IMMUNOPATHOGENESIS OF IBD: INSUFFICIENT SUPPRESSOR FUNCTION IN THE GUT?

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IMMUNE ACTIVATION AND SUPPRESSION IN INFLAMMATORY BOWEL DISEASES

The intestinal immune system is in a constant state of controlled inflammation, and there is substantial evidence that loss of control is an important pathogenic mechanism in inflammatory bowel diseases (IBD). A major current working hypothesis defines Crohn's disease as a dysregulated immune response towards components of the intestinal flora, leading to chronic intestinal inflammation.[1] The causes for this inappropriate response can be attributed to (a combination of) defects in the epithelial barrier, the innate immune response or the adaptive immune response.

Animal experiments as well as clinical data indicate that the immunopathogenesis of Crohn's disease and ulcerative colitis differ at the level of T cell differentiation and activation although the governing mechanisms responsible for these differences have been incompletely defined. In both diseases, activation of T cells is evident but pathogenic T cells in Crohn's disease predominantly produce interferon γ (membrane bound), tumour necrosis factor (TNF)α and interleukin (IL)-23 whereas ulcerative colitis is characterised by production of IL-5 and IL-13.[2 3]

The increased production of "Th1" type cytokines in Crohn's disease is probably related to increased activation of mucosal dendritic cells and macrophages, and the pivotal function of membrane associated (Toll-like receptor (TLR)) and intracellular (nucleotide oligomerisation domain (NOD) family) receptors in the activation of these antigen presenting cells (APC) has now been well established. Both receptors are key mediators of innate host defence, crucially involved in maintaining intestinal homeostasis.[4] In healthy subjects, the colonic mucosa harbours "non-inflammatory" dendritic cells, expressing low levels of TLR2 and TLR4 and producing cytokines such as IL-10, contributing to a non-inflammatory environment,[5 6] but in the mucosa of patients with Crohn's disease the production of IL-12 is greatly increased.[7–9] Dendritic cells in both ulcerative colitis and Crohn's disease have an activated phenotype with higher levels of the activation markers CD40 and CD86, and produce more IL-12 and IL-6 compared with controls.[5 10]

The causes for this excessive activation are presumably diverse and have been incompletely defined. A minority of patients with Crohn's disease have inactivating mutations within the susceptibility gene NOD2. The NOD2 protein is normally stimulated by its natural ligand muramyl dipeptide, a degradation product of bacterial peptidoglycan.[11–13] Some studies have shown increased activation of nuclear factor κB, which in antigen presenting cells causes increased transcription of IL-12. Indeed, dendritic cells from patients with Crohn's disease with NOD2 mutations produce increased amounts of IL-12 after stimulation with peptidoglycan, most likely via loss of inhibition of the simultaneously activated TLR2 pathway (Zelinkova *et al*, submitted). It should be noted that other studies have shown impaired activation of nuclear factor κB in patients with Crohn's disease with NOD2 mutations, suggesting decreased activation.[14 15] Hence it remains unclear what mechanism is responsible for the excessive Th1 profile in Crohn's disease and whether the underlying genetic defects lead to initial decreased immune activation with failure to clear pathogens, or whether these mutations directly increase activation of immune cells such as dendritic cells. Abnormal activation and expression of TLR receptors may also be linked to IBD: associations of TLR4 and TLR5 signalling with their bacterial ligands lipopolysaccharide (LPS) and flagellin, respectively, have been reported,[16 17] enhanced expression of TLR2 and TLR4 on dendritic cells has been observed[5] and recent studies suggest that Crohn's disease is also associated with TLR9 promoter polymorphisms.[18]

In addition, defective apoptosis of T cells has been suggested to play a role in the pathogenesis and chronic state of inflammation in Crohn's disease. Lamina propria T cells from patients with Crohn's disease were shown to be resistant to activation induced cell death whereas lamina propria T cells from healthy controls readily underwent apoptosis.[19 20] The latter would clarify the effective therapeutic action of anti-TNFα agents such as infliximab in Crohn's disease, as this reagent was shown to induce apoptosis through binding to membrane bound TNFα.[21]

Although the precise cellular and molecular pathways involved remain to be elucidated, these findings give solid and abundant evidence for increased stimulation or dysregulation of the innate immune system in IBD that, in the case of Crohn's disease, results in induction of hyperactive T cells which is probably necessary for the initiation of chronic mucosal inflammation.

Increased activation of the innate and adaptive mucosal immune systems is tightly controlled by various regulatory circuits, and it is possible that defects in such mechanisms that normally downregulate intestinal inflammation are insufficient in IBD.

This review discusses evidence for abnormal regulation of T cell activation in Crohn's disease, as well as data pertaining to the existence and functional activity of regulatory T cells (Treg) in the intestinal mucosa. We also consider the potential therapeutic application of Treg in IBD.

