from positive selection and deletion [33].

The growing number of inhibitory mechanisms ascribed to Tregs suggests that these cells take a multipronged approach to immune regulation. It is likely that the relative importance of each inhibitory mechanism is context dependent and modulated by the inflammatory milieu and the magnitude of the immune response. Taken together, these mechanisms provide a potent arsenal with which Tregs can act [34, 35].

4.1. Metabolic Disruption. Because of the presence of the alpha chain of IL-2 receptor (CD25), Tregs could be scavengers of IL-2, inhibiting the activation of other T-cells in the same environment, reducing the IL-2 availability. They do not produce IL-2, thus depend on IL-2 production by other cells. Reducing IL-2, Tregs induce apoptosis by IL-2 deprivation, which has been shown in several clinical conditions, including HIV disease [36].

Besides, these cells express also the transcriptional repression factor FoxP3 which is involved in the inhibition of cellular activity. So Tregs could consume IL-2 without activating their immune function and preventing, at the same time, the activation of other T-cells.

Moreover, because of the presence of the ectoenzymes CD39 and CD73 on the cellular surface of Tregs, these cells are also able to modulate cellular inhibition, through the production of pericellular adenosine (Ado) from ATP. ATP and Ado are important endogenous signaling molecules in immunity and inflammation. Extracellular ATP is a danger signal released by damaged cells; ATP behaves as chemoattractant for lymphocytes, activator of proinflammatory response, and inducer of local pain [37].

4.2. Cell-to-Cell Inhibition. One additional possibility to explain Tregs function is based on observation by Vignali [38], who referred that Tregs can mediate their inhibitory function by direct cell-to-cell contact. Wing et al. described the role of cytotoxic T-lymphocyte antigen-4 (CTLA-4), a protein highly expressed by Tregs, which inhibits the immune response [39]; CTLA-4 binds the costimulatory molecules CD80 (B7-1) and CD86 (B7-2) with higher affinity than CD28, inhibiting the second signal, necessary to immune activation [40]. Constitutive expression of CTLA-4 among $CD4^+$ cells was restricted primarily to Tregs, suggesting that CTLA-4 expression by these cells is involved in their immune-suppressive function. These findings raise the possibility that Treg cell function contributes to the immune suppression characteristic of CTLA-4 signaling [41]. In vivo blockade of CTLA-4 for a limited period in normal mice leads to spontaneous development of chronic organ-specific autoimmune diseases, which are immunopathologically similar to human counterparts [42]. CTLA-4 is constitutively expressed on Tregs that express the transcriptional factor FoxP3. Furthermore, ectopic FoxP3 expression can phenotypically convert effector T-cells to regulatory T-cells [43]. Horwitz et al. revealed an important relationship between TGF-beta, CTLA-4, and FoxP3 in the generation of iTreg, but not nTreg [44].

Tregs also express the glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR, also known as TNFRSF18), a member of the tumor necrosis factor-nerve growth factor (TNF-NGF) receptor gene superfamily, which plays a key role in dominant immunological self-tolerance [45]. Stimulation of GITR abrogate $CD4^+CD25^+$ T-cell-mediated suppression and administration of a monoclonal antibody to GITR produce organ-specific autoimmune disease in otherwise normal mice [46].

One other cell surface molecule, Lymphocyte activation gene-3 (LAG-3), marks regulatory T-cell populations and contributes to their suppressor activity [47]. LAG-3 is a CD4-related transmembrane protein expressed by regulatory T-cells that binds MHC II on APCs. During Tregs-DCs interactions, LAG-3 engagement with MHC class II inhibits DC activation [48].

Another possibility of inhibition cell-to-cell contact-mediated of Tregs is based on the interactions, in lymphoid tissues, between Tregs and dendritic cells (DCs). In fact activated professional APCs (DCs, B-cells, and macrophages) can attract Tregs, through the secretion of common chemokines, such as CCL4, which is the most potent chemoattractan of Tregs [49]. Then, the cellular relation with DCs and Tregs helps to restrict DCs ability to form stable contacts with self-reactive T-cells, resulting in only transient interactions between DCs and naïve $CD4^+$ T-cells [50].

4.3. Cytotoxic Activity. Tregs can suppress immune response also inducing apoptosis; this requires, in the target T-cells, the proapoptotic protein Bim, involved in the activation of caspases cascade [51]. Apoptotic mechanisms are used to regulate the development of thymocytes, the shaping of T cell repertoire, its selection, and the coordinate events leading to

immune responses in the periphery [52]. Tregs can also control the immune response mediating the perforin/granzyme pathway; in fact Grossman et al. have shown that human adaptive Treg cells preferentially express granzyme B and can kill allogeneic target cells in a perforin-dependent manner. In humans, when Tregs are activated, they express granzyme A, but very little granzyme B [53]. This evidence shows that Tregs objectify their regulatory activity also through suppression by cytotoxic activity.

Granzyme B is also important for the ability of NK cells and CD8$^+$ T-cells to kill their targets. Recent data in the mouse has reported the important role of granzyme B in the suppression of immune system against tumor. In fact granzyme B has not been shown to be expressed in naive Treg cells but it is highly expressed in 5%–30% of Tregs in the tumor environment. Thus, in mouse, both granzyme B and perforin are relevant for Treg cell-mediated suppression of tumor clearance in vivo [54].

4.4. Inhibitory Soluble Factors. In contrast to these hypotheses of cell-contact suppression, there are quite a few reports indicating that Tregs could secrete several cytokines, as TGF-beta and IL-10, for mediating suppression reviewed in [55], limiting effector T-cells function, and inhibiting recruitment of inflammatory myeloid cells such as neutrophils, eosinophils, and monocytes [50]. For instance, IL-10 is required for the control of colitis and homeostatic maintenance of the T-cell number by Tregs review in [55]. However, the production of IL-10 and TGF-beta by Tregs has been challenged by others [56].

IL-35, an inhibitory cytokine of recent description, may be specifically produced by Tregs and is required for maximal suppressive activity [57].

4.5. Peripheral Tolerance. Moreover Tregs are also involved in the induction of peripheral tolerance to self-antigen. Tolerance to self-antigens is generated through two fundamental mechanisms, which included the elimination of self-reactive cells in the thymus during selection (central tolerance) and generation of a variety of peripheral regulatory cells to control self-reactive cells that escape the thymus (peripheral tolerance) [43]. So, at the peripheral mechanisms of tolerance in CD4$^+$ T-cells, which included functional anergy and deletion [58], it is also possible to include the function of suppression by Tregs. Thus, the existence of Tregs that actively suppress the function of conventional T-cells is a key mechanism by which the immune system limits inappropriate or excessive response [15].

5. T$_H$17 Cells

The heterogeneity of CD4 T-cells was further expanded by the recent description of the novel subpopulation T$_H$17, characterized by potent proinflammatory properties. These cells, together with T-cells populations, which mainly produce a T$_H$1 or T$_H$2 pattern of cytokines, INF-gamma, and IL-4, respectively, regulate the overall immune response. Since their discovery, efforts have been made in characterizing

human T$_H$17 cells and the factors involved in their differentiation and in understanding the role that these cells play in inflammation, protective immunity, and autoimmune diseases [59].

