Current Trends in Immunology
Vol. 12, 2011

Review

Phenotype and function of regulatory T cells in the genital tract

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ABSTRACT

T cells with the specialized ability to suppress both adaptive and innate immune responses have been identified and called T regulatory cells (Tregs). The primary function of Tregs is to maintain a balance between immunity (foreign Ag) and tolerance (self Ag) to tissues. Tregs prevent autoimmune disease, maintain immune homeostasis and modulate protective responses against infection. Tregs function in two ways; 1) limiting the magnitude of effector responses which influence the adequate control of infection and 2) control collateral tissue damage caused by vigorous antimicrobial responses against pathogens. Initially, the immune suppressive ability of CD4 T cells was predicted by expression of the forkhead box p3 (Foxp3) transcription factor. However, many reports have demonstrated immune suppressive function in an array of other T cells which include iT_R35, CD8+, NKT cells, especially in mucosal tissues. The immune suppressive mechanisms of Tregs include contact-dependent, cytokine secretion and regulation of immune cell migration. The expanded group of Tregs is crucial for protecting the function of mucosal tissues such as the gut, respiratory and genital tracts, as these tissues are routinely exposed to foreign pathogens.

KEYWORDS: suppressor cells, Tregs, Foxp3, nTreg, iTreg

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INTRODUCTION

T cell-mediated immune suppression of adaptive immune responses is important for the homeostatic function of tissues. Compelling evidence has found that the normal immune system produces T cells specialized in immune suppression called regulatory T cells (Tregs). The majority of Treg cells express the transcription factor, Foxp3, and play a pivotal role in the maintenance of immune tolerance preventing autoimmunity and rejection of transplanted tissue [1, 2]. Recently Tregs have also been implicated in preventing inflammatory diseases. Extensive evidence has shown that Tregs exacerbate and suppress inflammatory responses in various diseases, including the human multiple sclerosis model, experimental autoimmune encephalomyelitis (EAE) [3] and chronic inflammatory bowel disease [4]. Tregs also play major roles in regulating immunity to infections of viral, bacterial or parasitic pathogens. Generally, Tregs restrain immune responses to benefit the individual. However, tumors and microbes commandeer the immune suppressive properties of Treg cells to evade host immunity and cause disease. This is especially prominent at mucosal tissues since they are exposed to a plethora of pathogens. In this review, we will introduce the broad category of Tregs with focus on their phenotype, function and role in maintaining mucosal tissues, especially of the genital tract.

Classification and origin of T regulatory cells

As comprehensive human biological systems, immune regulatory mechanisms are complex; and

effective at containing immune responses to self and foreign antigens, as well as to commensal microorganisms, which are carried out mainly by CD4+Foxp3 expressing Tregs. Presently, Tregs are classified into two subsets: “natural” CD4+Foxp3+ Tregs (nTregs) which emerge from the thymus as a distinct lineage [5, 6]; and “induced” CD4+CD25+ Tregs (iTregs). iTregs have a different developmental program compared to nTregs and develop outside the thymus from CD4+CD25- T cell precursors. They are then converted to Tregs by antigenic stimulation and the surrounding cytokine milieu [7, 8].

Experiments have found that CD25, the high-affinity subunit of the IL-2 receptor, is an important marker of the thymic-derived Tregs. CD4+CD25+ Tregs were capable of preventing autoimmunity not only in neonatal thymectomized mice [9], but also in the lymphopenic animal infused with pathogenic effector T cells [5]. Adoptive transfer of CD25+ T cell-depleted splenocytes into lymphopenic hosts induced a multi-organ autoimmunity syndrome with similar characteristics of neonatal thymectomized mice [5]. Later on, the transcription factor, Foxp3, was found by three independent laboratories to be expressed constitutively by CD25+ Tregs [10-12]. Foxp3 is a forkhead transcription factor family member and mutations in the Foxp3 coding gene were identified as responsible for the immune dysregulation [13]. It was concluded that Foxp3 was mandatory for nTreg development in the thymus and its expression constituted a valuable marker for this independent lineage of T cells [14]. Data has shown that adoptive transfer of nTregs isolated from normal wild type mice significantly prevented disease and related mortality in the Foxp3 mutant mice [14].

Even though iTreg may be phenotypically similar to nTregs, they differ in their developmental requirements and function. iTreg cells differentiate outside of the thymus under more varied conditions. During induction of oral tolerance, iTreg cells first are induced in mesenteric lymph nodes (MLN) in response to microbial and food antigens [15]. iTregs also continuously differentiate in peripheral tissues such as the lamina propria of the gut [16], tumors [17], chronically inflamed tissues [18] and transplanted tissues [19]. The microenvironments that support the development of iTregs are not yet completely understood. However, it was determined that TCR stimulation and the cytokines TGF-β and IL-2 are required [7, 20, 21]. Studies on the gene expression of Foxp3 between the two subtypes of Tregs identified that the Foxp3 locus of nTregs show complete demethylation within an evolutionary conserved region and maintain Foxp3 expression and suppressive functions in the absence of TGF-β stimulation. In contrast, iTregs lose both Foxp3 expression and suppressive functions without TGF-β re-stimulation [22, 23]. Thus, iTregs can be viewed as “transient” suppressive cells.

