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Working out mechanisms of controlled/physiologic inflammation in the GI tract

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Abstract

The mucosal immune system is distinct from its systemic counterpart by virtue of its enormous antigenic exposure (commensal flora, food antigen, pathogens). Despite this, the mucosal immune system maintains a response defined as controlled or physiologic inflammation. This is regulated by many different mechanisms, among which there are physical, cellular and soluble factors. Our laboratory has focused on unique Tregs in the gut controlled by, in one instance, intestinal epithelial cells that serve as non-professional antigen-presenting cells. We believe that intestinal epithelial cells, expressing classical and non-classical MHC molecules, serve to activate Tregs and thus maintain controlled or physiologic inflammation. In this review, we describe regulatory cytokines and T cells that are one part of the emphasis of our laboratory.

Keywords

Mucosal immunity; Oral tolerance; Lamina propria lymphocytes; Tregs; Epithelium

Introduction

The intestinal immune system: major components

The GI tract is the main site of contact with the external environment and, as such, is constantly exposed to antigenic stimulation. Stimuli can be harmful, such as pathogens (bacteria, protozoa, fungi, viruses) and toxic substances, or beneficial to the host, such as food or commensal flora [1]. The mucosal immune system has a complex scheme of defenses involving a physical barrier in addition to innate and adaptive immune mechanisms to create a delicate balance between mounting protective responses against pathogens and tolerance to harmless antigens.

Epithelial cells lining the mucosal surface of the GI tract comprise a dynamic physical interface between the external luminal environment and the body's interior. Epithelial integrity is crucial for the maintenance of a selective physical barrier. Its function is maintained by specific intercellular structures including tight junctions, adherens junctions and desmosomes. This cell monolayer creates a trans-epithelial resistance that is indicative of the integrity of the epithelium [2]. The cells that make up the mucosal barrier are a self-renewing system undergoing continuous replacement from pluripotent stem cells located at the base of the crypts. Daughter cells undergo terminal differentiation into the absorptive or

secretory lineage. Epithelial cells actively participate as a frontline defense response to external stimuli by playing an integral role in both innate and adaptive mucosal immunity. Epithelial host defense functions include secretion of fluid, electrolytes and mucus (flushing noxious substances from the bowel lumen) as well as the production of chemokines, cytokines, growth factors and antimicrobial peptides. Epithelial cells are also able to process and present antigens to immune cells located in the lamina propria (LP) [3, 4].

The mucosal immune system samples antigens and is responsible for their clearance. Sampling of antigens from the lumen of the gut is accomplished through different routes. Antigens can be taken up, processed and presented by classical and non-classical MHC molecules on intestinal epithelial cells (IECs). Antigens can also pass in between the intestinal epithelial cells and either get absorbed into capillaries that drain into the portal vein en route to the liver or get captured in the LP by resident antigen-presenting cells (APCs) [5–8]. Antigens can also be sampled from the gut lumen via fluid-phase endocytosis by specialized microfold cells (M cells) that overlay Peyer's patches (PPs) [9]. Antigens within the PPs are presented by APCs to naïve T cells, and these T cells traffic to the draining mesenteric lymph nodes (MLNs) where classical APC presentation can occur [10, 11]. In the distal small bowel, antigens can be sampled by intestinal dendritic cells (DCs), which express tight junction proteins and can insert their dendrites through the epithelium and directly sample antigens from the lumen [12]. Effector cells traffic to the LP via contact with chemokines and integrins. Chemokines such as CCL25 and CCL28 are produced by IECs (from the small and the large intestine, respectively) and regulate trafficking of specific T cell subsets that express CCR9 or CCR10 to the mucosa. The expression of the $\alpha 4\beta 7$ integrin by activated lymphocytes correlates with the recruitment to its ligand, mucosal addressin cell molecule (MAdCAM-1), which is expressed on the surface of high endothelial venules in the intestinal tissue and focuses homing of effector cells to the PP and the LP [4, 13, 14].