REGULATORY MECHANISMS IN THE GUT

The immune system controls activation of the innate as well as the adaptive arms through various means, primarily including induction of anergy, apoptosis of activated immune cell and the activities of regulatory $CD4^+$ T cells. In addition, several other regulatory mechanisms are operational in the gut mucosa, including CD8 T cells, γδ T cells and NKT cells, that are highly correlated with their surrounding epithelial cells, and IL-10 secreting B cells, immature dendritic cells and plasmacytoid dendritic cells.

Intraepithelial CD8 T lymphocytes, γδ T cells and NKT cells are mucosal T cell subsets with a restricted T cell receptor repertoire that are in close contact with mucosal epithelial cells. There is evidence that these interactions can lead to induction of T cells with regulatory capacities: for example, interactions between human intestinal epithelial cells and peripheral blood T cells cause expansion of $CD8^+CD28^-$ T cells with regulatory activity.[22] These cells are present in the lamina propria of healthy individuals but not in the lamina propria of patients with IBD,[23] suggesting that these cells prevent the pathogenesis of IBD. Intraepithelial lymphocytes have been reported to downregulate excessive inflammation caused by infection and autoimmunity in epithelial tissues and their protective ability has been shown in several murine models of colitis.[24–28]

Accumulating evidence implicates additional cell types in immunoregulation. Recent studies have revealed a protective role of IL-10 producing B cells in murine $CD4^+$ T cell colitis[29] and inhibition of antigen specific T cell proliferation by plasmacytoid dendritic cells. This dendritic cell population has a marked presence in mucosal tissue and is, in common with steady state lamina propria immature dendritic cells, able to induce a non-anergic state of T cell unresponsiveness that involves the differentiation of Treg[30 31]

These data indicate that mucosal immune activation is regulated at various levels by different cells that downregulate immune responses. A rapidly expanding body of evidence indicates that the most important among these regulatory cells reside within the $CD4^+$ T cell population, and these will be the further focus of this review.

REGULATORY $CD4^+$ T CELLS: PHENOTYPE, FUNCTION AND REGULATION

Once T cells are activated through engagement of the T cell receptor, they do not distinguish between "self" and "non-self". It is now clear that the human immune system regards antigens expressed by the normal gut flora as "self". Because activated T cells that recognise self-antigens induce significant tissue damage, it is important to either prevent their activation or control proliferation. It has long been known that most high

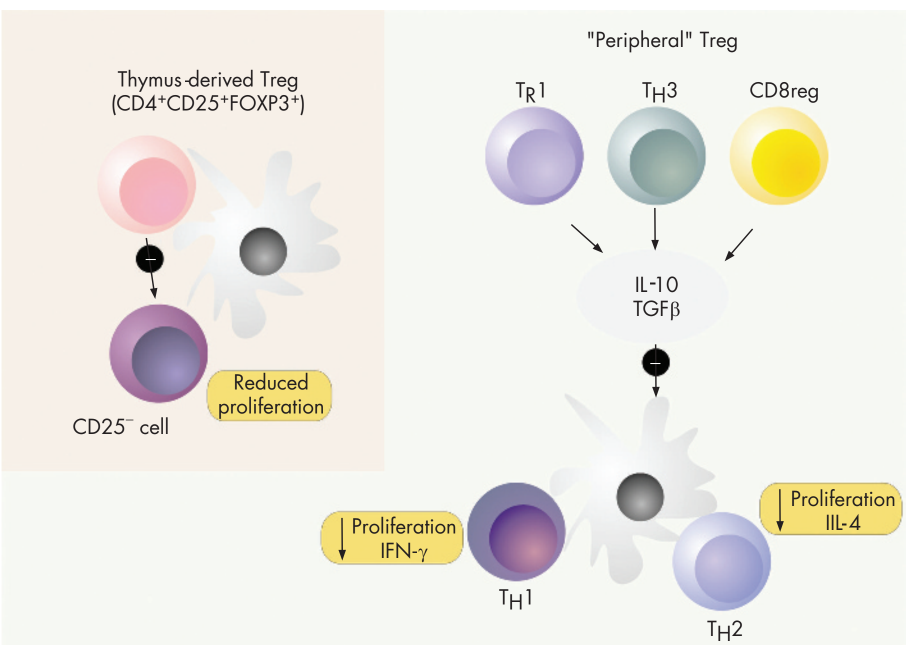

Figure 1 Regulatory T cells and their function. Several types of regulatory T cells (Treg) have been identified and the mechanisms of suppression may differ. Thymus derived regulatory T cells, also known as naturally occurring regulatory T cells, are a subset of $CD4^+CD25^+$ T cells and are thought to suppress activation of T cells at level of antigen presenting cell. Adaptive peripheral induced regulatory T cells include Tr1, Th3 and CD8. These cells produce the immunosuppressive cytokines interleukin (IL)-10 and/or transforming growth factor (TGF)β and function in a cytokine dependent manner. IFN-γ, interferon γ.