Transformation of induced T regulatory cells

Naïve CD4+Foxp3- cells can be converted to functional regulatory CD4+CD25+ by cytokines in the environment and are called iTregs. In general, there are two types of iTregs that have been described based on the cytokines which are responsible for their conversion to iTregs: TGF-β+ iTregs and IL-10+ iTregs. Both types of iTregs have suppressive properties *in vitro* and *in vivo* [7, 24, 25]. However, they are quite distinctive on molecular level. TGF-β+ iTregs express Foxp3 and secrete mainly TGF-β whereas IL-10 iTreg do not express Foxp3 after conversion and secrete IL-10.

T cells that are exposed to TGF-β, IL-2 and co-stimulation through the TCR are converted to TGF-β+ iTregs. Chen *et al*. has shown that addition of TGF-β to TCR-stimulated naïve CD4 T cells, induced the transcription of Foxp3, acquisition of anergic and suppressive activities *in vitro*, and the ability to suppress inflammation in an experimental asthma model [7]. Further it has been disclosed that TGF-β induces transcription of Foxp3 and involves cooperation of the transcription factors STAT3 and NFTA at a Foxp3 gene enhancer element [26]. Consistently, *in vivo* neutralization of TGF-β inhibited the differentiation of antigen-specific Foxp3+ iTreg [15] and also blocked iTreg cell-dependent tolerance to tissue grafts in an experimental model [19]. The ability of cells to be converted to iTregs occurs in a finite time frame and depends on the

presence of TGF-β. Conversion takes place only when TGF-β is added within a 2-3 day window of TCR stimulation, and withdrawal of TGF-β results in the loss of Foxp3 within 4 days [27]. Thus, microenvironments commonly found to contain TGF-β, such as the genital tract, have the propensity to produce iTregs.

IL-2 appears to be essential for iTreg cell generation and/or homeostasis. *In vitro*, stimulation of naïve CD4 T cells with anti-CD3 and TGF-β found that IL-2 was required to release the TGF-β-mediated inhibition of proliferation [7]. By neutralizing IL-2 and using IL-2 deficient T cells, Zheng *et al*. has shown that IL-2 is required *in vitro* for TGF-β induction of Foxp3 transcription and suppressor activity [21]. Unlike TGF-β, IL-2 is not required to maintain Foxp3 expression, since iTreg cells transferred into RAG-deficient recipient mice did not lose their suppressive functions [20].

Expansion of T regulatory cells to include other lymphocytes with immune suppressive properties

Recent findings have shifted attention to other types of Tregs which do not fit into the traditional classification scheme described above. One of them is IL-35 induced Tregs (iT_R35) found in both human and animal models [28-30]. IL-35 belongs to the IL-12 cytokine family, including IL-12, IL-23 and IL-27. IL-35 is a heterodimeric cytokine composed of an alpha chain (p19, p28 or p35) and a beta chain (p40 or Ebi3). IL-35 signals through any of five receptor chains (IL-12Rβ1, IL-12β2, IL-23R, gp130 and WSX-1) [31]. Although IL-12, IL-23, IL-27 and IL-35 belong to one family, their tissue source, activity, function and kinetics of expression are quite different. IL-12, IL-23 and IL-27 share the common feature of inducing IFN-γ, promoting Th1 differentiation and proliferation. In contrast, the function of IL-35 is solely suppressive [30]. It has been shown in humans, that IL-35 is required for maximal suppressive capacity of Tregs by upregulating Epstein-Barr-virus-induced gene 3 (EB13) and IL-12A. This was not found to occur with TGF-β or IL-10 exposure. Thus, iT_R35 mediates contact-independent suppression which is IL-35 dependent [32].

Accumulating evidence demonstrates that Tregs are not only defined by markers but also more precisely by their ability to regulate immune responses. CD8+Treg can exercise non-contact dependent regulatory function by secreting IL-10 or increasing IL-4 mRNA to generate more IL-4 [33, 34]. In addition, our group and others have shown that natural killer T (NKT) cells can regulate immune responses and prevent extensive tissue damage [35] (Jiang, J. *et al.*, submitted). Seino, *et al*. reported that NKT cells expressing the invariant chain, Valpha 14, were necessary to produce cardiac allograft acceptance and prevent graft rejection [35]. We have found that CD1d-restricted NKT cells can regulate the number of effector T cells during inflammatory responses by inducing the production of multiple inflammatory cytokines and chemokines and causing accumulation of chronic inflammatory cells in a murine model of chlamydial genital infection (Jiang, J. *et al*., submitted). Thus, there are numerous examples of non-Foxp3 expressing T cells with regulatory functions that are important for controlling immune responses against microbial and alloantigens to prevent excessive inflammation in peripheral tissues.