An interesting feature distinguishing the systemic and mucosal immune systems is that the reaction to antigens is geared toward hyporesponsiveness of the mucosal immune system, which is responsible for tolerance to commensal flora and food antigens. Different components of the mucosal immune system are responsible for maintaining the tolerant state (mucosal homeostasis). First, regulatory mucosal lymphocytes including regulatory T cells, DCs and macrophages are abundant in the intestinal LP. One more feature contributing to hyporesponsiveness in the mucosal immune system is the impaired signal transduction through the T cell receptor/CD3 complex in LP lymphocytes (LPLs). LPLs, unlike peripheral blood lymphocytes (PBL), can proliferate in response to anti-CD2 stimulation but not to anti-CD3 [15]. Furthermore, upon stimulation (via CD2), LPLs display increased levels of apoptosis [16]. LP macrophages, in contrast to peripheral blood monocytes, do not support proliferation of either LPL or PBL [17]. LP-DC populations, expressing CD103, induce the de novo differentiation of naïve T cells into CD4⁺ CD25⁺ Foxp3⁺ T cells, via a TGF- β - and retinoic acid (RA)-dependent mechanism. The LP-DC expressing the surface markers CD11b^{lo}CD11c^{lo} (although expressing high levels of TLRs) fail to produce proinflammatory cytokines when challenged. DCs isolated from PP can induce gut-homing properties in T cells, B cells and Tregs via an RA-dependent mechanism and can drive IgA class switching [18]. IgA is secreted by LP-resident plasma cells into the lumen and plays a role in neutralizing antigens. IgA-B cell development is usually T cell dependent; however, studies on the intestine have shown that IgA-producing B cells can develop in a T cell-independent manner, as part of the innate immune system. TLR signaling via NF- κ B elicits B cell-activating factor (BAFF) and proliferation-inducing ligand (APRIL) expression in DCs, monocytes, macrophages, granulocytes and epithelial cells, including IECs. In the presence of appropriate cytokines, BAFF and APRIL initiate IgA-B cell development [19, 20].

Some of the regulatory mechanisms employed in the intestine to promote the tolerogenic environment by innate immune cells, comprising mucosal DC, monocytes and IECs, include the preferential secretion of the inhibitory cytokines such as interleukin (IL)-10, TGF- β and the expression of indoleamine-2,3-dioxygenase (IDO). IDO mediates tryptophan depletion, an essential nutrient for T cell proliferation and survival [21].

In addition, the hyporesponsiveness to antigens by IECs is mediated by aberrant expression of toll-like receptors (TLR) and the nucleotide-binding oligomerization domain (NOD) family of proteins that recognize conserved viral and bacterial motifs—pathogen-associated molecular patterns (PAMPs). TLR function can be regulated by the location of the TLRs and the differential expression of co-stimulatory molecules such as CD14 and others [22]. TLR5 is only expressed on the basolateral side of IECs, allowing for the recognition of bacterial flagellins that are able to cross the barrier [23].

Immune regulation

One of the key components in regulating mucosal immune responses is the inhibitory pathways that are in place in the gut. These are divided into two groups: cytokines and regulatory cells.

Regulatory cytokines

Transforming growth factor β (TGF- β)

TGF- β is a highly pleiotropic cytokine mediating several physiological processes including morphogenesis, embryonic development, adult stem cell differentiation, immune regulation, wound healing and inflammation. The human TGF- β family includes six isoforms: TGF- β , BMPs (bone morphogenetic proteins), GDFs (growth and differentiation factors), MIF (Müllerian inhibitory factor), activins or inhibins. This family of proteins is expressed in distinct temporal and tissue-specific patterns. Interestingly, all immune cell lineages (including B cells, T cells, DCs and macrophages) secrete TGF- β . While the production of TGF- β is well programmed, the TGF- β receptor complex is widely expressed on hematopoietic and non-hematopoietic cells. The TGF- β receptor complex is composed of two serine/threonine kinase receptor subunits, TGF- β receptor 1 and TGF- β receptor 2 (TGF- β R1 and TGF- β R2, respectively), which form a heteromeric complex in the presence of the dimerized ligand [24, 25].

TGF- β is synthesized as part of a large precursor molecule containing a propeptide in addition to TGF- β . TGF- β is cleaved from a large precursor before its secretion from the cell but remains attached to the propeptide. Once secreted, the propeptide is stored in the extracellular matrix (ECM) proteins and integrins. The attachment of TGF- β to its binding protein (latency-associated protein—LAP) prevents it from binding to its receptor. The bioavailability of TGF- β depends on proteolytic processing that releases the cytokine. A number of mechanisms have been described for mediating this activation: high heat, pH, chaotropic agents, proteases, integrins and thrombospondin 1. Only integrin α v β 6 and thrombospondin 1 have been implicated by both in vivo and in vitro experiments [26, 27].

