

Review article

T cell-mediated immunoregulation in the gastrointestinal tract

In the intestinal tract, only a single layer of epithelial cells separates innate and adaptive immune effector cells from a vast amount of antigens. Here, the immune system faces a considerable challenge in tolerating commensal flora and dietary antigens while preventing the dissemination of potential pathogens. Failure to tightly control immune reactions may result in detrimental inflammation. In this respect, 'conventional' regulatory CD4⁺ T cells, including naturally occurring and adaptive CD4⁺ CD25⁺ Foxp3⁺ T cells, Th3 and Tr1 cells, have recently been the focus of considerable attention. However, regulatory mechanisms in the intestinal mucosa are highly complex, including adaptations of nonhaematopoietic cells and innate immune cells as well as the presence of unconventional T cells with regulatory properties such as resident TCRγδ or TCRαβ CD8⁺ intraepithelial lymphocytes. This review aims to summarize the currently available knowledge on conventional and unconventional regulatory T cell subsets (Tregs), with special emphasis on clinical data and the potential role or malfunctioning of Tregs in four major human gastrointestinal diseases, i.e. inflammatory bowel diseases, coeliac disease, food allergy and colorectal cancer. We conclude that the clinical data confirms some but not all of the findings derived from experimental animal models.

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There is compelling evidence that regulatory T cells (Tregs) play a fundamental role in controlling immune responses, and our knowledge on established and potentially novel subsets of Tregs and their immunoregulatory mechanisms is still expanding rapidly. While experimental models and an increasing amount of clinical data imply an important function for Tregs in multiple organs and many settings of disease, including autoimmune diseases, transplantation and – from a different perspective – cancer, maintenance of local tissue homeostasis may be of particular importance in the intestinal mucosa. Here, only a single layer of epithelium separates the inner from the outer, antigen-rich environment and the immune system must be able to effectively discriminate pathogens from dietary antigens and commensal flora. Failure to establish or maintain immunological tolerance against innocuous intestinal antigens that have breached the epithelial barrier may result in local and often also in systemic inflammation with potential deleterious consequences. It is therefore hardly surprising that at this particular site, a variety of distinct T cell subsets with regulatory functions have been identified. This review will first discuss the potential immunoregulatory functions of unconventional, mostly CD8⁺ T cells resident in the intestinal mucosa. A second chapter will deal separately with the currently known conventional subsets of CD4⁺ Tregs that are not necessarily restricted to the intestinal mucosa but are most certainly involved in immune regulation at this particular site. Finally, as a wealth of publications have

addressed the role and mechanisms of Tregs in intestinal inflammation in experimental animal models, yet limited information is available with respect to human disease, this review specifically aims to summarize our currently available knowledge on the function of human conventional and unconventional Tregs under pathologic conditions in four selected major gastrointestinal diseases, i.e. inflammatory bowel diseases (IBD), coeliac disease, food allergy and colorectal cancer (CRC). A last chapter will then try to critically discuss novel Treg-based therapeutic approaches and the main associated problems with respect to treatment of human disease.

Unconventional T cell subsets with assigned regulatory functions

Intestinal T cells reside in two distinct compartments, i.e. the lamina propria (including solitary follicles and Peyer's patches) and the epithelium where they are present in the intraepithelial space in a basolateral position. These intraepithelial lymphocytes (IEL) are likely involved in the local immunosurveillance and in the human small intestine, on average, 10–20 IEL are present per 100 enterocytes. IEL subsets differ in their phenotypic and functional properties from T cells in the systemic immune system. Whereas almost all circulating CD8 T cells and the CD8 T cells in secondary lymphoid organs express a heterodimeric CD8 molecule composed of an α and β

chain, the IEL populations are more complex. In mice, about 50% of the TCR $\alpha\beta$ IEL express a CD8 $\alpha\alpha$ homodimer on their cell surface whereas most of the remaining TCR $\alpha\beta$ IEL express a CD8 $\alpha\beta$ heterodimer. A substantial portion of CD8 $\alpha\alpha$ TCR $\alpha\beta$ IEL seem to be autoreactive and are functionally more related to TCR $\gamma\delta$ T cells rather than TCR $\alpha\beta$ T cells (1). In addition to classical MHC class I molecule-restricted CD8 $\alpha\alpha$ TCR $\alpha\beta$ IEL, some of these unconventional CD8 T cells seem to be nonclassical MHC class I, and even class II-restricted (2).

Our knowledge of IEL function in the human intestine is still very limited. Cytotoxic activity of CD8 intraepithelial T cells has been described in some reports (3, 4) but not by others (5, 6). Upon antigen-specific activation in peripheral lymphoid organs, some CD4 and CD8 TCR $\alpha\beta$ T cells migrate to the intestinal wall and transiently accumulate in the intraepithelial compartment (7–9).

In contrast to the CD8 $\alpha\beta$ TCR $\alpha\beta$ T cells, mouse CD8 $\alpha\alpha$ TCR $\alpha\beta$ IEL, even after potent Ag-specific activation *in vivo*, remain noncytotoxic and, intriguingly, appear to express the immunomodulatory cytokine TGF β (10). The adoptive transfer of a polyclonal population of CD8 $\alpha\alpha$ TCR $\alpha\beta$ IEL successfully prevented the subsequent induction of colitis in SCID recipients of colitogenic CD4 CD45RB^{hi} T cells (11). TCR $\gamma\delta$ T cells represent a substantial fraction of 'unconventional' T lymphocytes in the intestinal epithelium. In humans, approximately one-third of the resident IEL of the noninflamed large intestine are TCR $\gamma\delta$ T cells (12) expressing predominantly the TCR V δ 1 chain, whereas in the peripheral blood human TCR $\gamma\delta$ T cells preferentially display TCR V δ 2 (12). Intriguingly, an increased appearance of V δ 1-positive TCR $\gamma\delta$ T cells was noted in the peripheral blood of some patients with active IBD (13). The production of keratinocyte growth factor (KGF) by a subset of intraepithelial TCR $\gamma\delta$ T cells of the skin and the intestine suggests an involvement of TCR $\gamma\delta$ T cells in wound repair and immunosurveillance (14–16). In general, however, $\gamma\delta$ T cells represent a functionally

