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Song-Guo Zheng *Editor*

T Regulatory Cells in Human Health and Diseases

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Regulatory T Cells: Concept, Classification, Phenotype, and Biological Characteristics

1

Yang Du, Qiannan Fang, and Song-Guo Zheng

Abstract

Regulatory T cells (Treg) play an indispensable role in maintaining the body's immune nonresponse to self-antigens and suppressing the body's unwarranted and potentially harmful immune responses. Their absence, reduction, dysfunction, transformation, and instability can lead to numerous autoimmune diseases. There are several distinct subtypes of the Treg cells, although they share certain biological characteristics and have unique phenotypes with different regulatory functions, as well as mechanistic abilities. In this book chapter, we introduce the latest advances in Treg cell subtypes pertaining to classification, phenotype, biological characteristics, and mechanisms. We also highlight the relationship between Treg cells and various diseases, including autoimmune, infectious, as well as tumors and organ transplants.

Keywords

Treg cells · Immune response · Classification · Immunological diseases · Cell therapy

1.1 Introduction

Five decades ago, a subset of T cells with immunosuppressive properties was described by Dr. Richard Gershon and colleagues at Yale University (Gershon and Kondo 1970, 1971). However, the existence of this suppressor T cell was questioned due to lack of reliable markers. However, two decades later, Sakaguchi and his colleagues demonstrated that a CD4+ subpopulation that constitutively expresses CD25 (IL-2 receptor alpha chain) in mouse thymus showed inhibitory activity. This discovery led scientists to revisit the study of suppressor or regulatory T cells (Treg) (Sakaguchi et al. 1995). These thymus-derived naturally occurring CD4+CD25+ (tTreg) cells migrate to the periphery 3 days after birth and constitute 5–10% of the peripheral CD4+ T cells in normal naïve mice. Moreover, these cells have critical functional abilities since the removal of these CD4+CD25+ T cells causes a discrete autoinflammatory clinical phenotype: gastritis, thyroiditis, and type 1 diabetes (T1D). These manifestations were similar to which was

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seen in the neonatal thymectomy (NTx) mice. For a limited time after thymectomy, adoptively transferring normal CD4+CD25+ T cells can prevent the development of autoimmunity in these mice (Kojima and Prehn 1981; Sakaguchi et al. 1995; Itoh et al. 1999). Thus, CD4+CD25+ was reestablished as a Treg population. However, since CD25 is also an activation marker for CD4 + cells, its expression on CD4+ cells cannot be viewed as a specific marker for Treg cells.

A decade later, a milestone observation was made; describing the expression of the transcription factor Foxp3 (mice) or Foxp3 (human) in CD25+CD4+ in rodents and humans, respectively, can specifically define the Treg population (Fontenot et al. 2003; Khattri et al. 2003; Hori et al. 2003). Functional deletions and mutations in the human Foxp3 gene can cause severe autoinflammatory disease. The clinical syndrome resulting from this deletion was described much earlier and was called immune dysregulation, polyendocrinopathy, enteropathy, and X-linked (IPEX) syndrome (Powell et al. 1982). Thus, Foxp3 is a specific marker for these cells, and CD4+CD25+Foxp3+ cell population is designated regulatory T cells. Indeed, Treg cells, which naturally express Foxp3 in the nucleus and CD25 on the cell surface, are present in normal individuals and actively participate in suppressing aberrant or unwarranted immune responses against self, microorganisms, and bacteria (Martín-Orozco et al. 2017; Grant et al. 2015). Additionally, recent studies have established that the Treg cell lineage requires not only the transcription factor Foxp3 but also the establishment of Treg cell-specific CpG hypomethylation pattern (Ohkura et al. 2012).