Table 1 Characteristics of the different regulatory T cell subsets

Feature	Naturally Treg	Adaptive Treg	
Subpopulations	CD4+CD25+	Tr1	Th3
Site of induction	Thymus	Periphery	
Mechanism of action	Cell–cell contact, cytokine independent	Cytokine dependent	
Characterisation	CD25+ and Foxp3+	IL-10	TGF-β
Specificity	Self-antigens in the thymus	Tissue specific antigens and foreign antigens	
Protection demonstrated	Transfer colitis, SCID	Transfer colitis, SCID	Neutralising TGF-β antibodies

IL, interleukin; TGF-β, transforming growth factor β; Treg, regulatory T cells.

affinity self-reactive T cells are clonally deleted within the thymus but this system is leaky and by itself insufficient to prevent autoreactivity. Hence prevention of autoreactivity is also continuously controlled outside the thymus, and this "peripheral tolerance" is critically dependent on the presence of Treg.

Although, in general, microbes mount strong immune responses, the resident gut flora is unable to activate T cells in healthy individuals. Thus there should be a mechanism by which potentially detrimental immune responses in the gut are prevented.

Regulatory $CD4^+$ T cells represent a population of lymphocytes with the ability to suppress both adaptive and innate immune responses (fig 1),[32–34] and these characteristics make them important for both maintenance of immunological tolerance and control of antimicrobial responses. Various types of Treg have been identified (table 1), but because specific phenotypic markers have long been lacking, it is uncertain to what extent these Treg constitute separate lineages. Nonetheless, Treg can be divided into two major groups, the so-called "naturally occurring" Treg and "adaptive" Treg, containing the so-called Tr1 and Th3 cells.[35]

"NATURALLY OCCURRING" REGULATORY T CELLS

Most $CD4^+$ T cells that recognise autoantigens in the thymus with high affinity are either clonally deleted or differentiate into a "naturally occurring" Treg. This cell is characterised by a unique phenotype and potent suppressive function towards autoreactive peripheral T cells. Thymus derived Treg constitute about 5–10% of mouse and 1–2% of human peripheral $CD4^+$ T cells. Initially, these cells were identified by their $CD4^+CD25^{high}$ phenotype but an increasing number of markers has been recently reported (table 2). Several membrane expressed molecular markers such as CD25 (IL-2 receptor α chain), glucocorticoid induced TNFR family related protein (GITR) and

Table 2 Cell surface and intracellular markers constitutively expressed by thymus derived natural regulatory T cells

Species	Cell surface	Intracellular
Murine	$CD25^+$, $CD122^+$, $CD69^+$, $CD44^+$, $CD45RB^{low}$, $GITR^+$, $CD103^+$(αE-integrin), $CD134^+$(OX-40), $CD54^+$(ICAM)	CTLA-4^+, *Foxp3*$^+$
Human	$CD25^{high}$, $CD122^+$, HLA-DR^+(50%), $CD45RO^+$(80%), $CD95^{high}$, $CD45RB^{low}$, $CD38^{low}$, partly $CD62^{low}$, $GITR^+$,$CD127^{low}$	CTLA-4^+, *FOXP3*$^+$

cytotoxic T lymphocyte associated antigen 4 (CTLA-4) are constitutively expressed on Treg but can also be observed on activated non-regulatory T cells, and it was not until the discovery of the *foxp3* gene (*FOXP3* in humans) that a unique marker for murine Treg was identified. Mutations in *Foxp3* result in severe autoimmune reactivity in both mice and humans, leading to, respectively, the scurfy or IPEX (immune dysregulation, polyendocrinopathy, enteropathy, x-linked) syndrome. The *foxp3* gene was identified as a master regulatory gene; it is constitutively and specifically expressed in natural Treg and plays an indispensable role in their development and function. Furthermore, forced expression of *foxp3* can convert naïve peripheral blood T cells to Treg cells.[36] The specificity of *foxp3* in mice is clear; it is solely expressed in Treg and the scurfy mutation is always related to defective suppressive function. Conversely, the expression of *FOXP3* in humans is not restricted to Treg and can be induced on activation of conventional T cells, albeit at much lower levels than in natural Treg. To add to the confusion, it has been reported that IPEX patients have varying degrees of disease severity and not all patients have dysfunctional Treg.[37] Even with these restrictions, there is general consensus that *FOXP3* expression is highly correlated to the suppressive function of $CD4^+$ $CD25^{high}$ T cells.

It has recently become apparent that expression of the α-chain of the IL-7 receptor, CD127, allows an unambiguous flow cytometry based distinction of Treg ($CD127^{low}$) and non-regulatory T cells ($CD127^{high}$) within the $CD4^+CD25^+$ populations. $CD127^{low}$ cells were strongly suppressive in functional suppressor assays and expression of *FOXP3* protein was highly correlated to a $CD127^{low}$ phenotype.[38 39] These findings are important because human Treg can now be accurately identified and isolated.