heterogeneous T cell population and evidence for both an inflammation exacerbating, and peripheral tolerance maintaining, role of intestinal TCR $\gamma\delta$ T cells has been reported (17, 18). In addition to the existence of resident T cell subsets with potential regulatory activity in the intestinal epithelium, the lamina propria further harbours 'unconventional' T cell subsets with possible regulatory functions. Accordingly, a human CD8⁺ T cell subset, which recognizes an epithelial-derived antigen (gp180) in a CD1d-dependent manner, exerts regulatory functions *in vitro* and was found to be reduced in patients with IBD (19). A role for epithelial-expressed CD1d was further described with respect to the α -galactosylceramide-mediated protection by NK T cells (NKT) cells in the dextran sodium sulphate model of colitis (20). A (incomplete) summary of these 'unconventional' T cells (as opposed to the classical MHC class II and MHC class I restricted CD4 and CD8 $\alpha\beta$ T cells, respectively) and their regulatory activities is provided in Table 1. Importantly, the lamina propria is also home to and site of action of various subsets of 'conventional' MHC class II-restricted CD4 T cells (summarized in Table 2). While some of these subsets were effectively discovered and characterized based on their functions in the intestinal mucosa (Tr1, Th3 cells), many studies have now investigated the phenotypical and functional characteristics of conventional Treg subsets in various tissues and settings of disease. The following chapter will therefore provide a general overview of our currently available knowledge on 'conventional' Treg subsets.

'Conventional' subsets of Tregs

Regulatory T cells of a CD4⁺ phenotype have been categorized into two main classes based on their ontogeny: naturally occurring Tregs (nTregs), which develop in the thymus and are present in mice and healthy humans from an early postnatal period, and Tregs which can arise in the periphery (or *in vitro*) under various experimental conditions (21). The latter can further be subdivided into

Table 1. 'Unconventional' T cell subsets with ascribed regulatory functions in intestinal tissue homeostasis

T cell subset	Main experimental findings	Suggested main suppressive mechanism
iNKT cells	Activation of NK1.1 ⁺ cells by α GalCer injection protects mice from DSS colitis (20)	ND; IL-10?
CD8 ⁺ LPL (CD103 ⁺ CD101 ⁺)	Expansion of CD8 ⁺ Treg from human PBMC by IEC in a CD1d and gp180-dependent manner; presence in LPL from healthy controls but not IBD patients (19, 140)	Cell-contact-dependent, perforin-independent (<i>in vitro</i>)
TCR $\alpha\beta$ CD8 $\alpha\alpha$ CD4 ⁺ IEL	Reconstitution of SCID recipient mice with self-specific TCR $\alpha\beta$ CD8 $\alpha\alpha$ IEL protects from CD45RB ^{high} -induced colitis (11)	IL-10
TCR $\alpha\beta$ CD8 $\alpha\alpha$ CD4 ⁺ IEL	Reconstitution of RAG ^{-/-} recipient mice with TCR $\alpha\beta$ CD8 $\alpha\alpha$ CD4 ⁺ IEL (recovered from recipients originally reconstituted with splenic CD4 T cells) protects from Th1-induced colitis (77)	IL-10
TCR $\gamma\delta$ IEL	Contribute to oral tolerance (18), produce KGF (14), promote epithelial turnover (15), prevent exaggerated inflammatory responses after parasitic infection (141), protective role in colitis (16), regulatory potential in human coeliac disease (81)	KGF-mediated epithelial repair (14, 16) TGF β (81)

iNKT cell, invariant NKT cell; ND, not determined; LPL, lamina propria lymphocytes; IEC, intestinal epithelial cell; IEL, intestinal intraepithelial lymphocyte; KGF, keratinocyte growth factor.

three putatively separate subsets of Th3 cells (22), Tr1 cells (23) and iTregs (as defined below). A very simplified overview of the four subsets is presented in Table 2. nTregs are characterized by their high expression of CD25 (CD4⁺ CD25^{+/high}) and co-expression of the forkhead box P3 transcription factor (Foxp3⁺) (24). These CD4⁺ CD25⁺ Foxp3⁺ T cells have been shown to suppress proliferation of other T cells *in vitro* and to protect from autoimmune and other inflammatory diseases (including colitis) in several animal models. Generally, nTregs are believed to suppress by a cell-cell contact-dependent manner *in vitro* (possibly by granzyme or perforin-mediated cytotoxicity or by cAMP), while their regulatory effects *in vivo* are assumed to be mainly cytokine-mediated (25). In this regard, the particular role of TGFβ remains highly controversial; while some investigators have described membrane-bound expression or secretion of TGFβ by nTregs (26), and TGFβ-neutralizing antibodies abrogated suppression both *in vitro* and *in vivo* (26, 27), others were able to isolate functionally suppressive nTregs from neonatal TGFβ-deficient mice (28). Additional mechanisms have also been postulated for nTregs, including the consumption of IL-2 (29), the expression of IL-35 (30) and indirect suppressive effects via CTLA-4 (31) or LAG3 (32) signalling and modulation of antigen presenting cells (APC) function. Mice carrying a loss-of-function mutation of Foxp3 (scurfy mice) completely lack nTregs and develop lethal autoimmune disease (33, 34), which identifies Foxp3 as a master regulator of Treg differentiation and function and nTregs as vital mediators in the control of immune homeostasis.

Functional CD4⁺ CD25⁺ FOXP3⁺ nTregs have also been isolated from humans (35). Here, mutations in FOXP3 are associated with an autoimmune syndrome termed IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) (36). However, unlike the Foxp3 mutant scurfy mice, human IPEX patients have a less severe and more heterogeneous phenotype depending on the site of the particular mutation in the FOXP3 gene (37). Moreover, IPEX patients do not necessarily present

with a complete lack of FOXP3⁺ nTregs but rather have nTregs with impaired suppressive functions (37).