Much progress has been made in demonstrating the role of Treg cells in the pathogenesis and development of many autoimmune diseases (Yang et al. 2019a, b). Furthermore, the role of Tregs in the tumor microenvironment is being closely examined since their numbers are increased in tumors (Kashima et al. 2020; Solis-Castillo et al. 2020). Whether these Treg cells belong to tTreg or they are induced locally is not clear (Horwitz et al. 2008; Lin et al. 2013). In addition to tTreg, induced Treg in the periphery (pTreg) or those in ex vivo with TGF-β and IL-2 (iTreg) represents a new development in the history of Treg cells (Zheng et al. 2002, 2004, 2007). In both immune responses to self-antigens and to tumor antigens, Foxp3+ Treg plays an inhibitory role. While the former, inhibition of immune response to self-antigens, is a beneficial; the latter is deleterious. Consistent with this concept is the observation that large numbers of Treg cells infiltrating a tumor tissue are often associated with poor prognosis (Tanaka and Sakaguchi 2017). More researches are needed to delineate in more details such as the role and mechanism of Treg cells in various immune responses in autoimmune diseases and in tumors. Enhancing the suppressive role of Treg cells in immune responses in autoimmunity and dampening those responses in malignancy are likely to become novel treatments for these diseases.

1.2 Treg and the Immune Response

Regulatory T cells (Tregs) are necessary for the maintenance of immune self-tolerance and homeostasis (Josefowicz et al. 2012). Treg cells have unique surface expression profiles, including CD4, CD25, CD62L, and specific CD45 isoforms. With the discovery of the specific transcription factor Foxp3 in Treg cell population, the concept of a regulatory immune cell has changed from a rare CD4+ T-cell subtype to an important immune homeostatic regulator (Hori et al. 2003). Although it is formally named as a regulatory T cell, its role mostly relates to immune suppression. Treg cells inhibit various immune responses, thus a defect or reduction of Treg number and/or function; or other Treg biological changes have been observed in various autoimmune diseases, such as RA (Prakken et al. 2013), SLE (Scheinecker et al. 2010), and IPEX