Treg were originally thought to be anergic when stimulated ex vivo, yet adoptive transfer studies using (DO11.10) T cell receptor (TCR) transgenic $CD4^+CD25^+$ cells have clearly demonstrated the ability of these cells to expand in vivo on TCR stimulation.[40] Recently, it has been shown that human Treg can be greatly expanded ex vivo by TCR stimulation in the presence of high concentrations of IL-2 as CD25 is functionally essential as a key component of the high affinity IL-2 receptor,[41 42] largely increasing their potential for therapeutic manipulation. The exact mechanism of suppression by Treg remains uncertain. In vitro, the suppressive function can be assessed by coculturing Treg with conventional CD4 (or CD8) T cells ("responder" T cells) in a mixed leucocyte reaction. The proliferation of conventional T cells in such assays is induced via TCR stimulation by allogeneic peripheral blood mononuclear cells (PBMCs) or agonistic anti-CD3 antibodies. In the presence of Treg, in ratios below 1/10, the proliferation of the responder T cells and their cytokine production is strongly suppressed. In these in vitro studies, the suppressive function is cell contact dependent and independent of cytokines. However, mouse studies have proven the suppression to be dependent on cytokines such as transforming growth factor (TGF)β and IL-10. The mechanisms responsible for these differences between in vivo and in vitro results remain to be fully explained.

Treg execute suppressive functions as soon as they are activated via the TCR, aspecifically (CD28, CD3), by a natural (HLA class II presented) ligand or by foreign antigens that are cross reactive to self-antigen receptors[43] in the periphery. Treg not only suppress proliferation but also downregulate activation, differentiation and even effector function of multiple immune cells, including CD4+ and CD8+ T cells, natural killer cells and dendritic cells.[44–46]

APC are able to regulate Treg activation by differential expressed costimulatory molecules and MHCII molecules. Although the precise mechanism of CTLA-4 expression and its involvement on Treg is not known,[47] it is thought that engagement of CTLA-4 by CD80/CD86 on dendritic cells activates Treg, whereas interaction with CD28, in the context of TCR activation, downregulates suppression. In addition, activation of GITR by GITR ligand (which is expressed on dendritic cells) downregulates Treg function.[48] An integrin that is expressed by dendritic cells, CD103, is also involved in T cell polarisation, promoting a positive balance of Treg over effector T cell activity in the intestine.[49] Interestingly, the very same stimuli (ie, LPS) that cause APC such as dendritic cells to become activated and present antigen, have a direct effect on Treg. Murine $CD4^+CD25^+$ Treg express TLR1, TLR 2 and TLR 4–8, and activation of TLR-4 and TLR-5 by LPS and flagellin, respectively, activates Treg and increases suppressor function in vitro.[50–52] Therefore, TLR activation of Treg seems to counteract uncontrolled activation of T cell proliferation. Conversely, natural ligands for TLR8 and TLR2 can reverse Treg function.[53 54]

The functional importance of Treg is underscored by many observations in mice where depletion of the $CD4^+CD25^+$ population precipitates diseases characterised by autoreactive T cells.[55–59]

''ADAPTIVE'' REGULATORY T CELLS

Apart from the $CD4^+CD25^+$ thymus derived Treg, there is evidence for the existence of Treg that are induced in the periphery, the so-called ''adaptive'' Treg. In mice and humans, peripheral conventional CD4+ T cells were shown to differentiate into $CD4^+CD25^+$ Treg under the influence of TGFβ in addition to TCR mediated signals.[60–62] Alternatively, adaptive Treg which are phenotypically distinct from Treg from intrathymic origin have been identified, and known as the Th3 and Tr1 T cell subsets. These generally do not express CD25 or *foxp3* and are characterised by the secretion of the immunosuppressive cytokines TGFβ and IL-10, respectively. Although their functions are complex and incompletely understood, it seems that their suppressive activity is critically dependent on the production of regulatory cytokines.

A classical example of peripheral regulatory cells is the Th3 Treg that secretes predominantly TGFβ together with varying amounts of IL-4 and IL-10, and mediates oral tolerance.[63 64] The main immunosuppressive effect of TGFβ is inhibition of Th1 responses via downregulation of IL-12β2 chain expression, and TGFβ itself is required for differentiation of TGFβ producing cells. Th3-like cells have been shown to be important in some cases of allergy and in autoimmune diseases.[65 66]

Tr1 cells were initially isolated from human SCID chimera and subsequently derived by culturing naïve T cells in the presence of high concentrations of IL-10. They secrete high levels of IL-10, a cytokine that inhibits Th1 induction by downregulation of IL-12, and suppress the production of proinflammatory effector cytokines. Tr-1 cells are anergic, functionally suppressive in vitro, generally produce low levels of TGFβ and IL-5 but no IL-4, and are critically dependent on IL-10 for their function and development.[67] In SCID patients transplanted with HLA mismatched haematopoietic stem cells, the number of Tr1 cells correlated with tolerance of the host to the graft.[37]

Proliferation of murine Tr1 cells in vivo is induced by plasmacytoid dendritic cells that express low numbers of CD11c and costimulatory molecules, and secrete large amounts of IL-10.[68] Human Tr1 cells can be induced ex vivo with the pharmacological immunosuppressant vitamin D3 and dexamethasone[69] and by immature ($CD83^-$) dendritic cells.[70] In contrast with the latter observations, we have demonstrated that induction of Treg that result from activation of monocyte derived $CD11c^+$ dendritic cells by probiotic bacteria requires full maturation of the dendritic cells (Braat H *et al*, submitted). Induction of these Treg is dependent on production of IL-10 by the mature dendritic cells, and although these Treg also secrete IL-10, this is not required for their regulatory function.