CD4$^+$ T-helper (T$_H$17) cells are characterized by the production of IL-17A and IL-17F [60]. T$_H$17 cells have recently been defined as a unique subset of proinflammatory helper T-cells whose development depends on signaling initiated by IL-6 and TGF-beta, autocrine activity of IL-21, activation of STAT3, and induction of the retinoic acid related orphan nuclear receptor RORgammat [61].

T-helper cells produced from the thymus develop into different differentiation program, controlled by cytokines produced in response to microbial products by innate immune cells. Each differentiation program, other than being characterized by unique cytokine profile, INF-gamma for T$_H$1 cells and IL-4 for T$_H$2, can also be identified by specific transcription factor, as pivotal regulator of the T-cells differentiation. These transcription factors include T-bet for T$_H$1 cells, and GATA-3 for T$_H$2 cells [62].

RORgammat does not function in isolation but coordinates the activity of a series of other essential transcription factor in guiding the differentiation of T$_H$17 cells [62]. RORgammat induces transcription of the genes encoding IL-17 and the related cytokine IL-17F in naive CD4$^+$ T-helper cells and is required for their expression in response to IL-6 and TGF-beta, the cytokines known to induce IL-17. TGF-beta together with IL-6 or IL-21 initiates the differentiation, while the IL-23 stabilizes the generation of T$_H$17 cells [63].

Moreover, T$_H$17 cells are also characterized by the surface expression of CCR6, IL-23R, IL-12Rbeta2, and CD161. They also can express the transcription factor T-bet in addition to RORgammat.

The origin of T$_H$17 cells is controversial: in human, T$_H$17 cells originate from CD161$^+$ naive CD4$^+$ T-cells precursor, which constitutively express RORgammat and IL-23R, in response to the combined activity of IL-1beta and IL-23. These findings implicate CD161 as a novel surface marker for human T$_H$17 cells and demonstrate the exclusive origin of these cells from a CD4$^+$ CD161$^+$ T-cell progenitor [64].

T$_H$17 cells play a role in various human diseases associated with inflammation and destruction such as rheumatoid arthritis, psoriasis, Crohn's disease, and multiple sclerosis, where IL-17 can be seen as a therapeutic target. These diseases have in common the local chronic inflammatory reaction with production of inflammatory cytokines, leading to matrix destruction and defective repair [65]. Infectious disease mouse models indicate that T$_H$17 cells mediate protection against extracellular pathogens [66]. T$_H$17 may also contribute to tumor progression due to the role of their cytokines in inflammation and tissue repair [67].

6. Plasticity of CD4$^+$ T-cells: Connection with Tregs and T$_H$17 Cells

The differentiation of T-cells into several differentiation lineages with distinct effector function is well established in the literature. However, after the investigation of other

CD4$^+$ T-cell populations, such as T$_H$1 and T$_H$2 cells, nTregs and iTregs, and T$_H$17 cells, it is not more possible to enunciate with absolute certainty that these CD4$^+$ T-cells subsets represent irreversibly differentiated endpoints [5]. The development into T$_H$17 cells has not been considered to represent a terminal product of its developmental program [68]. Moreover, it appears that expression of FoxP3 by iTregs or IL-17 by T$_H$17 cells may not be stable and that there is a great degree of flexibility in their differentiation options [5].

There is a close relation between Tregs and T$_H$17 cells. While in mice these cells originate from a common precursor, in human it has been reported a developmental link between Tregs and T$_H$17 cells, in which TGF-beta is essential for the generation of both cells [63]. TGF-beta orchestrates T$_H$17 and Tregs differentiation in a concentration-depended manner [62]. At low concentration, TGF-beta synergizes with IL-6 and IL-21 to promote IL-23R expression, favoring T$_H$17 cell differentiation. High concentration of TGF-beta represses IL-23R expression and favors FoxP3$^+$ Treg cells [69]. Besides, there is also an inverse correlation with the transcription factors that identify each of these two T-cell subpopulations. In fact in the presence of TGF-beta, CD4$^+$ T-cells express both RORgammat and FoxP3, but RORgammat function is antagonized by FoxP3. Rather in the presence of proinflammatory cytokines and low concentration of TGF-beta, RORgammat expression is further upregulated, whereas FoxP3 expression and function are inhibited. These evidences show the importance of cytokines environment in the differentiation of CD4$^+$ T-cell subsets, depending upon the balance of expression of the transcriptional factors RORgammat and FoxP3.

Moreover, several recent publications have demonstrated the importance of retinoic acid in the plasticity between Tregs and T$_H$17 cells. Retinoic acid has been demonstrated to be a key regulator of TGF-beta dependent immune responses, capable of inhibiting the IL-6-driven induction of proinflammatory T$_H$17 cells and promoting anti-inflammatory Treg cell differentiation [70].

7. Tregs and T$_H$17 Cells in Gastrointestinal Tumors

Tregs play also a crucial role in the modulation of the immune response to tumor. In particular Tregs are inversely correlated with prognosis of human neoplasias. An increase of their number is associated to a more high risk of incidence of tumors. Compared with healthy patients, patients with gastrointestinal malignancies had a higher proportion of CD4$^+$ CD25$^+$ T-cells in peripheral blood [71].

Most available studies are related to the number of Tregs in peripheral blood of patients with solid tumors. However the most critical point could be to evaluate the presence of Tregs at the site of tumor. It has been shown that many solid tumors are characterized by the infiltration of lymphocytes, and this presence of Tissue infiltrating lymphocytes (TILs). It is essential to determine the T-cell subsets involved in the immune response to understand the pathogenesis of immune diseases [72, 73].

Tregs have been associated with prevention of antitumor immunity and the evasion of efficient recognition of tumor antigens. TILs have been shown to possess a specificity to tumor-associated antigens (TAAs), which are often self-antigens. Analysis of the composition of the specific T-cell receptor (TCR) of TIL could thus provide information on the nature of the antigens recognized by TIL [74].

Many studies have revealed that Tregs are increased in several malignancies, such as esophageal cancer [75], gastric cancer [76], pancreatic cancer [77], and hepatocellular cancer (HCC) [78]. The increasing proportion of Tregs may be related to immunosuppression and tumor progression in patients with gastrointestinal malignancies. There are several evidences concerning the inverse correlation with the prevalence of Tregs and the prognosis of tumors. While it has been reported a direct connection with the increase of these cells and a poor prognosis in esophageal and gastric cancer, in pancreatic cancer and in HCC, there has already not been reported a significant difference by clinical stage in the prevalence of Tregs among patients with colorectal carcinoma [71].

Because of their recent discovery, the role of T$_H$17 cells in tumor is poorly understood. T$_H$17 cells increase in gastric cancer [79], colorectal cancer, and HCC [80].

The role of T$_H$17 cells has emerged as instrumental in cancer pathogenesis, since IL-17 promotes tumor growth, whether IL-23, also produced by T$_H$17, is considered a cancer-associated cytokine because it promotes tumor incidence and growth.