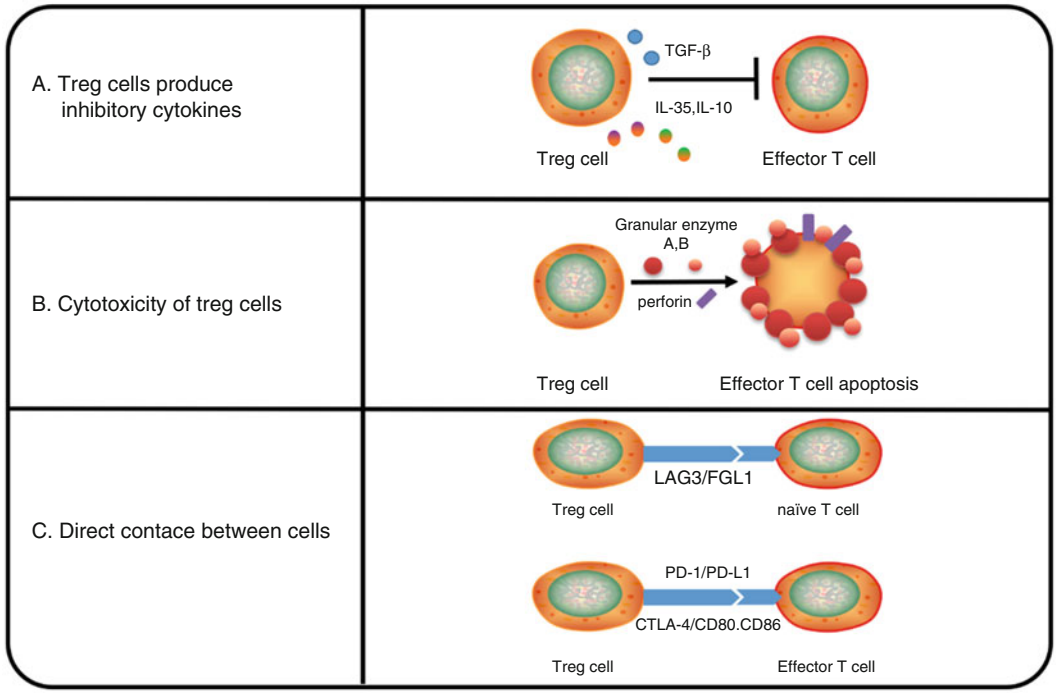

Fig. 1.1 Schematic diagram of the immunosuppressive mechanism of Treg

syndrome (Horino et al. 2014). Moreover, the changes in Treg in these various disorders are not incidental but have been shown to contribute to their development (Scheinecker et al. 2019). Conversely, an increase in Treg cells inhibits abnormal immune responses to autoantigens as well as antitumor immune responses. Large numbers of Treg cells infiltrating tumor tissue are often associated with poor prognosis (Tanaka and Sakaguchi 2017). In contrast, an increase in Tregs in the peripheral blood and in the graft microenvironment is considered important for inducing graft tolerance (Bahmani et al. 2018; Graca et al. 2002; Lee et al. 2005). Furthermore, an overabundance of Treg cells impairs the immune response in patients with infection (Costa et al. 2013). In general, Treg cells are part of the adaptive immune system playing a key role in maintaining homeostasis by exerting their immunosuppressive effects in normal and disease state (Mohr et al. 2019). In addition, we also describe various possible immunosuppressive mechanisms of Treg cells (Fig. 1.1).

1.3 Classification of Treg Cells

It is well known that there are many subpopulations of the Treg cell family (Fig. 1.2), including CD4+Foxp3+ cells, "Tr1" cells producing interleukin 10 (IL-10), CD8+ T suppressor cells, natural killer T cells, CD4−CD8− T cells, and γδ T cells. In this chapter, we focus on the first three types of Treg cells (Tang and Bluestone 2008; Zhou et al. 2011).

1.4 CD4+Foxp3+ Treg Cells

Among various Treg populations, CD4+Foxp3+ Treg cells are a key member of the Treg networks and display many important functional characteristics. Foxp3+ Treg cells are often classified as either "natural" or "induced" subsets. Natural tTregs are long-lived and develop in the thymus, while induced Tregs are thought to arise in the periphery after stimulation (pTreg). In

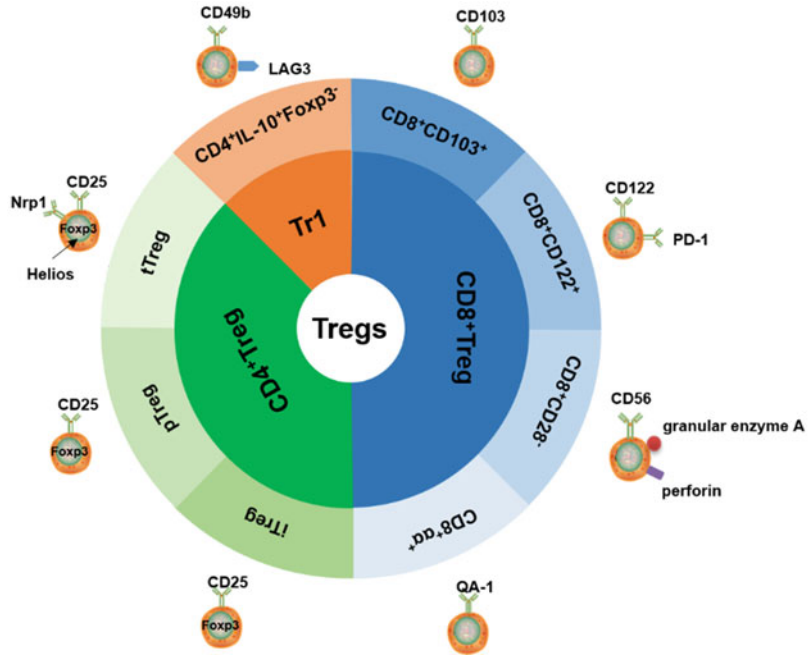

Fig. 1.2 Classification of Treg cells. The figure shows the classification of different subtypes of Treg cells and the characteristic markers of each type of Treg cells. Treg cells can be divided into CD4+ and CD8+ Treg cells. These two types of Treg cells can be further classified as various subpopulations as shown in the figure. In addition, in CD4+ Treg cells, another type of Tregs called Tr1 was identified. Unlike traditional CD4+Foxp3+ Treg, Tr1 cells were Foxp3− with high expression of IL-10