Differences with respect to Foxp3 expression and function in mouse vs human CD4 T cells are further apparent for induced or adaptive CD4⁺ CD25⁺ Foxp3⁺ Tregs (iTregs). Thus, in mouse and human CD4⁺ CD25⁺ Foxp3⁺ T cells, Foxp3 expression can be induced *de novo* under certain experimental conditions. In mice, Foxp3-expressing iTregs with suppressive activity *in vitro* and *in vivo* can be generated *in vitro* in the presence of IL-2 and TGFβ (38, 39), a process which is further enhanced by the vitamin A derivative all-trans retinoic acid (RA) (40). Moreover, oral administration of antigen to mice lacking nTregs also induces Foxp3⁺ iTregs (41, 42), which is likely attributable to the TGFβ-rich environment of the intestinal tract and the unique capacity of intestinal DC to produce RA (43). In humans, the mere stimulation of CD4⁺ CD25⁺ T cells *in vitro* is sufficient to (transiently) induce FOXP3 expression in the absence of exogenous TGFβ. However, supplementation of cultures with IL-2 and/or TGFβ results in an increased conversion of FOXP3⁺ T cells (44, 45). While in mice, RA depends on TGFβ for induction of Foxp3 (40–42, 46), in human CD4 T cells, RA alone supposedly promotes FOXP3 expression (47). Intriguingly, these human RA-induced, but not TGFβ-induced FOXP3⁺ CD4 T cells, express granzyme A, indicative of two separate pathways of differentiation (47). The most significant aspect of human (TGFβ-induced) iTregs is that the induced FOXP3 expression, unlike in mouse iTregs, does not consistently confer a regulatory phenotype (48). These findings imply that in humans great care must be taken when identifying Tregs solely based on their CD4⁺ CD25⁺ FOXP3⁺ phenotype in the absence of functional assays. Thus, CD25 will also be present at elevated levels on activated T cells and FOXP3 may have been induced by locally increased concentrations of TGFβ. Concomitant use of additional markers such as absence of CD127 (49, 50) or high CTLA-4 (51) and MHCII (52) expression could provide valuable information although neither will guarantee an unequivocal

Table 2. 'Conventional' Treg subsets

T-cell subset	Differentiation/experimental setting	Suggested main suppressive mechanism	Ascribed function in intestinal tissue homeostasis
nTreg (CD4 ⁺ CD25 ⁺ Foxp3 ⁺)	Thymus-derived; <i>ex vivo</i> / <i>in vitro</i> suppression studies, <i>in vivo</i> mouse autoimmune models (142), scurfy (33) / IPEX (36)	<i>In vitro</i> : cell-cell contact-dependent: cytotoxicity, cAMP cytokine-dependent: 'IL-2 -sink', IL-35, TGFβ, IL-10 (partially controversial) <i>in vivo</i> : multiple modes (25)	Prevent and cure colitis in mice (121) Oral tolerance?
iTreg (CD4 ⁺ CD25 ⁺ Foxp3 ⁺)	<i>In vitro</i> induction in mouse and human CD4 ⁺ CD25 ⁺ (IL-2, TGFβ ^{+/−} RA dependent) (38–40, 44, 45, 47); <i>in vivo</i> induction by antigen feeding (41, 42)	TGFβ? suppressive activity for human iTreg controversial	Prevent colitis in mice (40) Oral tolerance?
Th3 (CD4 ⁺ TGFβ ⁺ Foxp3 ⁺)	<i>In vivo</i> induction by antigen feeding in rodents (22)	TGFβ induction of iTreg?	Oral tolerance (22)
Tr1 (CD4 ⁺ IL-10 ⁺ Foxp3 [−])	Induction <i>in vitro</i> and <i>in vivo</i> in the presence of IL-10 (23)	IL-10 (and TGFβ)	Prevent colitis in mice (56) Oral tolerance?

Although in this table nTreg, iTreg and Th3 are listed as distinct lineages, there may be a significant degree of overlap and inter-relationship. nTreg, naturally occurring Treg; iTreg, induced (or adaptive) Treg; RA, retinoic acid.

identification of true Tregs. Alternatively, when functional assays are not applicable, analysis of the (de)methylation status of the Foxp3 locus might yield important cues regarding the actual suppressive capacity of the cells (53). It is self-explanatory that in *ex vivo* analyses of either peripheral or tissue-derived CD4⁺ CD25⁺ Foxp3⁺ cells irrespective of additional markers used, no discrimination between nTregs or iTregs is currently possible.

To add up to the general confusion with respect to TGFβ-induced Tregs and the general role of TGFβ in Treg function, another subset of induced Tregs which can use TGFβ as a growth/differentiation factor and secretes high levels of this factor has already long been identified. These so-called Th3 cells could be cloned from mice after feeding of antigen and have traditionally been implicated as principal mediators of oral tolerance (22). It remains curious whether the above described CD4⁺ CD25⁺ Foxp3⁺ iTregs identified after oral administration of antigen and Th3 cells are actually the same Tregs. However, recent studies have postulated the existence of two separate lineages and suggest that through their TGFβ production, Th3 cells may maintain nTregs and also contribute to the local induction of iTregs via a process of infectious tolerance (54, 55). Such a notion could reconcile the contradictory findings regarding the role of TGFβ in Treg function *in vitro* vs *in vivo*.

Tr1 cells, which also belong to the adaptive type of Tregs, require IL-10 for their differentiation and function *in vitro* and *in vivo* and human Tr1 cells additionally depend on signals provided by immature APC (23). While no cell-surface specific markers are known for Tr1 cells, these cells are characterized by the constitutive absence of FOXP3 (48), low expression of CD25 (CD25^{low}) and a unique cytokine profile (IL-4⁻, IL-5⁺, IL-10⁺, TGFβ⁺) (23). Hence, Tr1 cells appear to represent a truly distinct lineage of Tregs. In mice, Tr1 cells were among the first types of Tregs described to protect from experimental colitis (56). In human recipients of an allogeneic bone marrow transplantation, the presence of CD4⁺ T cell clones secreting high amounts of IL-10 but not IL-4 correlated with the absence of graft-versus-host disease (GvHD) and long-term tolerance (57). Of note, the possibility to induce antigen-specific Tr1 Tregs by repetitive stimulation on a large scale *in vitro* (an antigen-specific expansion of the rare nTregs present in the circulation would be much more difficult to accomplish and TGFβ-induced iTregs lack sustained suppressive functions) offers interesting prospects for the use of Tr1 cells in adoptive cell therapy of human disease (58).

Immunoregulation in gastrointestinal diseases

Inflammatory bowel diseases

The importance of the functional adaptations of the local intestinal immune system to its unique environment is

best illustrated in those instances where these mechanisms fail e.g. by the development of chronic intestinal inflammation in various mouse strains deficient for genes involved in the control of immune responses (59). Accordingly, deregulated immune response(s) against an otherwise harmless luminal microflora in genetically susceptible individuals are generally recognized as key factors in the pathogenesis of IBD with the two main clinical entities, i.e. ulcerative colitis (UC) and Crohn's disease (CD). In patients with UC, only the mucosa of the large intestine is affected, while in patients with CD, the deleterious inflammatory process can occur anywhere in the gastrointestinal tract and all layers of the bowel wall may become affected.

Given the increasing number of mutant mouse strains that spontaneously develop a progressive, chronic intestinal inflammation, particularly in the colon and when kept under conventional conditions of maintenance, the underlying causes of such an inappropriate immune response in patients with IBD are likely to be highly diverse (60). In support of this notion, the recently identified susceptibility gene locus for CD, NOD2/CARD15, is mutated only in a subpopulation of patients with CD (61–63).