In addition, T$_H$17 cells have recently been described to contribute to human tumor immunity by inducing T$_H$1 cytokines and recruit effector cells to the tumor environment [81].

8. Esophageal and Gastric Cancers

The direct relationship between the prevalence of Tregs in PBMCs and in TILs and the clinical outcome in patients with esophageal and gastric cancers is shown through several clinical evidences. In fact patients with these cancers have higher proportions of Tregs compared with healthy patients. The population of Tregs in patient with advanced esophageal and gastric cancers was significantly larger than that in patients with early esophageal and gastric cancers, both in PBMCs and TILs [75, 82]. In addition patients with these tumors, who have higher levels of Tregs, show more advanced stages and poorer survival rates.

9. Pancreatic Adenocarcinoma

In pancreatic cancer high levels of Tregs have been found as a marker of bad prognosis. The prevalence on Tregs is significantly higher in the peripheral blood, in the tumor microenvironment, and even in the lymph nodes TILs in the lymphatic metastasis, compared with normal donors [83]. Recent publication describes the expression of FoxP3 in pancreatic ductal adenocarcinoma cells, providing evidence that this could be an important tumor escape mechanism [84].

10. Hepatocellular Carcinoma (HCC)

It has been demonstrated that lymphocytic infiltration of the cancerous tissue of liver is indicative of a better survival after surgical resection of the tumor [85]. This evidence confirms the importance of the TILs in the favorable outcome of tumor. However patients with HCC have increased number of Tregs in their peripheral blood and there are also comparable high numbers of Tregs in TILs of the tumor microenvironment. This datum suggests that the increase in frequency of Tregs may play a role in modulation of the immune response against HCC and could be important in design of immunotherapeutic approaches [78].

11. Tregs in Liver Transplant

The liver is a privileged organ with a lower incidence of rejection than other organs. Recent evidence has pointed out that some transplanted patients can achieve operational tolerance, allowing withdrawal of immunosupression. This event is associated with increased levels of Tregs [86]. Tregs have been shown to play a pivotal role in transplant tolerance [87]; regarding their function of suppressive cells, Tregs may play a role in the regulation of alloreactivity.

12. Inflammatory Bowel Disease as Result of Imbalance of Proinflammatory and Regulatory T-Cells Responses

Inflammatory bowel diseases (IBDs), comprising Crohn's Disease (CD) and Ulcerative Colitis (UC), are characterized by chronic idiopathic intestinal inflammation, resulting from predisposing genetic (genes encoding proteins relevant to both innate and adaptive immunity: NOD2, STAT3, and IL-23 receptor, etc.) and environmental factors (specific Toll-like receptors (TLRs) ligands and antigens derived from commensal bacteria) acting on immunoregulatory system. IBD may be result of an imbalance of proinflammatory and regulatory T-cells responses.

Studies of Tregs in celiac disease are anecdotal. A recent work shows a higher percentage of circulating Tregs in patients with active disease than patients after dietary treatment; this experimental evidence suggests that Tregs could act in extinguishing the ongoing intestinal inflammation and the immune response to dietary gluten antigens [88]. In IBD, the inflammation is induced by many different cytokine-mediated pathways [89]. IL-23, IL-17, and the recently described IL-32 [90] have been linked to the pathogenesis of several inflammatory disorders, including IBD.

Data available before the full characterization of T_H17 cells implicated a T_H1 cytokine profile in the pathogenesis of Crohn's disease. More recently, mouse models revealed that tissue inflammation does not develop in mice deficient in the IL-23p19 subunit, whereas inflammation can be found in IL-12p35 deficient mice, suggesting that these models are associated with T_H17 responses [91].

Studies in humans are anecdotal, but, latterly an increased number of RORgammat expressing cells have been detected in the lamina propria of patients with Crohn's

disease [92]. In addition, T_H17 cells were isolated from inflamed lesions of Crohn's patients [93]. Subsequently, increasing interest in the pathogenesis of IBD has been attracted by the role of retinoic acid.

As Bai et al. put it [94], vitamin A and its metabolites, such as retinoic acid (RA), are active agents with a broad range of functions involving immune cell differentiation and maintaining immune homeostasis. For instance, vitamin A and its metabolites are capable of ameliorating various models of autoimmunity.

Deficiency of vitamin A can lead to exacerbated experimental colitis, and supplementation of vitamin A results in amelioration of diarrhea.

Elias et al. have shown that all-trans retinoic acid (ATRA) and other agonists of the retinoic acid receptor alpha (RARalpha) inhibit the formation of T_H17 cells and promote FoxP3 expression [95], while Benson et al. have reported that ATRA enhances Treg growth and differentiation [96].

The close link between IL-23 and IL-17 has been reported as IL-23/IL-17 axis. If an important role of IL-23 is emerging, the function of IL-17 in IBD remains still unclear. According to what was reviewed by O'Connor et al. [97], it has been verified that the absence of IL-17A or IL-17R in T-cells led to an accelerated and severe wasting disease accompanied by higher expression of genes encoding T_H1-type of cytokines. IL-17A can also modulate the T_H1 polarization in vitro in inhibitory sense, suppressing the induction of the transcription factor T-bet, the "master regulator" of T_H1 differentiation of T-cells. This datum indicates that the presence of IL-17 within its receptor (IL-17R) is a favorable prognostic factor in the modulation of the pathogenesis of IBD, diminishing the activation of T-cells in T_H1 sense.

These cytokines are produced by subsets of CD4+ T-helper (Th) cells: in contrast to UC that is considered more of a T_H2 response and CD that is associated with T_H1 and T_H17 profile [98–100]. But new T-cell subclasses Tregs exist independently of T_H1 and T_H2 and play a central role in modulating IBD. In fact, there are new evidences that defects in Treg cell function may underlie IBD and discuss evidence that altered T-cell dependent responses to bacterial proteins may be central to its aetiology [101]. In IBD there is a hyper-reactive immune response in the gut wall directed against the commensal intestinal bacterial flora, and the CD4 T-cells dominate the adaptive immune response [102].

13. Tregs in IBD

Treg cells expressing FoxP3 and/or IL-10 have a role in the immune homeostasis of the gut [103].

It has been well demonstrated in experimental colitis in mice that Tregs not only prevent colitis but also block the progression and reverse the pathology, via IL-10 and Transforming Growth Factor (TGF)-beta-dependent and -independent mechanisms [103], and they suppress both T-cell-dependent colitis and intestinal bacterial inflammation [104]. Furthermore, helminthes have been demonstrated to protect against colonic inflammation, and Tregs were suggested to be responsible for this protective effect; in

fact helminthes impede T_H1 and T_H2 responses generating an immunoregulatory environment via Treg, IL-10, and/or TGF-beta [105].

In humans, during active IBD, there is an inverse correlation between the frequency of peripheral Tregs and the severity of disease [106, 107], and there is an increased number of Tregs in the lamina propria, in mesenteric lymph nodes, and inflamed intestinal mucosa, compared to healthy controls [107, 108]. In addition, the frequency of Tregs decreases with treatment and correlates with clinical response [109]. It is possible that Tregs are recruited from peripheral blood to the inflammation's sites to regulate immune homeostasis [110].