addition, TGF-β and IL-2 are crucial cytokines to induce Treg ex vivo (iTreg) (Zheng et al. 2002, 2007). These cell populations all express CD25 and Foxp3; however, they also have some differences that will be discussed later in this chapter.

Foxp3 is an important marker for the development and function of tTreg cells. It is expressed in mostly CD4+CD25+ T cells and a small number of CD4+CD25− T cells. However, CD25 is a surface marker that effectively identifies live Treg cells while Foxp3 is an intranuclear transcription factor that require permeabilization of the cell membrane; therefore, it cannot be used as a marker in live cells (Fontenot et al. 2003). Initially, the three research teams found that Foxp3 was expressed in a large and stable amount in mouse CD25+CD4+ Treg cells, but not in naïve CD25−CD4+ T cells or activated CD4+ T cells (Fontenot et al. 2003; Khattri et al. 2003; Hori et al. 2003). These findings support the critical role of Foxp3 in the differentiation of Treg cells. Foxp3-mutant scurfy mice spontaneously die at 3 weeks of age of a systemic autoinflammatory diseases (Godfrey et al. 1991). In humans, the loss of function or mutation of Foxp3 also causes severe autoinflammatory disease, like IPEX. These are due to the deficiency or abnormal function of CD25+CD4+ natural tTreg (Powell et al. 1982; Bacchetta et al. 2018). All these observations suggest that Foxp3 is a functional molecule of Treg cells. Nonetheless, it has recently been reported that Foxp3 expression alone is not sufficient for Treg lineage commitment. It has been shown that demethylation of a Treg-specific demethylation region (TSDR) in the Foxp3 promoter plays a crucial role in the maintenance of tTreg lineage and that this demethylation of TSDR is associated with a stable tTreg cell phenotype (Ohkura et al. 2012).

tTregs are produced by immature Treg precursors of heat-stable antigen $(HAS)^{hi}$ CD4 single-positive (SP) stage when Foxp3 is induced and tTreg lineage commitment is established (Lee and Hsieh 2009). In the presence of IL-2 and TGF-β, peripherally derived Treg (pTreg) cells differentiate from naïve T cells in peripheral sites. Those induced by TGF-β and IL-2 in vitro are called induced Treg (iTreg) cells (Fig. 1.3). pTreg and iTreg share some similarities;