Until recently, the research interest on the role of immunopathological reactions in IBD mainly focused on adaptive immune reactions, e.g. pathogenic T cells, whereas the role of the innate immune system in IBD has received relatively little attention. However, over the past few years, several important advances were made expanding the knowledge on the role of the innate immune system, particularly macrophages and dendritic cells, in the intestinal mucosa and on their complex interactions with commensal microorganisms, which may also substantially affect the functional differentiation of local T cell subsets.

In mouse models of colitis, the preferential differentiation of reactive intestinal CD4 T cells either towards Th1 CD4 T cells (as exemplified in the CD4 T cell transfer model of colitis induction in immunodeficient mice), or towards Th2 T cells as observed in TCRα-deficient mice (64) may lead to fulminant inflammation of the large intestine under conventional conditions of maintenance. This situation is also seen in some patients with active CD with a predominance of IL-23 and IL-17 secreting cells at the site of the lesions, whereas in some patients with UC, IL-13 secreting T cells predominate (65).

In contrast to most T cell-mediated mouse models of colitis, where disease progression is often associated with a selective expansion of distinct CD4 T cell clones, T cell proliferation in the normal intestinal mucosa appears to be tightly regulated and T cells from normal intestinal mucosa display highly reduced proliferative activities upon TCR engagement (66). The observed attenuated CD4 T cell expansion and the protection from colitis induction upon co-transfer of CD4 CD45RB^{lo} T cells together with the otherwise colitogenic CD4 CD45RB^{hi} T cells in lymphopenic recipient mice indicated the presence of CD4 T cell with

regulatory activities (mostly CD4 CD25^{hi} Tregs) in the CD4 CD45RB^{lo} T cell subsets that is able to reconstitute a balanced local CD4 T cell reaction against intestinal lumen-derived antigens (67, 68).

The consequences of an impaired activity of resident regulatory cells is illustrated by the spontaneous development of progressive intestinal inflammation in mice deficient for IL-10 (69) or TGF- β (70) and upon adoptive transfer of CD4 CD45RB^{hi} T cells into immunodeficient mice (67, 68); because of the absence of Tregs in this CD4 T cell subset, transferred T cells expand rapidly in draining lymph nodes (DLN) and colonic mucosa in response to antigens derived from luminal bacteria. Transferred CD4 T cells subsequently induce an excessive activation and production of tumour necrosis factor (71). Combinations of gene defects in mouse models of colitis may result in intestinal inflammation that mimics more closely clinical and histopathological signs of human IBD. As an example, the combined deficiency of TGF- β -R2 and IL-10R2 leads to a fulminant onset of an ulcerative colitis-like disorder in mice (72).

Despite the evidence obtained in mouse models for a crucial involvement of aberrant Tregs functions in the induction of inflammatory disorders of the intestine as an aetiopathological factor, so far no direct indications for an impaired function of Tregs (Tr1, Th3) or a clear association with SNP in IL-10 and TGF β gene loci in patients with IBD have been reported. As a matter of fact, during active episodes of IBD, increased frequencies of IL-10 (73) TGF β (74) and FOXP3 (75) expressing cells were reported. In active IBD, the number of FOXP3 positive cells in the affected intestinal mucosa correlates with disease activity (Fig. 1). However, detailed functional analyses of these intestinal FOXP3 expressing iTregs will be required, including determining the extent of demethylation of CpG islands in the *Foxp3* gene locus (53). The presence of substantial numbers of Tregs not only in the normal colonic mucosa, but also in patients with UC and CD may thus offer an explanation for the relapsing-remitting course

of IBD, which is in sharp contrast to the fulminant colitis seen in those mouse strains where Tregs are absent.

A breakdown of local tolerance, i.e. loss of down-regulated T cell responses against the indigenous flora in the intestinal mucosa, which may initiate IBD, however, can be not only ascribed to impaired Tregs functions but may also result from alteration(s) in signalling pathways of pattern recognition receptors expressed on haematopoietic and nonhaematopoietic cells in the intestinal wall. As an example, mutations in the NOD2/CARD15 locus that represents a susceptibility locus for CD (61–63) may directly affect the signalling through Toll-like receptors (TLR). Therefore deregulation of microbe-associated molecular pattern – signalling is in some cases directly linked to chronic inflammation of the gut. Nevertheless, at present it cannot be excluded that in some patients with IBD, the timely induction of Tregs (e.g. via a RA and/or TGF β -dependent mechanism) (76), or an impaired recruitment of T cell subsets with regulatory functions during early stages of an inflammatory reaction, might contribute to the disease onset. Furthermore, impaired functional differentiation, activation, proliferation or recruitment of ‘unconventional’ T cell subsets with regulatory activities, such as TCR $\gamma\delta$ T cells or CD8 $\alpha\alpha$ TCR $\alpha\beta$ T cells, may also tip the balance towards excessive local immune reactions in some patients with IBD. The substantial contribution of unconventional T cell subsets with regulatory activities in maintaining gut homeostasis has been demonstrated in the mouse where the adoptive transfer of CD8 $\alpha\alpha$ TCR $\alpha\beta$ and of CD4 CD8 $\alpha\alpha$ TCR $\alpha\beta$ T cells attenuated, or even prevented from subsequent CD4 CD45RB^{hi} T cell induced colitis in lymphopenic recipient mice (11, 77).

Coeliac disease

Coeliac disease, also termed coeliac sprue or gluten-sensitive enteropathy, is a malabsorption syndrome that occurs in genetically susceptible individuals who are intolerant to dietary gluten. The disease is triggered by exposure to gliadin peptides, which are derived from protease-digested gluten and transported across the intestinal epithelial barrier to the lamina propria where they are deamidated by the enzyme tissue transglutaminase (tTG). In the small intestinal mucosa, tTG is preferentially expressed in the subepithelial area. These deamidated, gliadin-derived neoantigens are preferentially presented by HLA-DQ2 or HLA-DQ8 molecules to pathogenic CD4 T cells in the lamina propria (78).

Tissue sections of the affected small intestinal mucosa in florid coeliac disease show a hyperplasia of the crypt cells, a hypercellularity of the lamina propria with increased numbers of CD4 T cells and plasma cells, together with an atrophy of the villi leading to complete flattening of the mucosa. These morphological changes are typically reversible during a gluten-free diet (78).