In vitro functional analyses of Tregs from the peripheral blood or intestinal mucosa of IBD patients have shown that Tregs have normal cell-contact-dependent, cytokine-independent suppressive capacity, even against pathogenic T-effector cells derived from the inflamed mucosa [101]. In addition, Tregs derived from IBD inflamed mucosa suppress proliferation and cytokine production [111].

But, there are some difficulties to study mucosal Tregs because the markers used for Tregs from peripheral blood are not unique to Tregs from the lamina propria. Furthermore, nonsuppressive activated T effectors, increased in IBD mucosa, can express FoxP3 [112, 113]. Furthermore, functional in vitro assays are performed without the influence of the inflamed tissue, where diverse response to cytokines or costimulatory interactions may modify Tregs suppressive activity. For example, IL-23 inhibits the generation of colonic FoxP3$^+$ Tregs [114–116].

In recent years, much interest has been attracted by TLR that are essential in the development of antimicrobial responses, by inducing the production of antimicrobial proteins and peptides by a number of cells in the gut epithelium. Activation of macrophages through TLR results in the secretion of inflammatory cytokines and chemokines, thus leading to development of inflammatory responses.

In addition, decreased IL-10 production in T-cells from IBD patients or dendritic cells failure in inducing Tregs or presence of T effectors resistant to suppression can alter the Tregs suppressive activity in vitro [117].

Current IBD therapies have a role on the number and functions of Tregs. In children affected by CD, there is an increased number of colonic FoxP3$^+$ cells after infliximab therapy [118]. In children, in fact, in contrast to adults, there are reduced numbers of colonic Tregs compared to healthy subjects.

Probiotics stimulate the generation of Tregs that produce high levels of IL-10 and suppress the proliferation of effector T-cells in an IL-10-dependent manner [119].

Finally, recent evidence implicates T_H17 cells in the pathogenesis of Crohn's disease [91].

14. T_H17 in IBD

T_H17 cells have been shown to play a role in the pathogenesis of autoimmune and inflammatory diseases, including IBD. Although Treg cells are effectively recruited at inflamed mucosa in IBD, it is possible that Treg cells may have proinflammatory effects through their ability to differentiate into T_H17 in the presence of IL-6 and/or IL-23 at sites of inflammation [120].

T_H17 associated cytokines (IL-17A, IL-17F, IL-21, IL-22) are elevated in bioptic specimens from IBD inflamed colon and in the sera during active disease. While IL-17 expression is not detectable in normal colon, it is readily detectable in CD colonic specimens, is related to disease severity, and is significantly lower in UC than in CD [121].

The role of IL-17A in colitis is controversial: it has been shown to be proinflammatory (due to the production of proinflammatory cytokines, as IL-6 and IL-8, and chemokines, as MCP-1).

IL-21, with TGF-beta, drives T_H17 differentiation, contributing to the development of colitis. IL-22, derived from infiltrating T-cells and NK, is protective and induces antiapoptotic signaling pathways in the intestinal epithelial cells maintaining the integrity of the epithelial barrier [121].

Genome-wide association studies have linked CD to a number of IL-23 pathway genes, including the encoding gene for IL-23 Receptor. Similar associations in IL-23 pathway genes have been observed in UC. IL-23R is a key differentiation feature of CD4-T_H17 effector cells that are critical in mediating antimicrobial defenses, is highly expressed by T_H17 cells, and has a role in their maintenance. Several polymorphisms in the IL-23R gene locus were associated with either susceptibility or resistance to CD [122].

IL-23 is essential in mice for development of IBD and has the major proinflammatory role. In absence of IL-23, colonic levels of proinflammatory cytokines, as TNF-alpha, IFN-gamma, and IL-6, are reduced, while Tregs colonic population is greater.

IL-23 plays an essential role in the induction of intestinal inflammation by innate immune mechanism. Its expression is highly increased in the inflamed intestine but not in systemic sites as those of the spleen and the liver [122].

The differentiation of inflammatory T_H17 and suppressive Treg subsets is reciprocally regulated by relative concentrations of TGF-beta, with the concomitant presence of proinflammatory cytokines favoring T_H17 differentiation, such as IL-6, or Tregs differentiation, such as retinoic acid. The expansion and survival of T_H17 T-cells are subsequently mediated by IL-23 [121].

The homeostatic cytokine IL-7 is essential to the maintenance of colitogenic memory CD4 cells, critical to the maintenance of experimental colitis.

In summary, the IL-23/IL-17 axis is linked to chronic intestinal inflammation and has a beneficial role in intestinal protection and homeostasis.

15. Conclusions

Tregs and T_H17 cells are two important T-cell functionally subpopulations characterized by a specific pattern of cytokine production whose differentiation is modulated by the cytokine microenvironment that has been demonstrated to take part in the pathogenesis of autoimmune disease, cancer, and organs allograft.

So, if in tumor the increase of Tregs and T_H17 cells is inversely correlated with a favorable prognosis, in the IBD and celiac disease there are different situations: while the increase of Tregs prevents the autoimmunity reaction, an amount due of T_H17 cells promotes the outbreak of chronic inflammation. Finally an increase on the proportion of Tregs in the liver transplant correlates with a most favorable success of the transplant, preventing reject and also allowing to reduce the use of immune suppressives, reducing its numerous side effects. Further studies on the role of T_H17 cells in allografts might be performed to make clear the effective negative role of these cells in the organs transplants.

In summary, Tregs and T_H17 cells seem to represent useful and effective mediators in inflammation, autoimmunity, cancer, and allograft rejection, and their serum and tissue levels can be used in diagnosis, prognosis, and monitoring of several diseases.

References

[1] R. K. Gershon and K. Kondo, "Cell interactions in the induction of tolerance: the role of thymic lymphocytes," *Immunology*, vol. 18, no. 5, pp. 723–737, 1970.

[2] S. Sakaguchi, N. Sakaguchi, M. Asano, M. Itoh, and M. Toda, "Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25): breakdown of a single mechanism of self-tolerance causes various autoimmune diseases," *Journal of Immunology*, vol. 155, no. 3, pp. 1151–1164, 1995.

[3] T. R. Malek and A. L. Bayer, "Tolerance, not immunity, crucially depends on IL-2," *Nature Reviews Immunology*, vol. 4, no. 9, pp. 665–674, 2004.

[4] L. Borish and L. Rosenwasser, "T_{H1}/T_{H2} lymphocytes: doubt some more," *Journal of Allergy and Clinical Immunology*, vol. 99, no. 2, pp. 161–164, 1997.

[5] L. Zhou, M. M. W. Chong, and D. R. Littman, "Plasticity of $CD4^+$ T cell lineage differentiation," *Immunity*, vol. 30, no. 5, pp. 646–655, 2009.

[6] E. M. Shevach, "$CD4^+CD25^+$ suppressor T cells: more questions than answers," *Nature Reviews Immunology*, vol. 2, no. 6, pp. 389–400, 2002.

[7] H. Jonuleit, E. Schmitt, M. Stassen, A. Tuettenberg, J. Knop, and A. H. Enk, "Identification and functional characterization of human $CD4^+CD25^+$ T cells with regulatory properties isolated from peripheral blood," *Journal of Experimental Medicine*, vol. 193, no. 11, pp. 1285–1294, 2001.