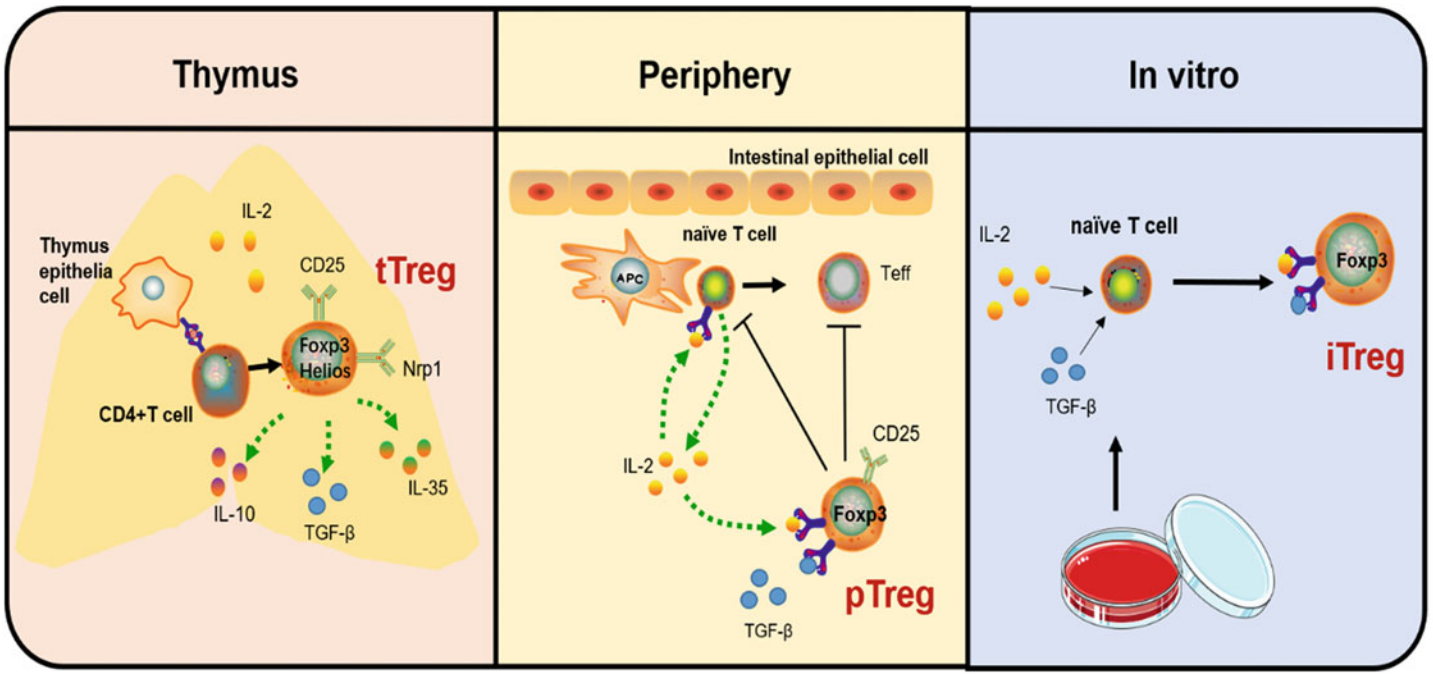

Fig. 1.3 Development and differentiation of Treg cells. The development and differentiation of Treg cells from thymus, peripheral, and in vitro are shown. Thymus-derived Tregs (tTreg) originates from the thymocytes in response to self-antigen stimulation during T cell development in vivo. In addition, induced Tregs develop in the periphery (pTreg), and induced Tregs (iTreg) differentiate from naïve T cells by means of T-cell receptor (TCR) stimulation through exposure to the IL-2 and TGF-β ex vivo

however, they also have some differences that will be discussed later. Human CD4+Foxp3+ T cells are composed of three subsets with different phenotypes and functions: CD45RA(+) Foxp3 (lo) inactive Treg cells (rTreg cells), CD45RA (−) Foxp3 (hi) activated Treg cells (aTreg cells), and cytokine-secreting CD45RA(−) Foxp3 (lo) non-inhibitory T cells (Miyara et al. 2009). It has been shown that the first two populations had inhibitory effects in vitro. The terminally differentiated aTreg cells die rapidly, and the rTreg cells proliferate and become aTreg cells in vitro and in vivo (Miyara et al. 2009).

Although CD4+Foxp3+ Treg population has attracted widespread attention for their role in maintaining immune homeostasis, studies have also found that CD4+ Tr1 and CD8+ Treg showed the immunomodulatory functions as well. The number and/or function of these cells are impaired in several autoimmune diseases and autoimmune experimental animal models (Roncarolo et al. 2018; Pellegrino et al. 2019), suggesting that immunotherapy targeting these cells can also improve autoimmune status management (Dinesh et al. 2010; Huang et al. 2017, 2018).