In summary, peripheral Treg comprise a heterogeneous group of T cells that secrete immunomodulatory cytokines that have been implicated in various inflammatory conditions.

REGULATORY T CELLS IN EXPERIMENTAL COLITIS

It is well known that adoptive transfer of T cells depleted of $CD4^+CD25^+$ cells in immunodeficient mice causes multiorgan autoimmunity in the recipient animals[71] and many studies have demonstrated that depletion of $CD4^+CD25^+$ T cells in mice aggravates T cell mediated models of inflammation,[72] including colitis.[73] Conversely, Treg clearly have anti-inflammatory effects in various murine models of IBD. For example, the induction of colitis that results from transfer of $CD4^+CD45RB^{high}$ T cells into immunodeficient mice can be prevented by cotransfer of the antigen experienced $CD4^+CD45RB^{low}$ T cells. Thymus derived Treg are $CD45RB^{low}$, and it is now thought that the $CD4^+CD25^+$ Treg present in the $CD4^+CD45RB^{low}$ subset are responsible for this regulatory activity.[74 75] Cotransfer with isolated $CD4^+CD25^+$ T cells prevents the induction of colitis which is reverted by the addition of monoclonal anti-CTLA-4, anti-IL-10R or anti-TGFβ antibodies. Not only do $CD4^+CD25^+$ T cells prevent the induction of colitis, they can also reverse established colitis and wasting disease, indicating their importance in controlling ongoing immune mediated inflammation.[76]

Peripheral Treg with a Tr1 phenotype also have the capacity to control colitis. Chronic activation of OVA specific naïve $CD4^+$ T cells in the presence of IL-10 induced Tr1 cells that produced large amounts of IL-10 after exposure to OVA. These cells were able to control colitis induced by pathogenic $CD4+CD45RB^{high}$ T cells in immunodeficient mice, and this

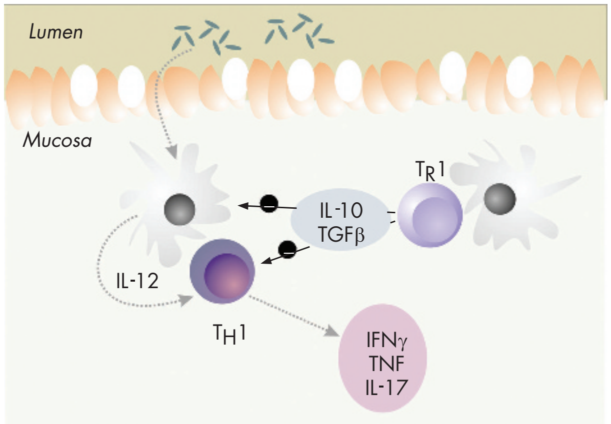

Figure 2 Bystander suppression. Presentation of bacterial antigens to naive T cells by dendritic cells results in the generation of Th1 effector cells that migrate into the intestine and cause an inflammatory response. Because the antigens that are involved in inflammatory bowel disease are unknown, therapeutic application of Treg requires antigen-non-specific suppression. Bystander suppression is the capacity of Treg to suppress immune responses that are caused by a different antigen. The mechanisms involved include the production of regulatory cytokines, deactivation of dendritic cells that attempt to stimulate effector T cells, or direct contact with the responding T cell. The concept of bystander suppression has been shown for Tr1, CD4+CD25+, Th3 and $CD8^+$ T cells. IFNγ, interferon γ; IL, interleukin; TGFβ, transforming growth factor β; TNF, tumour necrosis factor.

function was dependent on activation of the T cell receptor by OVA.[77 78] Mouse strains deficient in IL-10 spontaneously develop chronic enterocolitis, underlining the importance of IL-10 in controlling responses against the commensal flora.[79–81] Treatment with recombinant IL-10 in the T cell transfer model prevents but does not cure established colitis. In contrast, local mucosal delivery of recombinant IL-10 by the genetically modified bacteria of the strain *L lactis* seemed to be effective when disease activity was well established, and ameliorated DSS induced colitis and colitis in IL-10 deficient mice.[82]