T regulatory cell function in mucosal tissues

Tregs have been shown to function within inductive secondary lymphoid tissues but also at effector sites. This is particularly important in mucosal sites to maintain homeostasis, tolerance and control excessive immune responses to foreign antigens. Immune suppression has been widely studied in the intestinal mucosa where responses to food antigens must be suppressed. Many groups have shown that in the intestinal mucosa, iTregs are extremely important and critical for maintaining tolerance to food antigens, called oral tolerance [15, 18]. iTreg are induced in the MLN, which is the mucosal inductive site for the intestine and the lamina propria (LP), by TGF-β and retinoic acid-dependent mechanisms. However, this mechanism is restricted to the intestine and does not occur in the spleen or distant peripheral lymph nodes [16, 36, 37]. In contrast, TGF-β can also be involved in inflammatory responses since TGF-β in conjunction with IL-6 was shown to induce the

production of Th17 cells and IL-17 inflammatory responses [38]. However, activation in the presence of retinoic acid inhibits TGF-β/IL-6 mediated Th17 cells and promotes FoxP3 expression [36]. Thus, TGF-β is a central cytokine for regulating pro-inflammatory and immunosuppressive responses mediated by iTreg.

As mentioned above, Tregs can also perform immunosuppressive functions at inductive sites within draining lymph nodes. Homing properties of DCs are very important for the ability to induce iTregs. Production of retinoic acid occurs through CD103+ DCs. The activated CD103+ DC must first be able to induce expression of CCR7 and travel to a MLN in order to promote the production of iTreg during T cell activation. It was shown that the lack of *ccr7* gene in knockout mice prevents development of oral tolerance in CCR7-/- mice [39]. During activation of T cells, retinoic acid also induces the homing receptor, α4β7, and the chemokine which attracts cells to the intestinal mucosa, CCR9 [39-42]. The ability of Tregs to express tissue-specific homing properties appears to follow the same rules as effector T cells [43].

Function of T regulatory cells in the vaginal mucosa

The function of Tregs in periphery differs and depends on the particular microbe and the infection site. Reports have found that Tregs function by suppressing immune responses and limiting tissue damage which develops following pathogen-specific immunity [44-46]. This can be influenced by the infecting microbe. Different groups have shown that Tregs can lose their capacity to suppress immune responses by engagement of pathogen-associated molecular patterns or exposure to pro-inflammatory cytokines [47, 48]. Tregs also influence the composition of immune cells in the genital tract as Tregs were shown to regulate the trafficking of cells between vaginal tissue and the lymph node inductive site in a murine herpes simplex model. Specifically, Lund, *et al*. has found that Tregs influence chemokine secretion in secondary lymphoid organs which interfere with trafficking of immune cells to the vaginal mucosa and viral clearance in herpes simplex infected mice [49]. This implies that microbes activate Tregs which orchestrate immune responses.

Vaginal mucosa and gut mucosa share similarities but also differ in several ways. They both are in direct contact with the external environment and subject to constant assault of exogenous pathogens. Both sites harbor an extensive, yet distinct, bacterial, viral and fungal flora suggesting that immunologic tolerance occurs in genital tract. Unlike the gut mucosa, the vaginal mucosa lacks well organized lymphoid tissues or lymph nodules, such as Peyer's patches, and immune responses against bacterial (*C. trachomatis*), viral (HIV) are weak [50]. In addition, the reproductive tract is under influence of constant hormonal influx, which affects immune competence to fight infections [51].

Tregs in vaginal mucosa may play a dual role in the scenario of infection. They can limit potentially harmful immune activation by suppressing HIV-specific T cell responses and also contribute to viral dissemination as shown above [49]. In gut, evidence shows that the frequency of Tregs is positively correlated with viral load [52]. However, this phenomenon remains unknown in the HIV+ vaginal mucosa. Furthermore, during pregnancy, reproductive Tregs play a crucial role in maintaining tolerance to fetal tissue. Both CD4+CD25+ T cells and uterine NKT cells appear to participate in this process [53, 54]. A study of pregnant women provided evidence that Foxp3+ Tregs migrated from maternal blood to the maternal and fetal interface [53]. Taken together, reproductive mucosal Tregs are essential in maintaining immune system homeostasis and self integrity of epithelial surfaces.

CONCLUSION

T regulatory cells play a central role in adaptive and innate immunity by controlling immune responses and affecting the outcome of tissue inflammation. They initially comprised a phenotypic group of thymic-derived nTregs, which also expressed Foxp3. Currently, the group has been expanded to include a number of T cells (CD4+CD25+Foxp3+, iT_R35, CD8+, NKT cells) which can be induced (iTreg) to acquire immune suppressive function especially at mucosal surfaces. They have been shown to mediate

immune suppression by a number of mechanisms, both contact-dependent and through secretion of cytokines. In addition, T regulatory cells influence immunity at mucosal surfaces by orchestrating the composition of immune cells in response to microbial infection. The combination of phenotype, mechanism of suppression, influence on immune cell migration and type of microbial infection, impart T regulatory cells with a crucial function in mucosal tissues.

ACKNOWLEGEMENTS

This work was supported by a grant from the National Institutes of Health, AI026328 (K. K.).

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