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The Role of T Regulatory Cells in *Chlamydia trachomatis* Genital Infection

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1. 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 with a specialized function in immune suppression and this type of T cell is called a regulatory T cell (TRC). There are various types of TRCs with suppressive function. The majority of TRCs express the transcription factor called forkhead box p3 or Foxp3, and play a pivotal role in the maintenance of immune tolerance by preventing autoimmunity and rejection of transplanted tissue [Grazia Roncarolo et al., 2006; Sakaguchi et al., 2008]. Recently TRCs have also been implicated in preventing inflammatory diseases. Extensive evidence has shown that TRCs exacerbate and suppress inflammatory responses in various diseases, including the human multiple sclerosis model, experimental autoimmune encephalomyelitis (EAE) [Farias et al., 2011] and chronic inflammatory bowel disease [Veltkamp et al., 2011]. TRCs also play major roles in regulating immunity to infections of viral, bacterial or parasitic pathogens. TRCs dampen immune response which control pathogen replication. In many instances, these responses increase pathogen survival. Alternatively, TRCs can also limit collateral tissue damage caused by powerful immune responses directed toward microbes [Belkaid & Tarbell, 2009]. However, tumors and microbes commandeer the immune suppressive properties of TRCs 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 TRCs with focus on their phenotype, function and role in maintaining mucosal tissues, especially of the genital tract.

2. Characterization of T regulatory cells in mice and humans

Control of immune responses is critical to host survival and there are many mechanisms that can mediate control. Intrinsic control mechanisms exist which are programmed as the immune system develops. However, control also exists at the cellular level and involves interaction with specialized TRCs. TRCs are categorized into two general compartments based on their origin, mechanism of action, and generation; natural TRCs (nTRCs) or induced TRCs (iTRCs). The distinction between the two has been blurred by showing that regulatory function can be induced in previously non-regulatory T cells. T regulatory

functions can be induced by signals received in the environment such as; regulatory cytokines, immunosuppressive drugs and antigen presenting cells (APC) modified by infectious agents [Belkaid & Tarbell, 2009]. Thus, iTRCs can be further divided into Tr1 cells which secrete IL-10, TRCs which secrete TGF-β and TRCs which express Foxp3. However, all of these markers of T regulatory function, with the exception of Foxp3, do not always correlate with suppressive function. The finding that loss of function mutations in the Foxp3 gene of humans lead to a severe multi-organ autoimmune and inflammatory syndrome called immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX) and a similar disorder in scurfy mice allowed the definitive identification of TRC [Bennett et al., 2001; Brunkow et al., 2001; Chatila et al., 2000; Wildin et al., 2001].

Subsequent studies focused on the ability of $Foxp3^+$ cells to cause various tissue pathologies. Foxp3 is primarily expressed in CD4 cells. The first approach was in knock-in mice which showed that cell-intrinsic regulatory functions did not rely on Foxp3 but it was indispensible for lack of expression and was responsible for disease in humans and mice

[Chen et al., 2005; Fontenot et al., 2003; Fontenot et al., 2005; Hsieh et al., 2006; Wan & Flavell, 2005]. Further studies using anti-Foxp3 antibodies or conditional knock-out mice in the Cre-lox system to target various epithelial cells, proved without a doubt, that the suppressive function of Foxp3-dependent T cells was important for immune homeostatsis and tissue integrity [Kim et al., 2009; Liston et al., 2007; Rudensky, 2011].

The balance of TRCs with other immune cells has a bearing on immunity and immunopathology after infection. Evidence has been reported for two functions of TRCs in immunity against infection. In the first, increasing the number of TRCs interferes with pathogen elimination and supports survival and persistence in humans and mice. Examples are *Leishmania* [Belkaid et al., 2006; Belkaid et al., 2002; Campanelli et al., 2006], *Plasmodium*, [Amante et al., 2007; Hisaeda et al., 2004; Torcia et al., 2008; Walther et al., 2005] and *Mycobacteria* [Chen et al., 2007; Kursar et al., 2007; Scott-Browne et al., 2007]. The pathogen exploits TRCs to its advantage to persist in the host. Secondly, while decreasing the number of TRCs leads to better pathogen control, it also increases the immunopathology formed. Examples of this type of effect of TRCs include infection with herpes simplex virus (HSV) [Suvas et al., 2004] and *C. albicans* [Montagnoli et al., 2002].