A histopathological hallmark of florid coeliac disease is the increased frequency of IEL, in particular, TCR $\gamma\delta$

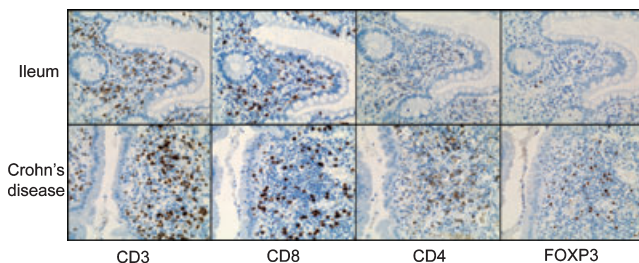


Figure 1. Number of FOXP3 expressing cells are increased in the affected lamina propria of patients with active inflammatory bowel diseases. Tissue sections from a non-inflamed ileum (upper row) and from the affected ileum (bottom row) of a patient with active Crohn's disease (CD) were stained for CD3, CD8, CD4 and FOXP3. The T cell-rich inflammatory infiltrate in the patient with CD contains numerous FOXP3 expressing T cells.

T cells, in the affected small intestinal segments. These intraepithelial TCR $\gamma\delta$ T cells are phenotypically, and possibly also functionally, heterogeneous. The interaction of the activating receptor NKG2D on a subset of small intestinal TCR $\gamma\delta$ T cells with the cognate NKG2D ligand on enterocytes, i.e. the class Ib molecules MICA/B, can induce cytotoxic effector functions in subsets of TCR $\gamma\delta$ T cells and CD8 TCR $\alpha\beta$ T cells, thus, contributing to the villous atrophy characteristically seen in patients with florid coeliac disease (79, 80). On the other hand, a regulatory role was very recently ascribed to CD8 $\alpha\alpha$ TCR $\gamma\delta$ IEL that express the inhibitory NKG2A receptor, which are more frequent in coeliac disease patients under gliadin-free diet. In co-cultures *in vitro*, these NKG2A positive TCR $\gamma\delta$ IEL inhibited the generation of cytotoxic TCR $\alpha\beta$ T cells (81). Hence, NKG2A positive TCR $\gamma\delta$ T cells in conjunction with gliadin-specific Tr1 CD4 T cells (82) may modulate locally the activity of the pathogenic CD4 T cells to maintain patients with CD in remission.

Food allergy

Considering the challenge that the immune system is facing at mucosal surfaces – discriminating a whole myriad of innocuous antigens against a handful of pathogens – the prevalence of food allergies is remarkably low and restricted to a few prominent allergens. The destruction of potentially immunogenic epitopes by gastric acids and luminal enzymes probably represents a mechanism efficient enough for ensuring immunological ignorance against dietary antigens. Nonetheless, it is commonly acknowledged that the immune system has evolved sophisticated mechanisms of so-called oral tolerance to actively induce and maintain tolerance against food antigens that pass the epithelial barrier in a nonsufficiently degraded form. It is also generally assumed that a failure to establish oral tolerance or a breakdown in pre-existing oral tolerance (or ignorance) provokes the induction of hypersensitivity reactions against food proteins.

While the concept of oral tolerance was extensively studied in mice (22), very limited evidence is available regarding the role of Tregs in oral tolerance in humans. Here, the existence of oral tolerance has also been implied by feeding experiments. Thus, volunteers who had received keyhole limpet hemocyanin (KLH) orally exhibited reduced KLH-specific proliferation upon subcutaneous immunization compared with controls who only received the immunization (83). Similarly, Zivny et al. observed reduced tetanus toxoid (TT)-specific proliferation of peripheral blood mononuclear cells (PBMC) from unmanipulated human volunteers when *in vitro* cultures besides TT additionally contained the food antigens ovalbumin and bovine gamma globulin (84). As the dietary antigen-mediated suppression could be reversed by the addition of exogenous IL-2, the authors concluded that anergy was the principal mediator of tolerance to food antigens (84). Circumstantial evidence for a role of Tregs in the control of

human oral tolerance, however, is provided by the presence of severe food allergy in a distinct familial form of IPEX syndrome (85). More direct support for an involvement of Tregs in tolerance against dietary antigens comes from a comprehensive study, which analysed the presence of circulating CD4⁺ CD25⁺ T cells in children that had either outgrown cow's milk allergy (termed tolerant) or remained allergic (86). Thus, tolerant children displayed increased frequencies of peripheral CD4⁺ CD25⁺ T cells and decreased *in vitro* proliferative responses against β -lactoglobulin but not PHA. Moreover, proliferation against β -lactoglobulin was augmented several-fold after depletion of CD25⁺ T cells from PBMC of tolerant children. Interestingly, these differences between allergic and tolerant children were not present when children were maintained on a cow's milk-free diet but only became apparent 1 week after re-introduction of cow's milk into their diet. This would indicate that during the long period of a milk-free diet, antigen-specific Tregs were residing in the gut-associated lymphoid tissues and only transiently entered the circulation upon the milk challenge. As the authors stated correctly, the study was not able to unequivocally discriminate CD4⁺ CD25⁺ Tregs from activated effector cells. FOXP3 was not analysed and for CTLA-4 expression, no differences were detected between the two groups of children. However, CD25⁺ cells from tolerant patients appeared to suppress in a cell-contact-dependent manner, yet a tendency for increased TGF β production after β -lactoglobulin stimulation was also noted. Circumstantial evidence for a potential role for TGF β was already put forward by two older publications, one describing reduced expression of TGF β by intestinal lymphocytes as well as intestinal epithelium of food allergic children (87), the other reporting the absence of TGF β (and IL-10) secretion by milk-specific compared with polyclonally-activated T cell lines from duodenal biopsies of allergic patients (88). Collectively, these studies suggest that similarly to what has been described in the mouse, Tregs and TGF β may participate in the control of oral tolerance also in humans.

Colorectal cancer

Colorectal cancer (CRC) represents the second leading cause of cancer-related mortality in developed countries. While genetic and environmental factors can predispose for CRC, increasing evidence also suggests a role for chronic inflammation in the pathogenesis (89). Thus, patients with IBD, particularly those with long-standing and extensive UC, carry an increased risk for CRC (90). Many neoplasms, however, are associated with persistent inflammatory conditions, indicative of a general link between chronic inflammation and potential development of tumours (91). Immune cells may contribute to tumour growth and progression by a complex interplay of a variety of mechanisms and mediators (91). For instance, the production of reactive oxygen and nitrogen species by

inflammatory leucocytes may cause or facilitate mutations in regenerating and proliferating epithelium. More importantly perhaps, an abundance of chemokines, cytokines and growth factors present at sites of inflammation and continuous tissue repair may promote tumour growth, angiogenesis and metastasis. Over time, chronic inflammation will certainly also induce compensatory anti-inflammatory mechanisms required to reduce collateral tissue damage. This is illustrated – at first sight seemingly paradoxical – by the increased frequency of functional colonic CD4⁺ CD25⁺ Tregs in patients with active UC compared with healthy controls (92). Although direct correlative evidence for increased numbers of Tregs in chronically inflamed tissues and a subsequent emergence of malignancies are lacking, it is tempting to speculate that local suppression in chronic inflammation may contribute to tumour growth by preventing effective early immunosurveillance.