[8] L. A. Stephens, C. Mottet, D. Mason, and F. Powrie, "Human $CD4^+CD25^+$ thymocytes and peripheral T cells have immune suppressive activity in vitro," *European Journal of Immunology*, vol. 31, no. 4, pp. 1247–1254, 2001.

[9] J. D. Fontenot, J. L. Dooley, A. G. Farr, and A. Y. Rudensky, "Developmental regulation of FoxP3 expression during ontogeny," *Journal of Experimental Medicine*, vol. 202, no. 7, pp. 901–906, 2005.

[10] C. Baecher-Allan, J. A. Brown, G. J. Freeman, and D. A. Hafler, "$CD4^+CD25^+$high regulatory cells in human peripheral blood," *Journal of Immunology*, vol. 167, no. 3, pp. 1245–1253, 2001.

[11] Q. Xu, J. Lee, E. Jankowska-Gan, et al., "Human $CD4^+CD25^+$low adaptive T regulatory cells suppress delayed-type hypersensitivity during transplant tolerance," *Journal of Immunology*, vol. 178, no. 6, pp. 3983–3995, 2007.

[12] N. Komatsu, M. E. Mariotti-Ferrandiz, Y. Wang, B. Malissen, H. Waldmann, and S. Hori, "Heterogeneity of natural FoxP3$^+$ T cells: a committed regulatory T-cell lineage and an uncommitted minor population retaining plasticity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 6, pp. 1903–1908, 2009.

[13] S. Sakaguchi and F. Powrie, "Emerging challenges in regulatory T cell function and biology," *Science*, vol. 317, no. 5838, pp. 627–629, 2007.

[14] S. Sakaguchi, "Regulatory T cells in the past and for the future," *European Journal of Immunology*, vol. 38, no. 4, pp. 901–937, 2008.

[15] C. J. Workman, A. L. Szymczak-Workman, L. W. Collison, M. R. Pillai, and D. A. A. Vignali, "The development and function of regulatory T cells," *Cellular and Molecular Life Sciences*, vol. 66, no. 16, pp. 2603–2622, 2009.

[16] S. Sakaguchi, R. Setoguchi, H. Yagi, and T. Nomura, "Naturally arising FoxP3-expressing $CD25^+CD4^+$ regulatory T cells in self-tolerance and autoimmune disease," *Current Topics in Microbiology and Immunology*, vol. 305, pp. 51–66, 2006.

[17] E. Bettelli, M. Dastrange, and M. Oukka, "FoxP3 interacts with nuclear factor of activated T cells and NF-κB to repress cytokine gene expression and effector functions of T helper cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 14, pp. 5138–5143, 2005.

[18] C. L. Bennett, J. Christie, F. Ramsdell, et al., "The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3," *Nature Genetics*, vol. 27, no. 1, pp. 20–21, 2001.

[19] D. Q. Tran and E. M. Shevach, "Therapeutic potential of FOXP3$^+$ regulatory T cells and their interactions with dendritic cells," *Human Immunology*, vol. 70, no. 5, pp. 294–299, 2009.

[20] S. Hori, T. Nomura, and S. Sakaguchi, "Control of regulatory T cell development by the transcription factor FoxP3," *Science*, vol. 299, no. 5609, pp. 1057–1061, 2003.

[21] W. Liu, A. L. Putnam, Z. Xu-Yu, et al., "CD127 expression inversely correlates with FoxP3 and suppressive function of human $CD4^+$ T reg cells," *Journal of Experimental Medicine*, vol. 203, no. 7, pp. 1701–1711, 2006.

[22] Y. Liu, P. Zhang, J. Li, A. B. Kulkarni, S. Perruche, and W. Chen, "A critical function for TGF-β signaling in the development of natural $CD4^+CD25^+FoxP3^+$ regulatory T cells," *Nature Immunology*, vol. 9, no. 6, pp. 632–640, 2008.

[23] S. Z. Josefowicz and A. Rudensky, "Control of regulatory T cell lineage commitment and maintenance," *Immunity*, vol. 30, no. 5, pp. 616–625, 2009.

[24] G. Borsellino, M. Kleinewietfeld, D. Di Mitri, et al., "Expression of ectonucleotidase CD39 by FoxP3$^+$ Treg cells: hydrolysis of extracellular ATP and immune suppression," *Blood*, vol. 110, no. 4, pp. 1225–1232, 2007.

[25] S. Deaglio, K. M. Dwyer, W. Gao, et al., "Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression," *Journal of Experimental Medicine*, vol. 204, no. 6, pp. 1257–1265, 2007.

[26] J. Lehmann, J. Huehn, M. de la Rosa, et al., "Expression of the integrin $\alpha E\beta7$ identifies unique subsets of $CD25^+$ as well as $CD25^-$ regulatory T cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 20, pp. 13031–13036, 2002.

[27] I. Suffia, S. K. Reckling, G. Salay, and Y. Belkaid, "A role for CD103 in the retention of $CD4^+CD25^+$ Treg and control

of Leishmania major infection," *Journal of Immunology*, vol. 174, no. 9, pp. 5444–5455, 2005.

[28] L. Chatenoud and J.-F. Bach, "Adaptive human regulatory T cells: myth or reality?" *Journal of Clinical Investigation*, vol. 116, no. 9, pp. 2325–2327, 2006.

[29] J. Wang, T. W. Huizinga, and R. E. Toes, "De novo generation and enhanced suppression of human CD4+CD25+ regulatory T cells by retinoic acid," *Journal of Immunology*, vol. 183, no. 6, pp. 4119–4126, 2009.

[30] X. Zhou, S. L. Bailey-Bucktrout, L. T. Jeker, et al., "Instability of the transcription factor FoxP3 leads to the generation of pathogenic memory T cells in vivo," *Nature Immunology*, vol. 10, no. 9, pp. 1000–1007, 2009.

[31] A. M. Thornton and E. M. Shevach, "Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific," *Journal of Immunology*, vol. 164, no. 1, pp. 183–190, 2000.

[32] A. Corthay, "How do regulatory T cells work?" *Scandinavian Journal of Immunology*, vol. 70, no. 4, pp. 326–336, 2009.

[33] M. S. Jordan, A. Boesteanu, A. J. Reed, et al., "Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide," *Nature Immunology*, vol. 2, no. 4, pp. 301–306, 2001.

[34] Q. Tang and J. A. Bluestone, "The FoxP3+ regulatory T cell: a jack of all trades, master of regulation," *Nature Immunology*, vol. 9, no. 3, pp. 239–244, 2008.

[35] D. K. Sojka, Y.-H. Huang, and D. J. Fowell, "Mechanisms of regulatory T-cell suppression—a diverse arsenal for a moving target," *Immunology*, vol. 124, no. 1, pp. 13–22, 2008.

[36] F. Pandolfi, M. Pierdominici, M. Marziali, et al., "Low-dose IL-2 reduces lymphocyte apoptosis and increases naive CD4 cells in HIV-1 patients treated with HAART," *Clinical Immunology*, vol. 94, no. 3, pp. 153–159, 2000.