A few studies have examined the involvement of TRCs in chlamydial infections. As described below, TRCs are found in a number of mucosal surfaces of which *Chlamydia* cause infection. The first study focused on *C. trachomatis* infection of the ocular mucosa. *C. trachomatis* infects the conjunctiva of the eye and eventually facilitates accumulation of inflammatory cells and organized follicle formation or clinical signs of progression to trachoma. The authors, Faal, N. et al. [Faal et al., 2006] divided individuals into three groups: Group 1: those with acute infection as defined as chlamydial PCR positive; Group 2: those with serological evidence of past chlamydial infection plus clinical disease signs of trachoma; and Group 3: those with serological evidence of past chlamydial infection and no signs of clinical disease. The authors showed that *FOXP*3 mRNA was found in Group 1 (only acute infection) and Group 2 (past infection and clinical disease) but not in Group 3 or those with past chlamydial infection but no signs of developing trachoma. The data clearly showed that during acute infection, *FOXP*3 mRNA was present. However, it was intriguing that Foxp3 transcripts continued to be elevated despite the fact that acute infection had resolved in the group that presented with clinical signs of trachoma development. The

authors speculated that since the presence of Foxp3 transcripts correlated with acute infection, Foxp3 was present to protect conjunctival tissue from immune damage during acute infection. However, its continued presence in the group which had resolved the disease but also showed signs of clinical disease, suggested that Foxp3 was unable to prevent tissue damage in certain individuals [Faal et al., 2006]. The mechanism of tissue protection has not been determined and one must exercise caution when interpreting data based on FOXP3 transcript levels as opposed to expression of protein in cells since these do not directly correlate [Probst-Kepper, 2006].

TRCs cells have also been identified in the lungs of mice infected with *C. pneumoniae* using Foxp3 protein expression in cells identified by flow cytometry. *C. pneumoniae* infection of the lung regulates the degree of T cell activation and can exacerbate development of asthma. In this model, depletion of TRCs increases T cell activation and lung tissue damage [Crother et al., 2011; Schröder et al., 2008]. These data further supported the hypothesis that TRCs prevent tissue from damaging immune responses.

TRCs are also present in the genital mucosa during chlamydial infection. Marks, E. *et. al.* [Marks et al., 2010] found Foxp3 expression in the upper genital tracts of mice following chlamydial genital infection. The expression of Foxp3 also corresponded to the number of Th1 cells in that site. These authors further noted that depleting TRCs increased immunopathology in the upper genital tract [Marks et al., 2007]. Similar to the trachoma study above, only transcripts of *FOXP*3 were followed in tissue as evidence of the presence of TRCs. Recently; we have shown that Foxp3+ TRCs are present in the genital tract after infection with the murine model of *C. trachomatis* infection, *C. muridarum*. TRCs were identified by expression of Foxp3 protein on the cell surface and quantitiated by flow cytometry. We found that $Foxp3^+$ TRCs peaked during early infection and correlated with the disappearance of Th1 cells in the genital tract [Moniz et al., 2010]. We have further investigated the role of TRCs during genital infection using Foxp3-EGFP-DTR mice (gift from T. Chatila). The $Foxp3^+$ TRCs can be depleted by administration of diphtheria toxin and followed by monitoring green fluorescent TRCs [Haribhai et al., 2011]. Our preliminary studies show that Th1 cell numbers inversely correlate with the number of TRCs. Further, the depletion of TRCs before and during early infection, at a time when TRCs peak in the genital tract, resulted in a decrease in tissue pathology in the oviducts (unpublished data). This suggests that TRCs do not protect upper genital tract tissue from pathology but instead contribute to tissue pathology by interfering with the eradicating function of Th1 cells in the genital tract.

The few studies reported in chlamydial infections have provided evidence that TRCs can both protect tissue and contribute to tissue damage as described above. These reports differ in the means TRCs were defined, by FOXP3 mRNA transcripts or cellular protein expression. In addition, they differ in the type of mucosal surface studied, such as conjunctiva, lung and genital tract. Although the mechanisms by which TRCs influence immune responses have been identified, none have been thoroughly examined in chlamydial infection. However, the data reported are consistent in that they all implicate TRCs with tissue pathology, either by prevention or exacerbation. Therefore, one can conclude that TRCs play a role in tissue pathology following chlamydial infection and additional studies are needed for a complete understanding of the mechanism(s). We would propose that TRCs form a third type of function in chlamydial infection; TRCs interfere with

the elimination of organism but do not enhance chronic persistence of the organism and instead contribute to tissue pathology by prolonging organism elimination from tissues.