An increasing wealth of studies indeed suggests a direct role for Tregs, if not in the initiation of tumour growth, at least in the prevention of immunity against established tumours (93). Thus, multiple mouse models of cancer, and to a lesser extent studies in human cancer patients, have demonstrated the presence of Tregs (mostly identified as CD4⁺ CD25⁺ and/or FOXP3⁺) in tumours and draining lymph nodes and an improvement of anti-tumour immunity after depleting Tregs or interfering with their suppressive function (93). Importantly, tumours themselves actively contribute to immunosuppression by a whole array of mechanisms (94). While it is beyond the scope of this review to discuss the complex interplay between tumour cells, tumour-associated APC, Tregs and T-effector cells into detail, some general aspects of tumour- and Treg-mediated immunosuppression are nonetheless briefly summarized below.

First, predominantly consisting of healthy 'self' and lacking costimulatory and danger signals, tumours are generally considered as poorly immunogenic and by failing to activate APC may 'passively' anergize effector T cells. More significantly, however, tumours seem to have evolved mechanisms to actively submerge and suppress potential anti-tumour immune responses (94). In human ovarian cancer, tumour cells and tumour-associated macrophages were shown to attract pre-existing Tregs to the tumour site by the secretion of CCL22 (95). There is also substantial evidence for a *de novo* conversion of T-effector cells into Tregs either at the tumour site or in local DLN by APC rendered tolerogenic by the tumour-environment (93, 94, 96). Interestingly, tumour cells and tumour-educated tolerogenic APC appear to share identical mechanisms for inducing Tregs. Both can secrete immunoregulatory cytokines such as TGF β and IL-10, and both have been shown to express IDO (indoleamine 2,3 dioxygenase) (93, 94, 96, 97). IDO catalyses the degradation of tryptophan, generating suppressive metabolites, which can anergize T cells or even induce the differentiation of new Tregs (97). By binding of CTLA-4 to B7-1 and B7-2, Tregs are capable

of triggering high levels of functional IDO expression in APC (31), which may account for the high IDO expression observed in some tumour-draining LN (97). Tregs are further able to convey suppressive activity to APC by inducing B7-H4 expression, which negatively regulates T-cell responses (98). In line with this, ovarian-tumour-associated macrophages, but not normal macrophages, were found to express B7-H4 (99). An additional positive feed-back loop for tumour-mediated suppression appears to be present with respect to the expression of the cyclooxygenase COX-2, a key enzyme in the biosynthesis of PGE₂. Thus, several tumour types overexpress COX-2 (100–102), and PGE₂ can either upregulate IDO expression in DC (103) or directly induce FOXP3 in human CD4⁺ CD25⁺ T cells (104). Of note, adaptively induced Tregs can themselves express COX-2 and suppress in a PGE₂-specific manner (105). Besides naturally occurring and induced CD4⁺ Tregs (which in most cases cannot be clearly discriminated by *in vivo/ex vivo* studies), CD8⁺ Tregs and suppressive type II NKT cells have also been implicated in tumour immune suppression (106, 107).

With respect to the role of Tregs in human CRC, fairly limited information is yet available. The presence of CD8⁺ T cells in cancer cell nests was found to positively correlate with patient survival (108). A recent comprehensive study, which analysed tumours from 415 patients, further described an improved clinical outcome for patients with high numbers of tumour-infiltrating CD3⁺ memory T cells, illustrating the importance of adaptive immunity in the control of CRC (109). Similarly, CRC patients with high intratumour infiltration of invariant (type I) V α 24 + NKT cells were reported to show higher overall survival rates (110). In agreement with studies of other human cancer types, several independent publications described increased frequencies of CD4⁺ CD25⁺ FOXP3⁺ T cells in PBMC, mesenteric lymph nodes and tumours of CRC patients compared with PBMC or colonic tissue from healthy controls (102, 111–114) or even IBD patients (113) (who in the particular study did not show augmented peripheral CD25⁺ T cells compared with normal individuals, suggesting that the increased frequency of Tregs in CRC is not simply a cause of chronic inflammation). Although – as discussed earlier – neither a CD25^{high} nor a FOXP3⁺ phenotype can be regarded as an unequivocal marker for Tregs, the putative Tregs appeared functional as tumour-infiltrating CD4⁺ CD25⁺ T cells suppressed their polyclonally-activated CD25⁺ counterparts *in vitro* or depletion of peripheral CD25⁺ T cells *in vitro* unmasked T cell responses to tumour-associated antigens while responses to the re-call antigen PPD (Purified Protein-Derivative) remained unaltered (111, 113, 114). The studies cited above did not systematically look at the mechanism of suppression by the identified Tregs. However, Yaqub et al. demonstrated that CD25⁺ peripheral Tregs from CRC patients suppressed carcino-embryonic antigen-specific proliferation in a COX-2 dependent manner, because inclusion of a COX-2 inhibitor