[37] M. J. L. Bours, E. L. R. Swennen, F. Di Virgilio, B. N. Cronstein, and P. C. Dagnelie, "Adenosine 5′-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation," *Pharmacology and Therapeutics*, vol. 112, no. 2, pp. 358–404, 2006.

[38] D. A. A. Vignali, "How many mechanisms do regulatory T cells need?" *European Journal of Immunology*, vol. 38, no. 4, pp. 908–911, 2008.

[39] K. Wing, Y. Onishi, P. Prieto-Martin, et al., "CTLA-4 control over FoxP3+ regulatory T cell function," *Science*, vol. 322, no. 5899, pp. 271–275, 2008.

[40] E. M. Shevach, "Immunology: regulating suppression," *Science*, vol. 322, no. 5899, pp. 202–203, 2008.

[41] S. Read, V. Malmstrom, and F. Powrie, "Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25+CD4+ regulatory cells that control intestinal inflammation," *Journal of Experimental Medicine*, vol. 192, no. 2, pp. 295–302, 2000.

[42] T. Takahashi, T. Tagami, S. Yamazaki, et al., "Immunologic self-tolerance maintained by CD25+CD4+ regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4," *Journal of Experimental Medicine*, vol. 192, no. 2, pp. 303–310, 2000.

[43] S. F. Ziegler, "FOXP3: of mice and men," *Annual Review of Immunology*, vol. 24, pp. 209–226, 2006.

[44] D. A. Horwitz, S. G. Zheng, and J. D. Gray, "Natural and TGF-β-induced FoxP3+CD4+CD25+ regulatory T cells are not mirror images of each other," *Trends in Immunology*, vol. 29, no. 9, pp. 429–435, 2008.

[45] R. S. McHugh, M. J. Whitters, C. A. Piccirillo, et al., "CD4+CD25+ immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor," *Immunity*, vol. 16, no. 2, pp. 311–323, 2002.

[46] J. Shimizu, S. Yamazaki, T. Takahashi, Y. Ishida, and S. Sakaguchi, "Stimulation of CD25+CD4+ regulatory T cells through GITR breaks immunological self-tolerance," *Nature Immunology*, vol. 3, no. 2, pp. 135–142, 2002.

[47] C.-T. Huang, C. J. Workman, D. Flies, et al., "Role of LAG-3 in regulatory T cells," *Immunity*, vol. 21, no. 4, pp. 503–513, 2004.

[48] B. Liang, C. Workman, J. Lee, et al., "Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II," *Journal of Immunology*, vol. 180, no. 9, pp. 5916–5926, 2008.

[49] R. S. Bystry, V. Aluvihare, K. A. Welch, M. Kallikourdis, and A. G. Betz, "B cells and professional APCs recruit regulatory T cells via CCL4," *Nature Immunology*, vol. 2, no. 12, pp. 1126–1132, 2001.

[50] A. Y. Rudensky and D. J. Campbell, "In vivo sites and cellular mechanisms of T reg cell-mediated suppression," *Journal of Experimental Medicine*, vol. 203, no. 3, pp. 489–492, 2006.

[51] P. Pandiyan, L. Zheng, S. Ishihara, J. Reed, and M. J. Lenardo, "CD4+CD25+FoxP3+ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4+ T cells," *Nature Immunology*, vol. 8, no. 12, pp. 1353–1362, 2007.

[52] A. Giovannetti, M. Pierdominici, A. Di Iorio, et al., "Apoptosis in the homeostasis of the immune system and in human immune mediated diseases," *Current Pharmaceutical Design*, vol. 14, no. 3, pp. 253–268, 2008.

[53] W. J. Grossman, J. W. Verbsky, W. Barchet, M. Colonna, J. P. Atkinson, and T. J. Ley, "Human T regulatory cells can use the perforin pathway to cause autologous target cell death," *Immunity*, vol. 21, no. 4, pp. 589–601, 2004.

[54] X. Cao, S. F. Cai, T. A. Fehniger, et al., "Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance," *Immunity*, vol. 27, no. 4, pp. 635–646, 2007.

[55] M. Miyara and S. Sakaguchi, "Natural regulatory T cells: mechanisms of suppression," *Trends in Molecular Medicine*, vol. 13, no. 3, pp. 108–116, 2007.

[56] E. M. Shevach, "Mechanisms of FoxP3+ T regulatory cell-mediated suppression," *Immunity*, vol. 30, no. 5, pp. 636–645, 2009.

[57] L. W. Collison, C. J. Workman, T. T. Kuo, et al., "The inhibitory cytokine IL-35 contributes to regulatory T-cell function," *Nature*, vol. 450, no. 7169, pp. 566–569, 2007.

[58] A. K. Abbas, J. Lohr, B. Knoechel, and V. Nagabhushanam, "T cell tolerance and autoimmunity," *Autoimmunity Reviews*, vol. 3, no. 7-8, pp. 471–475, 2004.

[59] F. Sallusto and A. Lanzavecchia, "Human Th17 cells in infection and autoimmunity," *Microbes and Infection*, vol. 11, no. 5, pp. 620–624, 2009.

[60] C. Dong, "Differentiation and function of pro-inflammatory Th17 cells," *Microbes and Infection*, vol. 11, no. 5, pp. 584–588, 2009.

[61] K. S. Voo, Y.-H. Wang, F. R. Santori, et al., "Identification of IL-17-producing FOXP3+ regulatory T cells in humans," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 12, pp. 4793–4798, 2009.

[62] L. Zhou and D. R. Littman, "Transcriptional regulatory networks in Th17 cell differentiation," *Current Opinion in Immunology*, vol. 21, no. 2, pp. 146–152, 2009.

[63] A. Awasthi and V. K. Kuchroo, "Th17 cells: from precursors to players in inflammation and infection," *International Immunology*, vol. 21, no. 5, pp. 489–498, 2009.

[64] L. Cosmi, R. De Palma, V. Santarlasci, et al., "Human interleukin 17-producing cells originate from a CD161⁺CD4⁺ T cell precursor," *Journal of Experimental Medicine*, vol. 205, no. 8, pp. 1903–1916, 2008.

[65] P. Miossec, "IL-17 and Th17 cells in human inflammatory diseases," *Microbes and Infection*, vol. 11, no. 5, pp. 625–630, 2009.

[66] H. J. P. M. Koenen, R. L. Smeets, P. M. Vink, E. van Rijssen, A. M. H. Boots, and I. Joosten, "Human CD25^{high}FoxP3^{pos} regulatory T cells differentiate into IL-17 producing cells," *Blood*, vol. 112, no. 6, pp. 2340–2352, 2008.

[67] J. L. Langowski, X. Zhang, L. Wu, et al., "IL-23 promotes tumour incidence and growth," *Nature*, vol. 442, no. 7101, pp. 461–465, 2006.

[68] Y. K. Lee, R. Mukasa, R. D. Hatton, and C. T. Weaver, "Developmental plasticity of Th17 and Treg cells," *Current Opinion in Immunology*, vol. 21, no. 3, pp. 274–280, 2009.