2.1 Defining T regulatory cells by expression of phenotypic markers

Complex human biological systems require immune regulatory mechanisms which are effective at containing immune responses to self and foreign antigens, as well as to commensal microorganisms. Presently, TRCs are classified into two subsets: "natural" $CD4^+Foxp3^+$ TRCs (nTRCs) which emerge from the thymus as a distinct lineage [Fontenot et al., 2005; Sakaguchi et al., 1995]; and "induced" $CD4^+CD25^+$ TRCs (iTRCs). iTRCs have a different developmental program compared to nTRCs and develop outside the thymus from $CD4^+CD25^-$ T cell precursors. They are then converted to TRCs by antigenic stimulation and the surrounding cytokine milieu [Chen et al., 2003; Curotto de Lafaille et al., 2004].

Experiments have found that CD25, the high-affinity subunit of the IL-2 receptor, is an important marker of thymic-derived TRCs. $CD4^+CD25^+$ TRCs were capable of preventing autoimmunity not only in neonatal thymectomized mice [Asano et al., 1996], but also in the lymphopenic animal infused with pathogenic effector T cells [Sakaguchi et al., 1995]. Adoptive transfer of $CD25^+$ T cell-depleted splenocytes into lymphopenic hosts induced a multi-organ autoimmunity syndrome with similar characteristics of neonatal thymectomized mice [Sakaguchi et al., 1995]. Later on, the transcription factor, Foxp3, was found by three independent laboratories to be expressed constitutively by $CD25^+$ TRCs [Fontenot et al., 2003; Hori et al., 2003; Wildin et al., 2002]. Foxp3 is a forkhead transcription factor family member and mutations in the Foxp3 coding gene were identified as responsible for the immune dysregulation [Brunkow et al., 2001]. It was concluded that Foxp3 was mandatory for the development of nTRCs in the thymus and its expression constituted a valuable marker for this independent lineage of T cells [Kim & Rudensky, 2006]. Data has shown that adoptive transfer of nTRCs isolated from normal wild type mice significantly prevented disease and related mortality in the Foxp3 mutant mice [Kim & Rudensky, 2006].

Even though iTRCs may be phenotypically similar to nTRCs, they differ in their developmental requirements and function. iTRCs differentiate outside of the thymus under more varied conditions. During induction of oral tolerance, iTRCs first are induced in mesenteric lymph nodes (MLN) in response to microbial and food antigens [Mucida et al., 2005]. iTRCs also continuously differentiate in peripheral tissues such as the lamina propria of the gut [Coombes et al., 2007], tumors [Liu et al., 2007], chronically inflamed tissues [Curotto de Lafaille et al., 2008] and transplanted tissues [Cobbold et al., 2004]. The microenvironments that support the development of iTRCs are not yet completely understood. However, it was determined that TCR stimulation and the cytokines TGF-β and IL-2 are required [Chen et al., 2003; Davidson et al., 2007; Zheng et al., 2007]. Studies on the gene expression of Foxp3 between the two subtypes of TRCs identified that the Foxp3 locus of nTRCs show complete demethylation within an evolutionary conserved region and maintain Foxp3 expression and suppressive functions in the absence of TGF-β stimulation. In contrast, iTRCs lose both Foxp3 expression and suppressive functions without TGF-β re-stimulation [Floess et al., 2007; Huehn et al., 2009]. Thus, iTRCs can be viewed as "transient" suppressive cells.

2.2 Types of T regulatory cells

There are multiple subsets of Foxp3$^+$TRCs that exist within an individual [Stephens et al., 2007]. The majority is CD4$^+$ but small number of CD8$^+$, CD4$^+$CD8$^+$ and CD4$^-$CD8$^-$ αβTCR^{hi} thymocytes and peripheral T cells are also found. Not all Foxp3$^+$TRCs are MHC class I or II restricted [Stephens et al., 2007]. In addition, Foxp3$^+$TRCs can be categorized as "natural". This subset is constitutively present and prevents development of immune responses against self-tissues. In contrast, subsets of Foxp3$^+$TRCs are also induced by inflammation or infection and are call "inducible or adaptive" Foxp3$^+$TRCs. It has been debated whether nTRCs and iTRCs are separate types of TRCs with differing function. [Bluestone & Abbas, 2003]. Both iTRCs and nTRCs have suppressive function as shown by their ability to prevent T cell activation [Fantini et al., 2006; Huter et al., 2008; Mottet et al., 2003]. However, the contribution of each cell type to peripheral tolerance is dependent on the model studied [Curotto de Lafaille et al., 2008; Haribhai et al., 2009]. Recent data has disclosed that each subset of TRCs have distinct functions; nTRCs prevent lethal disease while iTRCs prevent chronic inflammation and mostly have distinct TCR repertoires [Haribhai et al., 2011]. The TRCs that participate in chlamydial infection appear to be members of iTRCs and we will discuss them in depth.