TGFβ is also recognised for its ability to downregulate immune responses. TGFβ1 deficient mice develop a systemic inflammatory response, and blockade of TGFβ signalling in T cells results in T cell activation and induction of IBD in mice.[83] $CD4^+$ Th3 cells protect against uncontrolled inflammation in the gut, and in models of intestinal inflammation TGFβ producing mucosal T cells were shown to reduce disease activity.[84–86]

THE ROLE OF REGULATORY T CELLS IN INFLAMMATORY BOWEL DISEASES

Because it has long been difficult to reliably characterise Treg, all data about their presence and functional characteristics in humans are recent, and some of these data require confirmation. Several clinical observations indicate that the $CD4^+CD25^+$ population in patients with "autoimmune" diseases such as multiple sclerosis, uveitis and autoimmune polyglandular syndromes II is functionally defective.[87] In patients with Crohn's disease, approximately 6% of both peripheral blood and lamina propria T cells were found to be $CD4^+CD25^+$, and the fraction with a high expression of CD25 ($CD25^{bright}$) expressed CTLA-4 and GITR. In contrast with peripheral blood T cells, some expression of CTLA-4 and GITR was found on lamina propria $CD4^+CD25^-$ T cells. In agreement with this finding, *Foxp3* was predominantly transcribed by $CD4^+CD25^{bright}$ lamina propria T cells, and to a lesser extent by $CD4^+CD25^-$ T cells. When tested for functional properties, it was found that lamina propria $CD4^+CD25^{bright}$ T cells, but not $CD4^+CD25^-$ T cells, suppressed the proliferation (as well as cytokine production) of peripheral blood $CD4^+CD25^-$ T cells[88 89] but not of lamina propria $CD4^+CD25^-$ T cells. The inability of lamina propria Treg to suppress proliferation of lamina propria T cells may be related to the relative anergic and memory phenotype of the latter.[85] Likewise, $CD4^+CD25^+$ T cells isolated from human colonic mesenteric lymph node in ulcerative colitis display typical features of Treg cells and possess potent suppressor activity in vitro in spite of persistent mucosal inflammation {unpublished results, Saruta M *et al*, DDW 2006, abstract 599}.

At present, these sparse data on Treg in IBD suggest that the inflammatory pathology in patients with Crohn's disease does not result from an absence or altered functionality of the Treg population although the increase in Treg numbers and activity may be insufficient to suppress the inflammatory condition.[90]

There are no reliable data on the existence of Tr1 or Th3 cells in the human mucosa, or on their functional properties. IL-10 deficient mice develop IBD but patients with IBD do not have deficient IL-10 production.[91] Remarkably, isolated T cells from patients with IBD were found to express high levels of SMAD7, a negative regulator of TGFβ signalling, suggesting that impaired responsiveness to TGFβ may be involved in IBD.[92 93] The functional role of lamina propria Treg may be more complex than that of peripheral regulatory cells because of the necessity to specifically suppress immune responses to endogenous bacteria but not to bacterial pathogens.

THERAPEUTIC POTENTIAL OF REGULATORY T CELLS

As described above, Treg can prevent and even cure various experimental colitis models. Although their therapeutic potential is without dispute, translation of these data into therapeutic strategies is not straightforward. Furthermore, the ability to apply this therapeutic strategy in a human clinical setting will depend on techniques to isolate and transfer adequate numbers of cells. In most mouse models of autoimmune diseases, the antigens that induce T cell activation are known, and antigen specific Treg are able to potently suppress activation in an antigen dependent manner. For example, in the NOD model of autoimmune diabetes, islet antigen specific BDC2.5 Treg completely prevent diabetes. However, polyclonal Treg are at least 50-fold less potent than antigen specific Treg and can only be a viable therapeutic option in this context when sufficient numbers are applied.[94]

Although there is evidence for a role of peptidoglycan and flagellin at the level of dendritic cell stimulation, it is not known what antigens are involved in the pathogenesis of IBD, excluding the possibility to using antigen specific Treg. It is now clear that Treg do not need to be antigen specific in order to suppress immune responses as a result of so-called bystander suppression (fig 2). A clear example of bystander suppression was demonstrated in the SCID transfer model where OVA specific Tr1 cells suppressed the occurrence of IBD after administration of OVA although OVA is not involved in the immune mediated inflammation in this model.[95] Therefore, the OVA specific Tr1 cells were able to suppress responses induced by other antigens, very likely derived from intestinal bacteria, and this is known as ''bystander'' suppression. In various situations $CD4^+CD25^+$ Treg, once activated by their TCR, have been shown to be capable of such antigen-non-specific bystander suppression.[96]

Apart from bystander suppression, Treg inhibit the response of conventional CD4 T cells in a contact dependent manner and can even confer suppressive properties to such T cells. This process is known as ''infectious tolerance'' and results in the conversion of conventional T cells into IL-10 producing Tr1-like cells and TGFβ producing Th3-like cells.[97] These concepts of ''infectious tolerance'' and bystander suppression are instrumental in providing a context for using Treg as a potential therapy.