1.5 Tr1 Cells

Unlike other CD4+ T-cell subsets, Tr1 cells are a Treg cell type characterized by the expression of CD49b and LAG3 (Gagliani et al. 2013), as well as the lack or transient expression of the transcription factor Foxp3 and a significant expression of IL-10 (Vieira et al. 2004; Groux et al. 1997). Since their discovery, Tr1 cells have been shown to play an important role in maintaining immune homeostasis and preventing T-cell-mediated diseases (Vieira et al. 2004). Tr1 cells are made in the periphery after antigen exposure and under tolerogenic conditions. In vivo and in vitro studies have confirmed their inhibitory effects in mouse and human microenvironment. Specifically, Tr1 cells prevented and downregulated aberrant immune responses to pathogenic and nonpathogenic antigens and was also associated with long-term tolerance in humans (Bacchetta et al. 1994; Gianfrani et al. 2006; Serafini et al. 2009; Roncarolo et al. 2014; Globinska et al. 2018). Immunosuppression observed in some infectious diseases is associated with a higher frequency of Tr1 cells (Chang 2007; Koch et al. 2015).

Tr1 cells do not constitutionally express Foxp3 (Vieira et al. 2004), but once activated, they can rapidly upregulate Foxp3 (Levings et al. 2005; Brun et al. 2009, 2011), but this upregulation is not comparable to tTreg or iTreg. In addition to secreting large amounts of IL-10, Tr1 cells also secrete TGF-β, IL-5, GM-CSF, and IFN-γ and small amounts of IL-4, IL-17, and IL-2 (Bacchetta et al. 1990, 1994; Groux et al. 1997). A key aspect of Tr1 cell-mediated regulation is that these cells need to be activated by their TCR to show their regulatory activity, which reflects their antigen specificity. After activation, Tr1 cells secrete IL-10 and TGF-β, which directly and indirectly inhibit T-cell response (Roncarolo et al. 2014). Generally, IL-10 limits the magnitude of the immune response and inhibits the response of T effector cells by downregulating the expression of MHC II glycoproteins (de Waal Malefyt et al. 1991), co-stimulatory molecules, and pro-inflammatory cytokines that are produced by APC (Gregori and Roncarolo, 2018; Roncarolo et al. 2014).

The differentiation and function of Tr1 cells depend on the presence of IL-10, an effective immunosuppressive cytokine even though it has pleotropic effects. Tr1 cells can inhibit the response of effector cells in an IL-10-dependent manner by interacting with CTLA-4 and PD-1 (Roncarolo et al. 2014; Gregori et al. 2012) or by killing pro-inflammatory cells directly with a granzymes (Gagliani et al. 2013; Huber et al. 2011). Tr1 cells also express extracellular enzymes CD39 and CD73, which produce adenosine through enzymatic hydrolysis of extracellular ATP and disrupt the metabolic state of effector T cells (Mandapathil et al. 2010; Mascanfroni et al. 2015; Su et al. 2019). Moreover, the role of synergistic inhibitory receptors (such as LAG-3, TIGIT, and TIM3) in the regulation of Tr1 cells is under investigation. However, it was observed that injection of antibodies against

LAG-3 reversed the state of immune tolerance induced by Tr1 cells, and it was predicted that these co-inhibitory receptors may contribute to the suppressive function of Tr1 cells (Jofra et al. 2018). Interestingly, IL-27 also plays a key role in Tr1 cell induction in mice and provides an alternative mechanism for the generation of these cells. As such, it was demonstrated that the development of Tr1 cells in intestinal associated lymphoid tissues is independent of IL-10 (Maynard et al. 2007). Short-term activation of mouse T cells in the presence of IL-27 leads to the production of Tr1 cells both in vitro and in vivo (Awasthi et al. 2007; Batten et al. 2008; Fitzgerald et al. 2007; Iwasaki et al. 2013; Pot et al. 2009; Stumhofer et al. 2007).