2.2.1 Inducible or transient tregs

Naïve CD4$^+$Foxp3$^-$ cells can be converted to functional regulatory CD4$^+$CD25$^+$ by cytokines in the environment and are called iTRCs. In general, there are two types of iTRCs that have been described based on the cytokines which are responsible for their conversion to iTRCs: TGF-β$^+$ iTRCs and IL-10$^+$ iTRCs. Both types of iTRCs have suppressive properties *in vitro* and *in vivo* [Chen et al., 2003; Grazia Roncarolo et al., 2006; Groux et al., 1997]. However, they are quite distinctive on molecular level. TGF-β$^+$ iTRCs express Foxp3 and secrete mainly TGF-β whereas IL-10 iTRCs do not express Foxp3 after conversion and secrete IL-10.

T cells that are exposed to TGF-β, IL-2 and are stimulated by co-stimulation through the TCR are converted to TGF-β+ iTRCs. 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 [Chen et al., 2003]. Further it has been disclosed that TGF-β induces transcription of *FOXP*3 and involves cooperation of the transcription factors STAT3 and NFTA at a Foxp3 gene enhancer element [Josefowicz & Rudensky, 2009]. Consistently, *in vivo* neutralization of TGF-β inhibited the differentiation of antigen-specific Foxp3+ iTRCs [Mucida et al., 2005] and also blocked iTRCs cell-dependent tolerance to tissue grafts in an experimental model [Cobbold et al., 2004]. The ability of cells to be converted to iTRCs 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 [Selvaraj & Geiger, 2007]. Thus, microenvironments commonly found to contain TGF-β, such as the genital tract, have the propensity to produce iTRCs.

IL-2 appears to be essential for the generation and/or homeostasis of iTRCs. *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 [Chen et al., 2003]. By neutralizing IL-

2 and using IL-2 deficient T cells, Zheng et al. has shown that IL-2 is required for *in vitro* TGF-β induction of Foxp3 transcription and suppressor activity [Zheng et al., 2007]. Unlike TGF-β, IL-2 is not required to maintain Foxp3 expression, since iTRCs transferred into RAG-deficient recipient mice did not lose their suppressive functions [Davidson et al., 2007].

2.2.2 Antigen specificity

Recent findings have shifted attention to other types of TRCs which do not fit into the traditional classification scheme described above. One of them is IL-35 induced TRCs found in both human and animal models [Belkaid & Chen, 2010; Collison et al., 2010; Collison et al., 2007]. 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)[Collison & Vignali, 2008]. 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 [Collison et al., 2007]. It has been shown in humans, that IL-35 is required for maximal suppressive capacity of TRCs 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, IL-35 secreting TRCs mediate contact-independent suppression which is IL-35 dependent [Chaturvedi et al., 2011].

Accumulating evidence demonstrates that TRCs are not only defined by markers but also more precisely by their ability to regulate immune responses. $CD8^+$TRCs can exercise non-contact dependent regulatory function by secreting IL-10 or increasing IL-4 mRNA to generate more IL-4 [Gilliet & Liu, 2002; Zhou et al., 2001]. In addition, our group and others have shown that natural killer T (NKT) cells can regulate immune responses and prevent extensive tissue damage [Seino et al., 2001; 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 [Seino et al., 2001]. We have found that CD1d-restricted NKT cells, activated by antigens contained in chlamydial elementary bodies, can regulate the number of effector T cells during inflammatory responses by inducing the production of multiple inflammatory cytokines and chemokines. The prolonged induction of chemokines results in the accumulation of T cells dominated by Th1 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 which prevent excessive inflammation in peripheral tissues.