Upon TGF- β binding to the receptor complex, a series of phosphorylation events occur leading to phosphorylation of two intracellular proteins, SMAD2 and SMAD3. Once phosphorylated SMAD2 and SMAD3 associate with SMAD4 and translocate into the nucleus to induce transcription of target genes. Targeted disruption of SMAD3 is associated with diminished responsiveness to TGF- β [28]. SMAD6 and SMAD7 are negative regulators of this signaling pathway. They can bind to TGF- β R1 and block its ability to activate SMAD2 and SMAD3. SMAD7 has been shown to be upregulated in tissue and peripheral blood from IBD patients [29].

There has been a substantial amount of literature describing the role of TGF- β in numerous and sometimes contradictory biological processes. Alterations of the TGF- β signaling pathway may lead to a wide range of pathologic conditions. Defects in the TGF- β signaling pathway have been associated with multiple cardiovascular abnormalities by influencing vascular cell proliferation, angiogenesis, etc. High TGF- β levels have also been associated with several bone and muscle diseases, as well as with pulmonary diseases such as broncho-pulmonary dysplasia, emphysema and asthma [30]. The role of TGF- β in cancer is still unclear. Defective TGF- β signaling is reported in human cancer and is associated with a worse prognosis. However, TGF- β is also a known tumor promotor. This dual role of TGF- β happens as a result of a combination of genetic or epigenetic perturbations in tumor cells, along with an alteration in the tumor microenvironment [31].

The expression of TGF- β mRNA and the production of TGF- β are associated with a number of pro-fibrogenic pathologies, including pulmonary fibrosis, liver cirrhosis, fibrotic kidney disease, alcohol-induced and autoimmune hepatic fibrosis, systemic sclerosis and CD. Tissue damage often occurs during pathological immune responses as a result of inflammation. First neutrophils enter the wounded sites to remove pathogens and damaged tissue via phagocytosis. Macrophages continue this process and also secrete growth factors including TGF- β , which help the fibrotic process. The fibrotic reaction is characterized by an increased production of ECM components such as fibronectin, collagen as well as proliferation, migration and accumulation of mesenchymal cells. This leads to activation of fibroblasts and their differentiation into myofibroblasts. TGF- β induces connective tissue growth factor (CTGF) production. Moreover, CTGF binds to TGF- β and enhances its binding to its receptor [26, 30, 32].

TGF- β has multiple effects on the immune system. Since high levels of TGF- β are found in the intestine, it likely plays a significant role in mucosal immune homeostasis. TGF- β is a known immunosuppressive cytokine, yet it is also able to induce immune responses. TGF- β has an overall suppressive affect on B cells by inhibiting mature B cell proliferation. Mature splenic B cells from a TGF β R2 conditional B cell of KO mice have shown an inhibition in B cell proliferation. Moreover, TGF- β induces apoptosis in immature and resting B cells through decreased expression of c-myc and NF- κ B. Upon specific Ag stimulation, B cells can secrete TGF- β , which may be playing an autocrine role in inducing antibody production. TGF- β plays a role in T cell-dependent and T cell-independent IgA class switch in combination with CD40 ligand or BAFF and APRIL, respectively [33–35]. TGF- β attenuates isotype switching to IgG1 and IgE through the inhibition of signals induced by IL4 via enhancing SOCS1 and SOCS3 expression. Secretory IgA is crucial for the prevention of recognition of microbial flora by the host. TGF- β function in IgA class switching is associated with an increased transcription of germline α transcripts. Mice with a blockade of TGF- β signaling in B cells have almost no serum IgA [34, 36]. Other APCs, such as macrophages and DCs, are also affected by TGF- β in different ways. TGF- β is crucial in the development of Langerhans cells (skin resident DCs). Mice lacking TGF- β do not have Langerhans cells [37]. TGF- β inhibits the maturation of DCs by inhibiting MHC class II expression. DCs also produce TGF- β , which plays an autocrine role in the maintenance of the immature state of DCs as well as promotes T cell differentiation. TGF- β has conflicting roles in monocytes, since it stimulates cells in the resting state (monocytes), whereas it inhibits activated macrophages.