It has now become possible to produce and expand sufficient $CD4^+CD25^+$ and Tr1-like cells for therapeutic application, and clinical studies have been initiated. For example, in a currently ongoing clinical trial, Roncarolo *et al* (personal communication) used ex vivo induced Tr1 cells as post-transplant cellular therapy in haematological cancer patients undergoing HLA-haploidentical HSC transplantation. After a 10 day ex vivo culture of donor PBMC in the presence of irradiated host PBMC and IL-10, the IL-10 anergised donor T cells are infused into the host. The ultimate goal is to provide immune reconstitution with donor T cells that are anergic towards host antigens and contain precursors of host specific Tr1 cells. Although promising, the clinical usage of Tr1 cells for the cure of T cell mediated diseases is still in a developmental stage.[77]

A second important observation has been that regulatory functions can be imprinted in mouse and human T cells by genetic engineering. Unselected peripheral blood naïve mouse T cells become regulatory following transfection with a retroviral vector encoding IL-10, and these cells are able to suppress inflammation by a bystander mechanism.[98] Such cells can also be generated from human peripheral T cells.[99] Using similar techniques, FOXP3 can be overexpressed in human $CD4^+$ T cells but the data on the functional efficacy of these generated suppressor T cells are conflicting.[100, 101]

Finally, it may be possible to induce Treg in vivo by directing APC such as dendritic cells. It appears that the capacity of dendritic cells to induce regulatory T cells depends on the dendritic cell instruction and maturation state.[102] Different approaches to render ''regulatory'' dendritic cells[103, 104] include ex vivo genetic manipulation, anti-inflammatory cytokine exposure or by direct instruction with tolerogenic compounds. We have recently demonstrated that injection of the Bordetella pertussis derived filamentous haemagglutinin A reduced inflammation in a mouse model of IBD.[105] The experimental data of T cell as well as dendritic cell manipulation, along with future investigations, needs to determine the exact value of both approaches but recent advances are very promising.

When considering ex vivo manipulation or induction of Treg, two major hurdles need to be overcome.

Firstly, sufficient numbers of the manipulated T cells need to be directed to the gut mucosa, a process known as ''homing'', which is directed by specific integrins and by chemokines. It has been reported that cultured $CD4^+$ gut derived T cells that express high levels of the pivotal gut homing receptor α4β7 did not home to the gut following injection in healthy individuals.[89] However, we have reported that homing, at least in mice and rats, is greatly increased in inflammatory conditions. Also, it has become apparent that the isolation and expansion of $CD45RA^+$ naïve (instead of $CD45RO^+$) $CD4^+CD25^+$ T cells is the best strategy for adoptive Treg cell therapies.[106]

Secondly, it might be a problem that the bystander suppression of adoptively transferred Treg cannot be controlled. Treg may worsen inflammatory disease because they may interfere with the immune mechanisms that are necessary for clearance of microbial pathogens.[107] The non-specific immunosuppressive effects of Treg are a concern when considering therapeutic application. On the other hand, such effects may be limited because effective pathogen specific immune responses are shown to be Treg resistant.[108] However, from this perspective the use of natural occurring $CD4^+CD25^{high}$ Treg may be preferred, as in these cells, at least in vitro, TLR2 triggering results in a temporal loss of the suppressive Treg phenotype.[54]

CONCLUSIONS

Regulatory T cells are key players of immune regulation, and they have important functions in suppressing unwanted inflammatory responses towards self antigens, and the antigens of endogenous intestinal bacteria. Patients with IBD do not seem to have a primary absolute defect in regulatory cells but, apparently, the regulatory capacity of these cells is insufficient to downregulate inflammation.

None of the current therapies for IBD directly targets Treg function or generation but drugs that are used widely clinically may influence Treg function. For example, corticosteroids may enhance Treg function in asthma and allergic diseases[109–111] but future research needs to determine the exact role of corticosteroids on regulatory T cell function. Interestingly, it was recently shown that anti-TNF antibodies increased the FOXP3 mRNA and protein levels in the $CD4+CD25^{high}$ compartment and restored their tolerogenic function.[112] In mice and humans with diabetes, treatment with non-agonistic anti-CD3 antibodies resulted in prevention of progression of loss of islet cell function by immunosuppressive mechanisms that included the induction of Treg.[113]

None of the previously discussed possible clinical approaches uses a strategy that allows control over Treg activity. Are such approaches feasible?