3. Understanding mechanisms of T regulatory cell function

Regulatory T cells play a crucial role in self-antigen tolerance, tissue grafts, and suppression of autoimmune reactions. These cells modulate the intensity and quality of immune responses through attenuation of the activities of reactive immune cells. They modulate immunity by 1) secreting inhibitory cytokines, 2) direct killing cytolysis, 3) metabolic disruption of T cells and 4) modulation of dendritic cell maturation or function.

3.1 Suppression by inhibitory cytokines

TRCs cells produce immunoregulatory cytokines at the site of inflammation. Those regulatory cytokines, including IL-10, IL-35, TGF-β, directly affect the activity of cytotoxic T cells and antigen presenting cells (APCs).

3.1.1 IL-10

IL-10 released by TRCs down regulates the ability of APCs to produce IL-12 and further inhibits the differentiation and responses of Th1-type cells [Moore et al., 2001]. Interaction of T cells with APCs triggers IL-2 production, which acts to enhance reactive T cell proliferation. Thus IL-10 reduces the activity of APCs and indirectly lowers the intensity of entire immune reaction through inhibition of IL-2 production.

3.1.2 IL-35

IL-35 is a newest member of the IL-12 family. In the CD4 T cell population, IL-35 is expressed by resting and activated TRCs but not effector cells [Collison et al., 2007]. In addition, it has been suggested that IL-35 can suppress Th17 development *in vivo* and improve collagen-induced arthritis [Niedbala et al., 2007]. More studies are needed to define the mechanism.

3.1.3 TGF-β

TGF-β reduces cytokine secretion by activated CD4 T cells [Zheng et al., 2004], without limiting their capacity to expand and without inducing their apoptosis [Cottrez & Groux, 2001]. TGF-β also induces IL-10 production in Th1 cells, which further inhibits cytokine production and directly attenuates effector T cell function [Annacker et al., 2001]. In a correlative interaction, IL-10 also enhances the response of activated T cells to TGF-β [Cottrez & Groux, 2001]. Therefore, the combined effects of TGF-β and IL-10 inhibit the activity of effector T cells with minor changes on their expansion

3.2 Suppression by cytolysis

One other potential mechanism for regulatory T cell mediated suppression would be cytolysis of target cells. Many human $CD4^+$ cells display the ability to lyse other cells via cytotoxic mechanisms. Together, TRCs in certain contexts can differentiate and function as cytotoxic suppressor cells.

3.2.1 Granzyme A

It has been reported that human $CD4^+CD25^+Foxp3^+$ TRCs can be activated and lyse target cells which requires granzyme A and perforin [Grossman et al., 2004]. The authors further showed that granzyme A and perforin mediated target cell lysis through adhesion of CD18.

3.2.2 Granzyme B

Activation of mouse TRCs cells also lead to up-regulation of granzyme B expression [Gondek et al., 2005]. The up-regulation of granzyme B induced a reduction in contact mediated suppression by TRCs *in vivo* [Gondek et al., 2005].

3.2.3 Perforin

Although other cell types required granzyme B and perforin to mediate cytoxicity, it is not true for cytotoxicity mediated by TRCs. This is shown by the independent suppression of contact sensitivity by TRCs using perforin-/- mice [Gondek et al., 2005].

3.3 Suppression by metabolic disruption

There are several examples of TRCs mediating suppression by metabolic disruption. These include IL-2 cytokine deprivation and intracellular or extracellular release of adenosine nucleosides [Vignali et al., 2008a].

3.3.1 IL-2 cytokine deprivation

T effector cells require IL-2 for growth but TRCs do not and instead use IL-7. The hypothesis of IL-2 mediated suppression is that CD25 expression could cause the consumption of IL-2 and "starve" T effector cells [de la Rosa et al., 2004; Thornton & Shevach, 1998]. One study has reported this occurs by inducing apoptosis [Pandiyan et al., 2007].

3.3.2 Cyclic AMP-mediated inhibition

TRCs have been reported to transfer the ability of cyclic AMP (cAMP) to mediate suppression by passing on the cAMP to T effector cells through gap junctions [Bopp et al., 2007]. However, this is the only study which reports this mechanism and further reports are needed as confirmation that this is a general mechanism of suppression.

3.3.3 Adenosine receptor-2A

TRCs have been shown to express the ectoenzymes, CD39 and CD73 and generate adenosine secretion [Deaglio et al., 2007; Kobie et al., 2006]. Development of TRCs does not occur in the presence of IL-6 and also requires the presence of TGF-β. The binding of adenosine to the adenosine receptor-2A, not only suppresses T effector cell function but also produces additional TRCs by inhibiting IL-6 production and favoring TGF-β secretion [Zarek et al., 2008].