TGF- β exerts the greatest impact on T lymphocytes. TGF- β can inhibit T cell proliferation by inhibiting IL-2 transcription as well as by upregulating cyclin-dependent kinase inhibitors and downregulating c-myc. TGF- β is an important regulator of CD4⁺ T_H differentiation. It inhibits T_H1 and T_H2 differentiation by reducing T-bet and GATA3 expression, respectively. TGF- β induces the differentiation of two very different subsets of CD4⁺ T

cells. TGF- β contributes to immune suppression through the induction and maintenance of FoxP3 expressing CD4⁺ Treg cells (among other mechanisms). Nevertheless, TGF- β also has a pro-inflammatory effect through the differentiation of T_H17 cells by inducing retinoic acid receptor–related orphan nuclear receptor γ t TGF- β , in the presence or absence of IL-1 β , and IL-6 induces the development of T_H17 and Treg, respectively [38]. TGF- β plays a role in inhibiting CD8⁺ T cell differentiation and cytolytic activity. It inhibits perforin and FAS ligand expression.

The role that TGF- β plays in the mucosal immune system is still controversial. Animals with a homozygous mutation of TGF- β 1 have no gross developmental abnormalities, but within a few weeks after birth, they exhibit wasting disease, multi-organ inflammation and tissue necrosis (including the intestine) resulting in organ failure and death [39, 40]. Moreover, a specific abrogation of TGF- β signaling in CD4⁺ T cells (TGF β R2-dn transgenic mice) leads to inflammation in the colon and the lungs [41]. To support the important role of the TGF- β signaling pathway in controlling autoimmunity and intestinal inflammation, a genetic disruption of SMAD3 has been shown to exhibit abnormal immune functions leading to inflammation in multiple organs including the intestine [28]. Human biopsies from normal controls treated with anti-TGF- β show an increased production of pro-inflammatory cytokines such as IFN- γ , TNF- α , IL-6 and IL-17. Interestingly, colonic biopsies from IBD patients display increased levels of TGF- β [42, 43].

Interleukin-10 (IL-10)

IL-10 is a homodimeric, pleiotropic cytokine with an immunoregulatory function whose actions influence many cell types in the mucosal immune system. It is a cytokine with potent anti-inflammatory properties, produced by both hematopoietic and non-hematopoietic cells. IL-10 can suppress the activity of responder cells (T cells and APCs) or activates immunity as in IgA secretion by B cells. Most hematopoietic cells, lymphocytes and myeloid cells express the IL-10 receptor complex. Upon IL-10 binding to its receptor, Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2) are phosphorylated and lead to activation of STAT3. The STAT3 signaling pathway increases the transcription of anti-apoptotic and cell cycle progression genes [44]. Interestingly, IL-10R has been found to be a susceptibility locus for IBD [45]. This finding correlates with the phenotype of the IL-10 or IL-10R2 KO mice models. Both KO mice develop colitis-like inflammation only when maintained in conventional animal facilities [46, 47]. This inflammation is a direct response to bacterial stimuli and is mediated by CD4⁺ T cells [48, 49]. IL-10 KO mice reared under specific-pathogen-free conditions do not develop intestinal inflammation. Infection of these mice with *Helicobacter hepaticus* induces chronic colitis [50]. Different studies have shown that the addition of IL-10 leads to the recovery from specific infections, like *Leishmania*, and prevention of autoimmunity, such as autoimmune gastritis, psoriasis and rheumatoid arthritis [51–53].

STAT3 is a central player in mediating signaling from the IL-10 receptor complex. STAT3 KO mice die during early embryogenesis [54]; therefore, conditional STAT3 KO in different cell compartments are used. A myeloid-specific STAT3 ablation has led to the induction of macrophage activation and result in similar pathology to the one observed in IL-10 KO mice [55], while the loss of STAT3 in CD4⁺ T cells has been observed to prevent the development of inflammation [56].

Since the presence of IL-10 is crucial for mucosal immune homeostasis, potential therapies involving IL-10 have been tested. Injection of recombinant IL-10 into RAG mice prior to transfer with naïve CD4⁺ T cells has abrogated colitis, while transferring naïve CD4⁺ T cells from IL-10 transgenic mice has not led to colitis induction [57, 58]. Moreover, a genetically engineered IL-10 secreting *L. lactis* has been shown to cure colitis in IL-10 KO mice [59].

while IL-10 induction by polysaccharide A (product of an intestinal commensal) has been shown to prevent colitis [60]. However, human phase 3 trials using recombinant IL-10 in IBD have not been successful due to lack of efficacy. This indicates that increasing the levels of IL-10 is not sufficient to cure IBD [61]. In fact, IL-10 is increased in tissues from IBD specimens.