had a similar restorative effect on T cell responses as depletion of CD25⁺ Tregs (102). Importantly, in this study, CRC patients also showed elevated plasma PGE₂ compared with healthy controls. As tumour cells also expressed COX-2 at high levels, tumour cells, however, and not necessarily Tregs may have represented the main source of PGE₂ in patients (102). Nonetheless, these findings are in keeping with the beneficial effects of nonsteroidal anti-inflammatory drugs or COX-2 inhibitors, respectively, on disease progression in CRC patients (115). Sera from CRC patients were also identified to contain an increased concentration of ILT3 (inhibitory Ig-like transcript 3), which via induction of CD8⁺ Tregs inhibited proliferation in mixed leucocyte reactions (116). In agreement with observations from other tumour types, high IDO expression was further noted in CRC and was in fact associated with a significant reduction of CD3⁺ tumour-infiltrating T cells (117). Moreover, IDO-high immunoreactivity correlated with an increased frequency of liver metastases and a reduced survival rate (117). While in this study, IDO expression – as a potential marker of immunosuppression – could be employed as an independent prognostic variable (117), no correlation was found by others for an increased frequency of either tumour-infiltrating or peripheral Tregs and CRC patient survival (111, 112). Furthermore, controversial results were obtained with respect to clinical grading and the frequency of Tregs in CRC. Thus, while one study described slightly, but not significantly increased numbers of CD4⁺ CD25⁺ cells in either PBMC or tumour-infiltrating lymphocytes from Duke's C and D staged (compared to A and B staged) patients (114), another publication reported a significantly higher number of FOXP3⁺ Tregs in patients with limited disease compared to metastatic disease (112). The absence of any clear correlation in CRC contrasts with findings obtained from patients with gastric carcinoma or with ovarian and lung cancer, where an increased frequency of tumour-infiltrating Tregs or a decreased CD8/Treg ratio was evidently associated with a poorer prognosis (95, 111, 118, 119). Hence, despite the similarities regarding the overall increased frequencies of Tregs and the mechanisms of immunosuppression in different types of tumours, discrepancies appear to exist concerning the potential use of Tregs as prognostic disease markers. Hereby, the following caveats have to be taken into consideration: First, the extent of Treg infiltration may be the cause rather than the consequence of advanced disease. Secondly, restricting analyses to either PBMC or tumour-infiltrating cells does not take into account any potential migration patterns of Tregs between either compartment or to draining or nonDLN. Lastly, a CD4⁺ CD25⁺ or simply FOXP3⁺ phenotype cannot be taken as guaranteed marker for Tregs (48). Thus, while in CRC a potential role for Tregs in the pathogenesis and progression of disease may be implicated, more comprehensive studies that identify the phenotype and function of Tregs in all available compartments as closely as possible are clearly required.

Regulatory T cell-based therapeutic applications

Treg-based therapeutic approaches for the treatment of chronic inflammatory disorders and cancer obviously differ greatly in their aims – one situation appears to require the expansion of Treg numbers, or an enhancement of their regulatory functions, whereas the other condition calls for a depletion of Tregs or abrogation of their suppressive effects. Despite this apparent disparity, either approach is hampered by the same common difficulties. Thus, while a wealth of animal models has clearly demonstrated that Tregs prevent inflammatory disorders (including various forms of experimental colitis) and that depletion of Tregs has beneficial effects on anti-tumour immunity (including mouse models with established colorectal carcinoma tumours), many questions remain to be answered for a translation of the diverse experimental approaches to the clinic.

The major common obstacle with respect to a therapeutic use or targeting of human Tregs certainly is the current lack of markers that faithfully identify Tregs with *bona fide* regulatory functions (see also '*Conventional subsets of Tregs*'). To start with, both a missing or a false-positive identification of putative Tregs by *in situ* or *ex vivo* analyses, which are based on a phenotypical characterization only, may lead to a misconceived interpretation on a potential role of Tregs in a particular disease. While the currently available knowledge suggests that Tregs with suppressive functions are present in colorectal tumours and (paradoxically) in the lamina propria of patients with IBD, we currently do not know the true role of these cells in human disease. In addition, many conclusions from human studies have been based on the characterization of putative Tregs identified in the periphery, but not in the affected tissues. Secondly, even when assuming that the situation is identical to the one in rodents (i.e. that inflammatory conditions benefit from augmenting the functions of Tregs and anti-cancer immunity from abolishing their effects, respectively), the current lack of unequivocal human Treg markers does not allow a specific targeting *in vivo* (e.g. for a selective depletion of Tregs in cancer by drug-coupled mAbs). Similarly, the missing identification of true Tregs represents a severe drawback for a potential *ex vivo* expansion of Tregs (e.g. for adoptive cell therapy of chronic inflammatory disorders) as effector T cells could be co-expanded masked as CD25^{high} or even FOXP3⁺ cells. Another major difficulty is presented by the issue of (undefined) antigen-specificity and potential adverse effects of depleting or expanding bystander Tregs that are not involved in a particular disease. Accordingly, a general depletion or impairment of Tregs in cancer may lead to onset of autoimmunity, whereas a therapeutic unspecific expansion of Treg numbers or their functions in chronic inflammatory disorders could potentially lead to generalized immunosuppression with an increased risk for infections or even cancer.

For a Treg-based treatment of chronic inflammatory disorders, including allergies, autoimmune diseases and GvHD, two major strategies are currently being envisaged. One approach aims at an *ex vivo/in vitro* expansion or *de novo* induction of Tregs, which could be subsequently administered to patients as a cell-based immunotherapy. As discussed above, this approach is severely restricted by the low frequency and unknown antigen-specificity of nTregs, the overall difficult expansion of anergic Tregs and the risk of co-expanding putative Tregs without suppressive functions or potentially harmful effector T cells, when aiming at a *de novo* induction of human Tregs *in vitro*.

Possibly, the use of the immunosuppressive drug rapamycin, which appears to allow for expansion of Tregs while inhibiting the proliferation of effector T cells, may improve culture conditions for future Treg-based therapies to some extent (120). While the first clinical trials employing transfer of IL-10 anergized Tr1 cells for prevention of GvHD are ongoing (58), experts in the field are highly sceptical that cell-based immunotherapy will ever represent a major therapeutic strategy in the future. Too cumbersome is a large-scale culture of these cells and too uncertain their potential *in vivo* homing, durability, function and the overall benefit after adoptive transfer into humans with established disease. Of most importance perhaps, to date, only a handful of animal models have provided evidence that Tregs can not only prevent but also actually cure ongoing disease and some of these models required recognition of the specific antigen by the Tregs (121–123).

The second approach aims at restoring tolerance *in vivo* by repeated mucosal or parenteral administration of defined antigens. This so-called specific immunotherapy (which comprises very heterogeneous application procedures) has already been shown safe and effective in the treatment of a variety of allergic conditions and is believed to be based on the induction or expansion of antigen-specific Tregs, although in humans this assumption largely relies on circumstantial evidence. Oral and sublingual immunotherapy have also been applied to patients with food hypersensitivities with variable degrees of success as determined by parameters for desensitization such as lack of severe reactions upon specific food challenge, decrease in specific IgE or increase in specific IgG4, respectively (124). None of these studies has systematically looked at the presence and function of food-specific Tregs during the course of desensitization. Moreover, as – at least in children – allergic responses to the most common allergens milk and eggs are frequently outgrown naturally [by a process that likely also depends on the induction and presence of specific Tregs (86)], the actual beneficial effects of the desensitization in long-term studies using infants or children may have been masked. In food allergy, and also in coeliac disease, the responsible antigens are clearly identified and genetic/familial predispositions are often indicated, which would render these diseases seemingly ideal targets for a prophylactically applied specific immunotherapy. However, as established food allergy

and coeliac disease can be effectively kept in check by a controlled diet, the likely required life-long repeated administration of antigens and cumbersome clinical check-ups associated with specific immunotherapy may not make this a therapeutic approach of choice.