3.4 Suppression by modulation of dendritic cell maturation or function

There is evidence to support a function of TRCs which act directly on dendritic cells to influence the ability of dendritic cells to activate effector T cells [Bluestone & Tang, 2005; Tang et al., 2006].

3.4.1 Modulation of co-stimulatory molecules

Many studies have reported that TRCs reduce effector T cell function by acting on dendritic cells to influence their maturation [Lewkowich et al., 2005; Misra et al., 2004; Serra et al., 2003]. One of the prominent means for a dendritic cell to influence effector T cell function is modulation of co-stimulatory molecules. Studies have disclosed this function by showing that the use of antibodies which block the function of T-lymphocyte antigen-4 (CTLA4) or CLTA4-deficient T cells have a reduction in the suppression of effector T cells [Oderup et al.,

2006; Serra et al., 2003]. Alternatively, TRCs can also act on dendritic cells to decrease the expression of CD80 and CD86 [Cederbom et al., 2000].

3.4.2 Indoleamine 2,3-dioxygenase

TRCs have also been shown to alter effector T cell function by causing the dendritic cells to produce indoleamine 2,3-dioxygenase (IDO). IDO has the ability to regulate cellular function by encouraging apoptosis by producing precursors from the catabolism of tryptophan. This also results in the down-regulation of CTLA4, CD80 and CD86 [Fallarino et al., 2003; Mellor & Munn, 2004].

3.4.3 LAG/MHC class II

TRCs have also been reported to mediate suppression through the expression of lymphocyte activation gene-3 (LAG3). LAG3 molecules on murine [Liang et al., 2008)] TRCs bind to MHC II molecules on immature dendritic cells and suppress maturation by transducing an inhibitory signaling pathway. Alternatively, human TRCs have been shown to express a greater amount of LAG3 and potentially could interact directly with effector T cells [Baecher-Allan et al., 2006].

4. Induction of T regulatory cells

As described above, although many types of TRCs have been identified, two major subsets have emerge; nTRCs and iTRCs. The nTRCs mature in the thymus [Fontenot et al., 2005; Sakaguchi et al., 1995]. On the other hand, iTRCs have a different developmental program compared to nTRCs and develop outside the thymus from $CD4^+CD25^-$ T cell precursors. They are then converted to TRCs by antigenic stimulation and the surrounding cytokine milieu [Chen et al., 2003; Curotto de Lafaille et al., 2004]. However, both subsets are identified by the expression of Foxp3.

4.1 Expression of Foxp3

Foxp3 is a forkhead transcription factor family member and mutations in the Foxp3 coding gene were identified as responsible for the immune dysregulation [Brunkow et al., 2001]. The FOXP3 gene is essential for the ability of nTRCs to mature in the thymus and this subset always expressed Foxp3 [Kim & Rudensky, 2006]. This subset of TRCs is important throughout the life of the individual for preventing lethal autoimmune diseases [Kim & Rudensky, 2006]. Conversely, iTRCs only transiently express Foxp3 and Foxp3 expression correlates with suppressive function [Floess et al., 2007; Huehn et al., 2009]. Thus, expression of Foxp3 is necessary for TRCs to suppress immune responses.

4.2 Infections associated with T regulatory cell function

Pathogens are not the only culprits of tissue inflammation. Adaptive immune responses against host-antigens which have escaped deletion or control in the periphery provoke tissue inflammation [Rudensky et al., 2006]. This has been demonstrated during transplantation rejection and autoimmune diseases. Pathogens associated with chronic infections are hypothesized to encourage immune responses against host antigens.

Alternatively, microbial infections may simultaneously recruit regulatory cells to tissues to prevent inflammation while anti-microbial immune responses eliminate the pathogen [Sinclair, 2004]. The inability of a host to mount and recruit a sufficient regulatory response in tissues appears to result in tissue inflammation [Sather et al., 2007].