Regulatory/suppressor T cells

For years, immunologists have known that the immune system requires regulatory components that dampen immune responses after a pathogen has been cleared [62]. These include regulatory T cells, macrophages, DCs, B cells and stromal cells. In 1970, Gershon and Kondo first introduced the concept of T cells suppressing immune responses in a paper describing CD8⁺ suppressor T cells (Ts) [63]. The study of CD8⁺ Ts cells triggered great interest during the 1970s and 1980s, yet no surface or other markers were found to uniquely distinguish CD8⁺ Ts from cytolytic CD8⁺ T cells, and this area of research was largely neglected. In 1988, Sakaguchi et al. showed that autoimmunity developed in nu/nu mice after engraftment of a thymus from euthymic nu/+ mice treated with cyclosporin A (CsA) [64]. This could be prevented by co-administration of thymocytes prepared from normal nu/+ mice. The cells promoting this regulatory effect were later described as the CD4⁺ CD25⁺ FoxP3⁺ T cells [65]. Today, regulatory T cells have been shown to regulate immune responses, and several distinct regulatory T cell subsets have been described.

CD4⁺ regulatory T cells

The most studied population is the CD4⁺ CD25⁺ regulatory T cell, which exists as a naturally occurring Treg (nTreg) or induced (antigen activated) Treg (iTreg). nTregs develop in the thymus through their ability to recognize self and foreign antigens. They play a role in maintaining self-tolerance and preventing immune pathology following chronic inflammation [66]. iTregs differentiate from naïve CD4⁺ T cells in response to tolerogenic processes in peripheral tissues. This differentiation is TGF- β dependent. These cells upregulate FoxP3, CD25 and cytotoxic T lymphocyte antigen-4 (CTLA-4) [67, 68]. CD25 is not a specific marker for Treg cells because it is also expressed by all activated T cells. In 2003, the FoxP3 transcription factor was identified as both a marker and a lineage commitment factor for CD4⁺ CD25⁺ Tregs [69–71]. Patients with mutations affecting the FoxP3 gene develop immune dysregulation, polyendocrinopathy and enteropathy in an X-linked syndrome called IPEX. IPEX is a fatal autoimmune disorder, which develops early in life and affects multiple organs including the small intestine [72, 73]. Mice with a mutation in FoxP3 also develop fatal multi-organ inflammation similar to that found in IPEX patients [74]. In human, the expression of FoxP3 is not restricted to regulatory T cells, while in mice all of the evidence supports its role in a commitment toward a regulatory lineage [75]. nTreg protect from the induction of experimental colitis by CD4⁺ CD45RB^{high} expressing cells (transferred into SCID mice). Interestingly, intestinal inflammation is not generally associated with a decrease in Tregs (with the exception of IPEX patients) [76–78]. Moreover, Tregs have also been shown to be up-regulated in the mucosa of both UC and CD patients and to have intact suppressor activity (suppressing the proliferation of effector CD4⁺ CD25⁺ T cells) [79, 80]. Thus far, of all of the CD4⁺ Tregs shown to have regulatory function in vitro, there is little evidence that their deficiency is involved in the development of IBD. This can be due to the fact that these cells have a redundant function in regulating the immune system in the gut or alternatively that there may be other cell subsets that play a major role in regulating the mucosal immune system. It is important to note that IL2R KO mice (CD25 KO mice) have inflammation only in the colon, suggesting that other types of regulatory T cells are responsible for maintaining homeostasis in the small bowel. Scurfy mice that lack FoxP3 have only small bowel inflammation (if they are inflamed at all) [62].