[69] L. Zhou, J. E. Lopes, M. M. W. Chong, et al., "TGF-β-induced FoxP3 inhibits TH17 cell differentiation by antagonizing RORγt function," *Nature*, vol. 453, no. 7192, pp. 236–240, 2008.

[70] D. Mucida, Y. Park, G. Kim, et al., "Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid," *Science*, vol. 317, no. 5835, pp. 256–260, 2007.

[71] T. Sasada, M. Kimura, Y. Yoshida, M. Kanai, and A. Takabayashi, "CD4⁺CD25⁺ regulatory T cells in patients with gastrointestinal malignancies: possible involvement of regulatory T cells in disease progression," *Cancer*, vol. 98, no. 5, pp. 1089–1099, 2003.

[72] F. Pandolfi, G. Corte, and I. Quinti, "Defect of T helper lymphocytes, as identified by the 5/9 monoclonal antibody, in patients with common variable hypogammaglobulinaemia," *Clinical and Experimental Immunology*, vol. 51, no. 3, pp. 470–474, 1983.

[73] W. T. Bennett, F. Pandolfi, B. H. Grove, et al., "Dominant rearrangements among human tumor-infiltrating lymphocytes: analysis of T-cells derived from 32 patients with melanoma, lung, and renal cell carcinoma," *Cancer*, vol. 69, no. 9, pp. 2379–2384, 1992.

[74] M. Hishii, D. Andrews, L. A. Boyle, et al., "In vivo accumulation of the same anti-melanoma T cell clone in two different metastatic sites," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 4, pp. 1378–1383, 1997.

[75] K. Kono, H. Kawaida, A. Takahashi, et al., "CD4⁺CD25⁺high regulatory T cells increase with tumor stage in patients with gastric and esophageal cancers," *Cancer Immunology, Immunotherapy*, vol. 55, no. 9, pp. 1064–1071, 2006.

[76] Y. Mizukami, K. Kono, Y. Kawaguchi, et al., "Localisation pattern of FoxP3⁺ regulatory T cells is associated with clinical behaviour in gastric cancer," *British Journal of Cancer*, vol. 98, no. 1, pp. 148–153, 2008.

[77] U. K. Liyanage, T. T. Moore, H.-G. Joo, et al., "Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma," *Journal of Immunology*, vol. 169, no. 5, pp. 2756–2761, 2002.

[78] L. Ormandy, T. Hillemann, H. Wedemeyer, M. P. Manns, T. F. Greten, and F. Korangy, "Increased populations of regulatory T cells in peripheral blood of patients with hepatocellular carcinoma," *Cancer Research*, vol. 65, no. 6, pp. 2457–2464, 2005.

[79] B. Zhang, G. Rong, H. Wei, et al., "The prevalence of Th17 cells in patients with gastric cancer," *Biochemical and Biophysical Research Communications*, vol. 374, no. 3, pp. 533–537, 2008.

[80] J.-P. Zhang, J. Yan, J. Xu, et al., "Increased intratumoral IL-17-producing cells correlate with poor survival in hepatocellular carcinoma patients," *Journal of Hepatology*, vol. 50, no. 5, pp. 980–989, 2009.

[81] I. Kryczek, M. Banerjee, P. Cheng, et al., "Phenotype, distribution, generation, and functional and clinical relevance of Th17 cells in the human tumor environments," *Blood*, vol. 114, no. 6, pp. 1141–1149, 2009.

[82] F. Ichihara, K. Kono, A. Takahashi, H. Kawaida, H. Sugai, and H. Fujii, "Increased populations of regulatory T cells in peripheral blood and tumor-infiltrating lymphocytes in patients with gastric and esophageal cancers," *Clinical Cancer Research*, vol. 9, no. 12, pp. 4404–4408, 2003.

[83] N. Hiraoka, K. Onozato, T. Kosuge, and S. Hirohashi, "Prevalence of FOXP3⁺ regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its premalignant lesions," *Clinical Cancer Research*, vol. 12, no. 18, pp. 5423–5434, 2006.

[84] S. Hinz, L. Pagerols-Raluy, H.-H. Oberg, et al., "FoxP3 expression in pancreatic carcinoma cells as a novel mechanism of immune evasion in cancer," *Cancer Research*, vol. 67, no. 17, pp. 8344–8350, 2007.

[85] V. Chew, C. Tow, M. Teo, et al., "Inflammatory tumor microenvironment is associated with superior survival in hepatocellular carcinoma patients," *Journal of Hepatology*. In press.

[86] J. A. Pons, B. Revilla-Nuin, A. Baroja-Mazo, et al., "FoxP3 in peripheral blood is associated with operational tolerance in liver transplant patients during immunosuppression withdrawal," *Transplantation*, vol. 86, no. 10, pp. 1370–1378, 2008.

[87] K. J. Wood and S. Sakaguchi, "Regulatory T cells in transplantation tolerance," *Nature Reviews Immunology*, vol. 3, no. 3, pp. 199–210, 2003.

[88] G. Frisullo, V. Nociti, R. Iorio, et al., "Increased CD4⁺CD25⁺FoxP3⁺ T cells in peripheral blood of celiac disease patients: correlation with dietary treatment," *Human Immunology*, vol. 70, no. 6, pp. 430–435, 2009.

[89] T. Kanai, Y. Nemoto, N. Kamada, et al., "Homeostatic (IL-7) and effector (IL-17) cytokines as distinct but complementary target for an optimal therapeutic strategy in inflammatory bowel disease," *Current Opinion in Gastroenterology*, vol. 25, no. 4, pp. 306–313, 2009.

[90] P. Felaco, M. L. Castellani, M. A. De Lutiis, et al., "IL-32: a newly-discovered proinflammatory cytokine," *Journal of Biological Regulators & Homeostatic Agents*, vol. 23, no. 3, pp. 141–147, 2009.

[91] S. Brand, "Crohn's disease: Th1, Th17 or both? The change of a paradigmml: new immunological and genetic insights implicate Th17 cells in the pathogenesis of Crohn's disease," *Gut*, vol. 58, no. 8, pp. 1152–1167, 2009.

[92] J. Dambacher, F. Beigel, K. Zitzmann, et al., "The role of the novel Th17 cytokine IL-26 in intestinal inflammation," *Gut*, vol. 58, no. 9, pp. 1207–1217, 2009.

[93] J. Pene, S. Chevalier, L. Preisser, et al., "Chronically inflamed human tissues are infiltrated by highly differentiated Th17 lymphocytes," *Journal of Immunology*, vol. 180, no. 11, pp. 7423–7430, 2008.

[94] A. Bai, N. Lu, Y. Guo, Z. Liu, J. Chen, and Z. Peng, "All-trans retinoic acid down-regulates inflammatory responses by shifting the Treg/Th17 profile in human ulcerative and murine colitis," *Journal of Leukocyte Biology*, vol. 86, no. 4, pp. 959–969, 2009.

[95] K. M. Elias, A. Laurence, T. S. Davidson, et al., "Retinoic acid inhibits Th17 polarization and enhances FoxP3 expression through a Stat-3/Stat-5 independent signaling pathway," *Blood*, vol. 111, no. 3, pp. 1013–1020, 2008.