4.3 Dendritic cell populations

There have been many reports that TRCs target dendritic cells to mediate immune suppression. The interaction of TRCs with immature or activated myeloid dendritic cells or marrow-derived dendritic cells results in the down-regulation of co-stimulatory molecules CD80, CD86 and CD40 and MHC on dendritic cells [Tadokoro et al., 2006] as well as up-regulation of inhibitory factors [Mahnke et al., 2007], leading to impaired T cell stimulatory function of dendritic cells. Consistent with *in vitro* data, *in vivo* evidence in experiment models show that TRCs inhibit T cell immune response mediated by dendritic cells at various locations [Mahnke et al., 2007]. TRCs exert an early effect on immune responses by attenuating the establishment of stable contacts during priming of naïve T cells and dendritic cells and by forming synapses and aggregation with dendritic cells more frequently than with naïve T cells. Moreover, visualization of adoptively transferred TRCs in the lymph nodes of mice revealed that TRCs form stable associations with dendritic cells that in turn prevents subsequent strong interactions between dendritic cells and autoreactive effector T cells [Tang & Bluestone, 2006]. Together, strong evidence implies that dendritic cells are the primary targets of TRCs *in vivo* [Tadokoro et al., 2006; Tang et al., 2006].

Dendritic cells are needed to influence the development of adaptive TRCs. Production of Foxp3$^+$TRCs can be induced by plasmacytoid dendritic cells (pDC) by causing expression of Foxp3 in non-regulatory T cells within peripheral tissues. However, the precise dendritic cell subset is under debate and actively investigated [Tang & Bluestone, 2006]. The current consensus states that immature conventional dendritic cells and pDC can induce expression of Foxp3 in non-regulatory T cells within peripheral tissues. Although little is known regarding the interaction of pDCs and TRCs, recent evidence shows that this interaction occurs in draining lymph nodes and not spleen, and is specific for foreign antigens [Ochando et al., 2006]. Evidence suggests that newly activated Foxp3$^+$TRCs may act on conventional dendritic cells to limit production of T effector cells [Kim et al., 2007]. Plasmacytoid dendritic cells induce Foxp3 expression by interacting with T cells via certain co-stimulatory molecules such as ICOS-L [Akbari et al., 2002].

5. Migration of T regulatory cells

TRCs also influence the composition of immune cells in the genital tract as TRCs 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 TRCs influence chemokine secretion in secondary lymphoid organs which interferes with the trafficking of immune cells to the vaginal mucosa and viral clearance in herpes simplex infected mice [Lund et al., 2008]. This implies that microbes activate TRCs which orchestrate immune responses.

5.1 Chemokine receptor expression

As mentioned above, TRCs can also perform immunosuppressive functions at inductive sites within draining lymph nodes. Homing properties of dendritic cells are very important for their ability to induce iTRCs. Production of retinoic acid occurs through $CD103^+$ dendritic cells. The activated $CD103^+$ dendritic cells must first be able to induce expression of CCR7 and travel to a MLN in order to promote the production of iTRCs during T cell activation. It was shown that the lack of *ccr7* gene in knockout mice prevents development of oral tolerance in CCR7-/- mice [Mora et al., 2003]. During activation of T cells, retinoic acid also induces the homing receptor, α4β7, and the chemokine which attracts cells to the intestinal mucosa, CCR9 [Iwata et al., 2004; Mora et al., 2003; Papadakis et al., 2003; Svensson et al., 2002]. The ability of TRCs to express tissue-specific homing properties appears to follow the same rules as effector T cells [Siewert et al., 2007].

5.2 Control of migration of CD8+ cells

An additional function of TRCs that requires further investigation is their ability to migrate into tissue to prevent or control the activation of effector T cells. In a $CD8^+$ model of type I diabetes, transgenic expression of TNF-α was shown to be necessary for the TRCs to enter the pancreas, accumulate and prevent destruction and development of $CD8^+$ cells specific for islet cells [Green et al., 2003]. In support of this, a subset of TRCs has been shown to express CCR6 and accumulate in the CNS of mice which have EAE [Kleinewietfeld et al., 2005]. Taken together, these data suggest that a subset of TRCs may prevent the continued activation of effector T cells within peripheral tissues.

6. Suppression of specific cell populations by T regulatory cells

The majority of studies show that TRCs inhibit functions of effector T cells. However, TRCs have been reported to control B cells [Zhao et al., 2006]. In addition, TRCs have also been shown to prevent the killing of tumor cells [Cao et al., 2007]. It is anticipated that other types of cells will also be regulated by TRCs since they are important for controlling a plethora of diseases.