Both nTregs and iTregs have been shown to suppress proliferation of T cells. It is not clear whether FoxP3⁺ nTregs differ in phenotype or function from iTregs. When analyzing the mechanisms by which these subsets regulate immune responses, it is clear that both can specifically recognize a wide variety of antigens (auto-antigens in the case of nTregs). In fact, there is a requirement for Tregs to be activated, possibly by antigen stimulation via TcR, to exert Treg suppressive function in vitro. After activation by a specific antigen, Tregs mediate antigen-nonspecific suppression on the activation and expansion of other T cells [65, 81]. Survival of Tregs in the periphery is TGF- β and retinoic acid (RA) dependent [67, 82, 83]. Tregs have been demonstrated to suppress in a contact-dependent manner. Treg contact-dependent suppressor activity has been demonstrated in vitro and may involve signals from CTLA-4, glucocorticoid-induced tumor necrosis factor receptor (GITR) and membrane-bound TGF- β . Several groups have shown that membrane-bound TGF- β mediates this suppressor activity, while others have shown its dispensability. CTLA-4 expression has also been suggested by several groups to mediate suppression in nTregs and iTregs [84]. CTLA-4 is a member of the immunoglobulin superfamily and encodes a protein that transmits an inhibitory signal to T cells. CTLA-4 binds to B7 molecules with a higher affinity than CD28 molecules, thereby inhibiting the positive co-stimulatory signals that activate T cells. In responder cells, CTLA-4 plays a role in ending immune responses initiated by the CD28 co-receptor. CTLA-4 downstream signaling in Tregs has been suggested to promote the suppressor activity of the cells. GITR is a member in the TNF receptor superfamily and plays a role in T cell homeostasis. A major role of glucocorticoid hormones is both to induce apoptosis and to protect the cell from undergoing apoptosis under different stimuli. GITR expression appears to be dependent on the activation through the CD3 complex. Binding of GITR ligand to GITR abrogates the suppressive activity of Treg cells and therefore can be used as a marker to identify the FoxP3⁺ Treg cell [85, 86].

CD4⁺ CD25⁺ T cells do not produce IL-10, but there are some studies suggesting that IL-10 does play a role in their mechanism of suppression [80, 87–89]. Transferring CD4⁺ CD45Rb^{high} cells into SCID mice induces colitis that can be abrogated by co-transfer of CD4⁺ CD45Rb^{low} cells. Interestingly, administration of anti-IL-10-receptor antibodies completely abrogates the ability of CD4⁺ CD45Rb^{low} cells to protect. In addition, transferring CD4⁺ CD45Rb^{low} cells from IL-10-deficient mice were not able to protect the mice from colitis induction [90]. Furthermore, IL-10 administration benefits animals with colitis induced by CD4⁺ CD45Rb^{high} cells [57]. CD4⁺ CD103⁺ T cell is another CD4⁺ Treg cell subset (can either be CD25⁺ or CD25⁻) that expresses CTLA-4 and plays a role in preventing the development of IBD in mice. The molecule potentially involved in the suppression is CD101. It is expressed on 25–30 % of CD4⁺ CD25⁺ cells, and these cells have been shown to have a regulatory function in vitro, as well as in vivo (in a graft-vs-host disease model) [91]. Lack of expression of CD127 (the receptor for IL-7) is also seen in CD4⁺ CD25⁺ Tregs [13].

Tr1 cells do secrete IL-10 and are dependent on IL-10 but do not express FoxP3 and have low levels of CD25. Tr1 cells can proliferate in the presence of IL-2 and IL-15. The absence of Tr1 cells in IL-10-deficient mice is associated with the development of colitis, although there is no evidence that Tr1 cells play a direct role in the prevention of colitis in mice. Even though IL-10 KO mice have been shown to develop intestinal inflammation in the presence of commensal flora, the use of IL-10 as a treatment for human IBD has not proven efficacious.

T_H3 cells were first identified to be present after oral tolerance induction [92]. T_H3 express FoxP3 but not high levels of CD25. These cells produce TGF- β and respond to TGF- β . T_H3 suppress the activity of T_H1 and T_H2 cells. T_H3 are thought to promote the differentiation of

nTreg and iTregs [93]. However, there is no evidence to correlate defects in T_H3 cells and IBD [62, 94].

We have recently identified a novel population of CD4⁺ FoxP3/IL17 double-positive cells in the lamina propria of CD but not UC or normal patients. These cells share phenotypic characteristics of Th17 cells with secretion of IL17, IL22 and IL21 while expressing high levels of CCR6, CD161 and ROR γ t. However, unlike conventional Th17 cells and similar to FoxP3⁺ Tregs, they express high levels of CD101 and low levels of CD127, exhibit a similar TcR repertoire (V β usage) and are functionally suppressive in in vitro co-culture systems. FoxP3⁺ IL-17-producing cells are imprinted for gut homing, as indicated by high levels of CCR6, CD103 and the integrin α 4 β 7 expression. These cells secrete IFN γ but not IL10 or TGF- β . Thus, they represent a novel cell population that could provide useful insights into lineage commitment of Tregs versus Th17 cells. We propose that these cells sit at the crossroads between Treg and Th17 cells and that further commitment to either lineage results from microenvironmental cues present in the tissues. In an effort to define the microenvironmental cues present in the tissue that would give rise to these cells, we identified that UC LPL, but not normal or inflamed LPLs, in the presence of TGF- β gives rise to double-positive cells [95].