In human IBD, the situation for potential Treg-based therapies presents far more difficult despite an uncountable number of animal models demonstrating the importance of Tregs in preventing and ameliorating intestinal inflammation. As discussed above, the role of Tregs in human IBD remains unclear, especially as these cells do not appear to be impaired in their numbers and function in human patients and the frequency of IL-10 and TGF β -secreting cells is actually increased in the chronically inflamed mucosa (73, 74, 92). It is therefore not surprising that clinical trials with human recombinant IL-10 in CD patients have proven disappointing on the whole (125). In addition to the yet undefined role of Tregs in IBD, the precise antigens that drive inflammatory effector cells remain unknown, arguing further against a specific immunotherapy. A more promising approach may lie in the direct elimination of activated effector T cells. Accordingly, the administration of the humanized CD3-specific monoclonal antibody visilizumab to patients with steroid-refractory UC has shown potential efficacy despite adverse effects (126). As anti-CD3 treatment may in the long-term potentially also induce adaptive Tregs (127), the mechanism of visilizumab remains yet to be determined.

A similar discrepancy between experimental models and the clinical situation is apparent for CRC or cancer in general. Accordingly, while a variety of strategies targeting Tregs have been successfully applied to experimental tumour models, few of these approaches have been translated to the clinic with success. In various mouse tumour models, depleting or blocking mAbs against CD25, CTLA-4, GITR, or CCL22, as well as inhibitors of IDO, or ligands of TLR8 have all been shown to reduce tumour burden, probably by either depleting Tregs, interfering with their suppressive function or inhibiting tumour-directed migration, respectively (93, 96). In human renal- or ovarian carcinoma or melanoma patients, the administration of Ontak/Dinileukin difitox (a fusion protein between IL-2 and diphtheria toxin to selectively kill CD25-bearing T cells) induced a remission in some but not in all of the clinical trials (93, 96). Possibly, in the latter cases, Ontak eliminated not only Tregs but also activated effector T cells – a scenario, which could easily be envisaged. Similarly, ambiguous results were obtained from clinical trials employing anti-CTLA-4 mAbs (93). Hence, while two trials reported cancer regression in melanoma and ovarian carcinoma patients receiving combined cancer vaccination and anti-CTLA-4 mAbs (128, 129), a separate study showed that the positive effects could not be attributed to the depletion or inhibition of Tregs (130). Moreover, in the anti-CTLA-4 trials, some patients exhibited autoimmune manifestations (128).

Combined treatments encompassing both chemotherapy and immunotherapy (i.e. cancer vaccination in this context) are currently considered as the most promising strategy (131). Interestingly, the alkylating agent cyclophosphamide, which has long been used for the treatment of various forms of cancer, is now recognized to possibly act via impairment of Treg numbers or functions when administered at low doses, thereby facilitating vaccine-induced tumour rejection (132). However, although cyclophosphamide induced improved anti-tumour responses in various tumour models and in patients with end-stage metastatic melanoma (133–135), these effects were not seen in another clinical trial with carcinoma patients (136). In addition to these inconsistencies, several observations suggest that protocols concurrently employed to boost tumour vaccination may have unexpected adverse effects by expanding Tregs or augmenting their functions. Thus, although one clinical trial employing a combined regime of chemotherapy and recombinant IL-2 and GM-CSF in CRC patients reported a significant reduction in Tregs (137), the administration of recombinant IL-2 was shown to actually increase the frequency of FOXP3⁺ circulating Tregs in another study (138). Similarly, the use of CPG-ODN as adjuvants in cancer vaccines may, rather than fight, actually promote immunosuppression by the induction of IDO (139).

In summary, while similarities for Treg-mediated immunosuppression in various human tumours likely exist, there does not seem to be a Treg-based form of therapy, which is universally applicable. It appears that aiming at a general depletion of Tregs may be a too far-fetched approach and that a specific targeting of the suppressive mechanisms operative in a particular type of tumour may hold more promise with fewer side-effects for the patients. In this respect, the beneficial effects of nonsteroidal anti-inflammatory drugs in the treatment of CRC may indeed be partially in line with the reported COX-2-dependent suppressive activity of Tregs in CRC patients (102).

Concluding remarks

Over the past years, the characterization of human Tregs has made a tremendous progress and essentially

confirmed the existence of corresponding Treg subsets with very similar phenotypes and regulatory functions as described in the mouse. Accumulating clinical data also suggest an important role for Tregs in the maintenance of peripheral tolerance in humans, as perhaps most strikingly illustrated by the autoimmune manifestations in (IPEX) patients with a mutated form of FOXP3. Nonetheless, it appears that the situation in humans may be more complex than anticipated. For instance, FOXP3 expression by human T cells cannot necessarily be associated with stable suppressive functions, thus greatly impairing a conclusive analysis or a potential therapeutic targeting of these cells. Similarly, while animal models of intestinal inflammation suggest a clear cause in a defective immune regulation, in patients with IBD no substantial evidence for reduced numbers of Tregs or decreased expression of immunoregulatory cytokines is currently available.

Analysis of the potential pathogenetic factors in human gastrointestinal diseases may face particular hurdles because of the highly complex and likely redundant array of regulatory mechanisms present in the intestinal mucosa. Moreover, access to tissue specimens is limited and often only comprises samples of patients with fully active or even end-stage disease. To understand a potential malfunctioning of regulatory mechanisms and intervene therapeutically, however, it is crucial that we make the best possible use of these samples and not just restrict our analyses to the peripheral immune compartment. With the current lack of unequivocal markers for Tregs, investigators need to be aware of the limited informative content and potential misapprehension of strictly phenotypical analyses and include functional data wherever applicable. Alternatively, large-scale genetic analyses may provide valuable information and help to restrict functional investigations to the most relevant parameters. In this respect, the use of patient cohorts may prove invaluable for overcoming the limited power of small-scaled study groups.

Hopefully, the combined efforts of experimental and clinical studies as well as a long awaited novel exclusive marker for human Tregs will help to unravel the underlying molecular and cellular mechanisms for the distinct intestinal diseases and assist in the identification of the most adequate treatments.

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