[96] M. J. Benson, K. Pino-Lagos, M. Rosemblatt, and R. J. Noelle, "All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation," *Journal of Experimental Medicine*, vol. 204, no. 8, pp. 1765–1774, 2007.

[97] W. O'Connor Jr., M. Kamanaka, C. J. Booth, et al., "A protective function for interleukin 17A in T cell-mediated intestinal inflammation," *Nature Immunology*, vol. 10, no. 6, pp. 603–609, 2009.

[98] S. Ardizzone, A. Cassinotti, D. Trabattoni, et al., "Immunomodulatory effects of 1,25-dihydroxyvitamin D3 on T_{H1}/T_{H2} cytokines in inflammatory bowel disease: an in vitro study," *International Journal of Immunopathology and Pharmacology*, vol. 22, no. 1, pp. 63–71, 2009.

[99] J. H. Cho, "The genetics and immunopathogenesis of inflammatory bowel disease," *Nature Reviews Immunology*, vol. 8, no. 6, pp. 458–466, 2008.

[100] W. Strober, I. Fuss, and P. Mannon, "The fundamental basis of inflammatory bowel disease," *Journal of Clinical Investigation*, vol. 117, no. 3, pp. 514–521, 2007.

[101] M. E. Himmel, G. Hardenberg, C. A. Piccirillo, T. S. Steiner, and M. K. Levings, "The role of T-regulatory cells and Toll-like receptors in the pathogenesis of human inflammatory bowel disease," *Immunology*, vol. 125, no. 2, pp. 145–153, 2008.

[102] N. N. Kristensen, J. Olsen, M. Gad, and M. H. Claesson, "Genome-wide expression profiling during protection from colitis by regulatory T cells," *Inflammatory Bowel Diseases*, vol. 14, no. 1, pp. 75–87, 2008.

[103] P. Vieira and A. O'Garra, "Regula'ten' the gut," *Nature Immunology*, vol. 8, no. 9, pp. 905–907, 2007.

[104] K. J. Maloy, L. Salaun, R. Cahill, G. Dougan, N. J. Saunders, and F. Powrie, "$CD4^+CD25^+$ TR cells suppress innate immune pathology through cytokine-dependent mechanisms," *Journal of Experimental Medicine*, vol. 197, no. 1, pp. 111–119, 2003.

[105] J. Buning, N. Homann, D. von Smolinski, et al., "Helminths as governors of inflammatory bowel disease," *Gut*, vol. 57, no. 8, pp. 1182–1183, 2008.

[106] M. Saruta, Q. T. Yu, P. R. Fleshner, et al., "Characterization of $FOXP3^+CD4^+$ regulatory T cells in Crohn's disease," *Clinical Immunology*, vol. 125, no. 3, pp. 281–290, 2007.

[107] J. Maul, C. Loddenkemper, P. Mundt, et al., "Peripheral and intestinal regulatory $CD4^+CD25^+$high T cells in inflammatory bowel disease," *Gastroenterology*, vol. 128, no. 7, pp. 1868–1878, 2005.

[108] S. Makita, T. Kanai, S. Oshima, et al., "$CD4^+CD25^+$bright T cells in human intestinal lamina propria as regulatory cells," *Journal of Immunology*, vol. 173, no. 5, pp. 3119–3130, 2004.

[109] V. Muratov, A.-K. Ulfgren, M. Engstrom, et al., "Decreased numbers of FoxP3-positive and TLR-2-positive cells in intestinal mucosa are associated with improvement in patients with active inflammatory bowel disease following selective leukocyte apheresis," *Journal of Gastroenterology*, vol. 43, no. 4, pp. 277–282, 2008.

[110] K. Kamikozuru, K. Fukunaga, S. Hirota, et al., "The expression profile of functional regulatory T cells, $CD4^+CD25^+$high+/forkhead box protein P3$^+$, in patients with ulcerative colitis during active and quiescent disease," *Clinical and Experimental Immunology*, vol. 156, no. 2, pp. 320–327, 2009.

[111] Q. T. Yu, M. Saruta, A. Avanesyan, P. R. Fleshner, A. H. Banham, and K. A. Papadakis, "Expression and functional characterization of $FOXP3^+CD4^+$ regulatory T cells in ulcerative colitis," *Inflammatory Bowel Diseases*, vol. 13, no. 2, pp. 191–199, 2007.

[112] M. A. Gavin, T. R. Torgerson, E. Houston, et al., "Single-cell analysis of normal and FOXP3-mutant human T cells: FOXP3 expression without regulatory T cell development," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 17, pp. 6659–6664, 2006.

[113] D. Q. Tran, H. Ramsey, and E. M. Shevach, "Induction of FOXP3 expression in naive human $CD4^+FOXP3$ T cells by T-cell receptor stimulation is transforming growth factor-β-dependent but does not confer a regulatory phenotype," *Blood*, vol. 110, no. 8, pp. 2983–2990, 2007.

[114] R. H. Duerr, K. D. Taylor, S. R. Brant, et al., "A genome-wide association study identifies IL23R as an inflammatory bowel disease gene," *Science*, vol. 314, no. 5804, pp. 1461–1463, 2006.

[115] S. Hue, P. Ahern, S. Buonocore, et al., "Interleukin-23 drives innate and T cell-mediated intestinal inflammation," *Journal of Experimental Medicine*, vol. 203, no. 11, pp. 2473–2483, 2006.

[116] C. O. Elson, Y. Cong, C. T. Weaver, et al., "Monoclonal anti-interleukin 23 reverses active colitis in a T cell-mediated model in mice," *Gastroenterology*, vol. 132, no. 7, pp. 2359–2370, 2007.

[117] E. K. Boden and S. B. Snapper, "Regulatory T cells in inflammatory bowel disease," *Current Opinion in Gastroenterology*, vol. 24, no. 6, pp. 733–741, 2008.

[118] I. Ricciardelli, K. J. Lindley, M. Londei, and S. Quaratino, "Anti tumour necrosis-α therapy increases the number of $FOXP3^+$ regulatory T cells in children affected by Crohn's disease," *Immunology*, vol. 125, no. 2, pp. 178–183, 2008.

[119] H. H. Smits, A. Engering, D. van der Kleij, et al., "Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin," *Journal of Allergy and Clinical Immunology*, vol. 115, no. 6, pp. 1260–1267, 2005.

[120] L. A. Zenewicz, A. Antov, and R. A. Flavell, "CD4 T-cell differentiation and inflammatory bowel disease," *Trends in Molecular Medicine*, vol. 15, no. 5, pp. 199–207, 2009.

[121] A. Mizoguchi and E. Mizoguchi, "Inflammatory bowel disease, past, present and future: lessons from animal models," *Journal of Gastroenterology*, vol. 43, no. 1, pp. 1–17, 2008.

[122] K. J. Maloy, "The Interleukin-23/Interleukin-17 axis in intestinal inflammation," *Journal of Internal Medicine*, vol. 263, no. 6, pp. 584–590, 2008.

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