CD8⁺ T suppressor cells

The study of CD8⁺ T suppressor (Ts) cells is an evolving field of research, and the literature is somewhat puzzling and overlapping. It is still unclear how many distinct subsets there are and whether different groups have identified the same cell subsets utilizing different markers. Similar to CD4⁺ Tregs, CD8⁺ Ts cells can develop in the thymus (natural CD8⁺ Ts cells) or arise in the periphery upon stimulation. A number of subsets of naturally occurring CD8⁺ Ts cells have been described in humans and mice. Natural CD8⁺ Ts subsets have the ability to mediate suppression in an antigen non-restricted fashion. Human thymic CD8⁺ CD25⁺ Ts cell subsets share phenotypic similarities with CD4⁺ Treg. They express FoxP3, CTLA-4 and GITR. These cells mediate contact-dependent suppression, which can be blocked by anti-TGF- β - and anti-CTLA-4-neutralizing antibodies. CD8⁺ CD25⁺ Ts cells have also been shown to downregulate CD25 in responder cells [96]. Another subset is the CD8⁺ CD122⁺ Ts cell identified in mice and shown to mediate suppression via IL-10 production [97]. CD122-deficient mice spontaneously develop an experimental autoimmune encephalomyelitis (EAE), which can be prevented by adoptive transfer of CD8⁺ CD122⁺ Ts cells, but not CD4⁺ Tregs [98, 99]. Moreover, CD8⁺ CD122⁺ were able not only to prevent the development of EAE but also to mediate a recovery after the onset of the disease [100], by recognition of activated T cells through conventional MHC class I and $\alpha\beta$ TCR [101]. CD8⁺ CD122⁺ Ts cells have also been shown to play a role in regulating Graves' hyperthyroidism [102]. A microarray analysis of murine CD8⁺ CD122⁺ has revealed a high level of expression of CXCR3. Human and murine CD8⁺ CXCR3⁺ cells have been shown to have suppressor function in vitro and to secrete IL-10 similar to murine CD8⁺ CD122⁺ cells [103]. It is important to mention that CD8⁺ Ts cells can recognize self-peptides restricted by Qa-1 in mice (HLA-E in humans) and attenuate EAE and MS [104–108]. The mechanism of action utilized by these cells has been described as a direct killing of immune cells [109]. The interaction between the responder and the regulatory cells is mediated by the NKG2A receptor, and disruption of this interaction abolishes the ability of CD8⁺ Ts to protect from EAE development [110].

Multiple groups have shown that CD8⁺ Ts can be induced in the periphery. These can be separated into the ones that are activated in a specific cytokine milieu and the ones that are activated by TCR stimulation. Activation of CD8⁺ Ts cells has been shown to be IL-2 and GM-CSF dependent and found to be defective in multiple sclerosis patients. Moreover, the activated cells were not able to mediate suppression in the presence of anti-IFN- γ antibodies

[111]. Another group has shown that CD8⁺ Ts cells can be induced with IL-2 and GM-CSF from healthy subjects but not from systemic erythematosus (SLE) patients, underscoring the roles of IL-2 and GM-CSF. In this instance, the suppressive activity has been blocked by either anti-IFN- γ antibodies or anti-sense oligonucleotides targeting IL-6 [112, 113]. Defects in CD8⁺ Ts cell induction have been shown in several autoimmune diseases, including MS, SLE and systemic sclerosis, and in patients with chronic hepatitis C virus infection or HIV infection [112, 114, 115].