Intravenous administration of relatively large doses of regulatory cytokines is not effective (human recombinant IL-10 administration in IBD patients[114]) or found to be toxic (TGFβ). Only a small fraction of the total administered dose of such cytokines reaches the mucosa and results may be better when such cytokines are locally administered. Mucosal delivery of recombinant IL-10 by genetically modified bacteria such as *L lactis* addresses this problem and was indeed shown to ameliorate DSS colitis and colitis in $IL\text{-}10^{-/-}$ mice. Recently, we have demonstrated in a clinical phase 1 trial that this engineered cytokine excreting organism can be safely administered to patients with Crohn's disease and is biologically contained.[115] TGFβ can also be locally expressed, for example by delivery of TGFβ encoding plasmids to mucosal surfaces and this approach is in preclinical trials.[116]

Summary

- Activation of mucosal T cells occurs in IBD and is inhibited by various subsets of ''regulatory'' T cells, which can be functionally and phenotypically characterised.
- Regulatory T cells can be generated within the thymus or in peripheral tissues.
- In mice, loss of regulatory T cell activity results in IBD. Regulatory T cell activity has not been thoroughly studied in IBD but currently available data do not provide evidence for a loss of regulatory T cell activity in human IBD.
- Regulatory T cells can be generated or activated in vivo and in vitro using a variety of approaches, including specific small molecules, cytokine mediated activation, and gene therapy. These cells have therapeutic activity in the preclinical setting.

Contained immunosuppression can also be accomplished by modifying or expanding T cells with regulatory properties ex vivo. This strategy involves a harvesting step that yields peripheral blood T lymphocytes (eg, by apheresis) followed by forced differentiation by exposure to cytokines and tolerogenic compounds, or by genetic engineering with a ''regulatory'' gene. On readministration to the patient, such cells can downregulate inflammation by a bystander mechanism, following specific integrin mediated homing. It is technically feasible to specifically expand T cells with a predefined TCR that can be specifically activated by an orally administered antigen, allowing for control of the immune suppression. These strategies are attractive in view of the long lifespan of T cells, and are expected to have long term effects.

Treg are extremely potent and, with production of low amounts of IL-10 by a very small fraction of mucosal T cells (IL-10 engineered T cells that comprised only ~ 0.001% of all mucosal T cells were effective), a sufficient therapeutic effect can be achieved.[117] With the identification of more genes that determine Treg development, the ability to identify Treg using cell surface markers, and with improving transduction methods over time, the possibilities for such approaches will be significantly expanded.

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EDITOR'S QUIZ: GI SNAPSHOT

Answer

From the question on page 575

The CT scan (fig 1A,B in Questions section) revealed multiple adherent small bowel loops encased within a thickened enhancing peritoneal membrane (arrow) forming a sac-like structure, with localised fluid collection seen within this sac, features suggestive of an abdominal cocoon. The second and third parts of the duodenum were dilated (arrow) as far as and at the site of entry into the encapsulated sac-like structure. Peritoneum and omentum thickening (arrows) was also noted (fig 1C in Questions section). There was no ascites or lymphadenopathy. The liver, spleen, kidneys and pancreas were normal. A radiological diagnosis of sclerosing encapsulating peritonitis forming an abdominal cocoon was made, which was later confirmed at surgery.

Sections from gastric serosa showed fibrocollagenous tissue with multiple nodular and discretely scattered malignant cells (fig 1, arrows). UGI biopsy from the gastric ulcer revealed poorly differentiated adenocarcinoma.

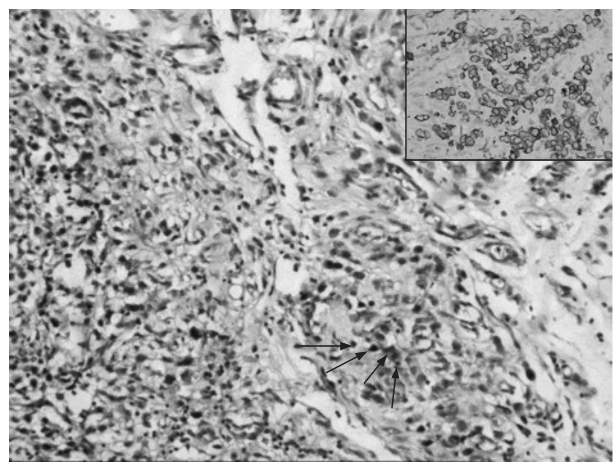

Figure 1 A photomicrograph (H&E) showing many malignant cells (arrows) surrounded by stroma and inflammatory cells. Inset: immunostaining for cytokeratin showing abundant malignant cells.

Abdominal cocoon, also referred to as sclerosing encapsulating peritonitis, is a rare condition characterised by fibrotic encapsulation of the bowel. The exact aetiology is unclear but has been previously described as a benign process in patients with a history of previous abdominal surgery or peritonitis, liver transplant, chronic ambulatory peritoneal dialysis, or prolonged use of the β-blocker practolol, and in patients with tuberculous pelvic inflammatory disease. Clinical presentation includes abdominal distension, acute intestinal obstruction and a palpable abdominal mass.

We describe a case of disseminated gastric adenocarcinoma (signet cell carcinoma) as a rare cause of abdominal cocoon.

Preoperative diagnosis is a challenge and diagnosis is usual made at laparotomy.

CT scan provides a more accurate diagnosis of this entity as well as its complications, and may also help to exclude other causes of intestinal obstruction.

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