6.1 Targeting dendritic cells

Several reports have demonstrated that TRCs modulate the maturation, activation and function of various subsets of human and murine dendritic cells both *in vitro* and *in vivo*. This gives further evidence that TRCs "educate" dendritic cells and impact outcomes of immune responses [Mahnke et al., 2007; Tadokoro et al., 2006; Tang et al., 2006]. *In vitro*, the interaction of TRCs with immature or activated myeloid dendritic cells or marrow-derived dendritic cells results in the down-regulation of co-stimulatory molecules CD80, CD86 and CD40 and MHC [Tadokoro et al., 2006] and up-regulates inhibitory factors [Mahnke et al., 2007]. TRCs also target dendritic cells and form stable interactions preventing naïve T cell activation [Tang & Bluestone, 2006]. Since TRCs regulate many types of immune responses it is plausible that dendritic cells are a major target of TRCs [Tadokoro et al., 2006; Tang et al., 2006].

6.2 CD4 effector T cells

A major target of TRCs is CD4 effector T cells. TRCs have been reported to prevent activation by directly acting on effector T cells to prevent clonal expansion and proliferation by limiting access to IL-2 [Thornton & Shevach, 1998], modulating T cell activation through exposure by suppressive cytokines such as IL-10 [Annacker et al., 2001] and TGF-β [Zarek et al., 2008]. These diverse ways to prevent T cell activation suggest that there are either multiple subsets of TRCs or one type of TRC with plastic development. This has been debated in recent review and likely will be the subject of ongoing research [Vignali et al., 2008b].

6.3 Innate immune cells

There are a few reports of TRCs modulating the functions of innate immune cells, particularly monocytes and macrophages [Taams et al., 2005; Tiemessen et al., 2007]. These reports use human cells and may be specific to humans.

7. Implications (implications) for vaccines and therapies to prevent reproductive tract inflammation

The primary function of TRCs is to suppress immune responses which are harmful for the individual and maintain survival. As we have reviewed, TRCs are important for numerous diseases and mediate suppression thorough a number of mechanisms. This suggests that certain TRCs as defined by function or phenotype can be exploited as therapeutics to prevent autoimmune disease. This has been accomplished in mice to prevent joint inflammation using two approaches; genetic transfer of TCRs from TRCs and the transfer of Foxp3 cells [Wright et al., 2009]. TRCs have also been proposed to prevent allergy and may replace tolerating injections which are effective for preventing allergic reactions [Robinson et al., 2004]. However, TRCs may also interfere with beneficial immune responses and inhibit tumor or pathogen eradication as described above. In the specific case of developing a vaccine for chlamydial infection, speculation on TRCs is premature, and will likely depend on the tissue site of infection as shown in the few studies to date. The ability of TRCs to prevent asthma may encourage preventative therapeutics. Chlamydial infection is widespread across the world and 92 million cases of genital infection and near 40 million cases of blindness due to chlamydial infection was reported around the beginning of the decade [Resnikoff et al., 2004; WHO, 2004]. This number of infections suggests that harnessing of TRCs could greatly reduce morbidity following infection.

8. Conclusion

T regulatory cells (TRCs) 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 natural TRCs (nTRCs), which also expressed Foxp3. Currently, the group has been expanded to include a number of T cells ($CD4^+CD25^+Foxp3^+$, IL-35 secreting TRCs, CD8+ and NKT cells) which can be induced 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, TRCs 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 TRCs with a crucial function in mucosal tissues.

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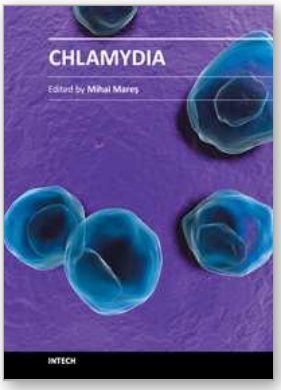

Chlamydia

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Nowadays, Chlamydia still represents a redoubtable pathogen. Among its consequences, the blindness in children and severe impairment of reproductive health in adults are the most mutilating. Worldwide, it is estimated that six million of people suffer from post-trachoma blindness and almost 90 million become sexually infected each year. Due to its silent evolution and sexually transmission, the chlamydial infection can occur in anyone. The book “Chlamydia - A Multifaceted Pathogen” contains an updated review of all-important issues concerning the chlamydial infection. It comprises 18 chapters grouped in four major parts dealing with etiology and pathogenicity, clinical aspects, diagnosis and prevention. The new molecular data about the pathogenicity and the exhaustive presentation of clinical findings bring novelty to the book and improve our knowledge about Chlamydia induced diseases.

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