A mixed lymphocyte reaction stimulating the induction of CD8⁺ Ts cells has been reported in multiple studies for over 20 years. A population of CD8⁺ CD28⁻ Ts cells has been induced by xenoreactive or allogeneic stimulation. These cells induce anergy in responder CD4⁺ T cells [110, 116–118]. Multiple groups have described a population of CD8⁺ CD103⁺ T cells. Allostimulation, viral peptides or TGF- β treatment have been shown to induce CD8⁺ CD103⁺ T cells. After the induction by viral peptides, these cells have been found to co-express FoxP3, GITR and CTLA-4, and the suppression is contact dependent [119–121]. Another cell population resembling CD4⁺ Tregs is the CD8⁺ CD25⁺ FoxP3 Ts-expressing cells. The cells are induced through incubation with different types of APCs and can also upregulate CTLA-4 expression, which is involved in mediating contact-dependent suppression [122, 123]. LAG3 and CCL4 have also been shown to be upregulated in CD8⁺ CD25⁺ FoxP3 Ts-expressing cells after activation [124]. A modified anti-CD3 antibody has been shown to induce CD8⁺ Ts cells [125, 126].

Several IBD animal models have demonstrated that CD8⁺ Ts cells have an in vivo suppressor activity and are able to prevent the development of colitis by naïve CD4⁺ T cells. These cells were not able to protect from colitis induction in this model when isolated from IL-10-deficient mice or when co-transferred with naïve CD4⁺ T cells that are defective in their ability to respond to TGF- β [127, 128]. In a mouse model of Crohn's disease, which features overexpression of tumor necrosis factor (TNF Δ ARE mice), CD8⁺ Tregs expressing CD44-CD103⁺ secrete TGF- β . CD8⁺ CD44-CD103⁺ T cells isolated from WT or TNFARE mice reconstitute the CD8⁺ compartment of RAG^{-/-} mice and attenuate the ileitis induced by adoptive transfer of CD4⁺ T cells from TNF Δ ARE mice [128]. Another CD11c⁻ and CD103⁺ CD8⁺ -expressing Ts cell population, isolated from the epithelium of the small intestine, is able to protect against the induction of colitis by naïve CD4⁺ T cells. Interestingly, the same cell population isolated from the colon has not been shown to protect the mice from induction of chronic inflammation [129]. Recent work has shown that TGF- β and RA can induce a CD8⁺ Ts cell population expressing FoxP3 and CTLA-4 with regulatory function. This cell population is reduced in UC patients when compared to normal controls [130].

IEC-activated CD8⁺ Ts cells

Our laboratory has previously shown that IECs can stimulate a specific subset of CD8⁺ T cells. This was first demonstrated using an in vitro IEC/T cell co-culture, showing that only a small fraction of PB T cells could be expanded⁴⁸. IECs express several antigen-presenting molecules, including classical class I and II molecules, non-classical class I molecules (CD1d, human leukocyte antigen E, MICA/B, FcRn) and co-stimulatory molecules (gp180, B7 h or B7H1) [131–133]. In vitro co-culture studies have shown that the presence of a CD1d/gp180 complex (a non-classical MHC class I molecule and a co-stimulatory molecule, respectively) is crucial for the expansion of CD8⁺ Ts cells. We have also shown that IECs isolated from IBD patients have a defect in inducing the expansion of this regulatory T cell population, likely a result of the absence of the CD1d/gp180 complex on the surface of IECs. The expanded CD8⁺ Ts cell populations have been shown to express CD101 and CD103 but not CD28 and to mediate suppression via cell-to-cell contact [134].

CD1d most likely presents glycolipid molecules that are generated from the cell walls of bacteria or host cells. The combination of a CD1d/gp180 complex with a glycolipid antigen might stimulate a unique LPL population. This concept is furthered by the observation that there is a clonal expansion of CD8⁺ VB5.1 T cells in the mucosa compared to peripheral blood. This unique expansion is thought to be a direct result of antigen presentation by IECs. Blocking CD1d and/or gp180 inhibits the expansion of Ts cells. Since LPLs from IBD patients promote inflammation, and IECs from IBD patients do not express the CD1d/gp180 complex, we propose that IBD LPLs lack CD8⁺ Ts cells in their gut. The absence of this population may allow for an unchecked inflammatory response against luminal flora [134].

Conclusion

Controlled/physiologic inflammation is mediated in the intestine by several mechanisms. Different antigens elicit distinct types of immune responses. It is imperative that the mucosal immune system can distinguish between benign versus pathologic insults. Tregs (CD4 and CD8) have the capacity to modify the immune response in the intestine, and defects in any of these cells can lead to disease. Failure to generate these cells can lead to mucosal pathology such as IBD.

Biography

Lloyd Mayer

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