IL-27 promotes IL-10 production in mouse CD4+ T cells by activating the STAT1 and STAT3 pathways (Awasthi et al. 2007; Fitzgerald et al. 2007; Stumhofer et al. 2007). However, the role of IL-27 in inducing human Tr1 cells remains unclear. Recent research has established that the functional development of Tr1 cells requires TCR/ITK signaling through the Ras/IRF4 pathway (Huang et al. 2017).

Like Foxp3+ Treg cells, Foxp3-IL10+ Tr1 cells also have a therapeutic potential in the treatment of inflammatory diseases. The overactivation of NLRP3 inflammasome is the basis of several common chronic inflammatory diseases (Robbins et al. 2014; Agostini et al. 2004). It was speculated that Foxp3+ Treg and/or Tr1 cells may play a role in regulating inflammasome activities. This was examined and only Tr1 cells can regulate this pathway (Yao et al. 2015). More studies are needed to compare the functional difference between Foxp3+ Treg and Tr1 cells.

1.6 CD8+ Treg Cells

CD8+ Treg subset is a subgroup of CD8+ T cells with inhibitory potential first discovered in 1972 (Gershon et al. 1972). However, the lack of unique markers to identify these inhibitory T cells led to long hiatus in this field of study. Interest in these studies began to re-emerge in the 1990s (Noble et al. 1998). In 2007, the function of these cells in the context of viral infections and tumorigenesis was highlighted (Guillonneau et al. 2007). Like CD4+ Treg cells, they also can be divided into multiple subtypes, including CD8+CD103+, CD8+CD122+, CD8+CD28−, and CD8+ Qa-1-restricted CD8αα+TCRαβ+ Treg cell populations.

1.6.1 CD8+CD103+ Treg Cells

CD8+CD103+ T cells are considered to be suppressive CD8+ T cells because they have been shown to control immune responses both in vivo and in vitro (Ma et al. 2015; Lerret et al. 2012). However, the exact properties of the CD8+CD103+ regulatory T cells, as well as their suppressive function and related mechanism in allogeneic transplantation, remain elusive.

CD103 (αE7β integrin) is an E-cadherin receptor that is present on the surface of CD8+ T lymphocytes in thymus, small intestine, bronchoalveolar fluid, and allografts in mice and humans (Pauls et al. 2001; Rihs et al. 1996; Sarnacki et al. 1992; Liu et al. 2014; Zhong et al. 2018; Zhang et al. 2019). CD103 has been described as the first affinity ligand that mediates cell adhesion and migration and has the function of directing T lymphocytes to epithelial cells that express E-cadherin (Cepek et al. 1994; Karecla et al. 1995). In humans, CD103 expression is limited to 1% of circulating memory T cells (Picker et al. 1990). In mice, CD103 is expressed in 40–60% of peripheral CD8+ T cells (Hadley et al. 1997), mainly in those showing a naïve phenotype (Wang et al. 2004).

The relationship between CD103 and CD8+ T cells has been established in studies of renal transplant rejection and intestinal graft versus host disease (El-Asady et al. 2005). It was found that CD103 expression was significantly upregulated in a TGF-β-dependent manner on CD8+ T cells entering kidney transplantation sites or intestinal epithelial cells. This demonstrated that CD103 characterizes CD8+ effector T cells (Wang et al. 2004; Hadley et al. 2001; El-Asady et al. 2005). However,

subsequent research found that these cells have regulatory activity. In these studies, alloreactive CD8+CD103+ T cells are produced by the stimulation using alloantigen but not anti-CD3/CD28 antibodies (Uss et al. 2006). CD8+CD103+ Tregs are mostly CD28+; they also lacked CD25, Foxp3, CTLA-4, LAG-3, and GITR expression. Moreover, the presence of TGF-β resulted in increased expression of CD103 (Hadley et al. 1997).

CD8+CD103+ Treg cells inhibit T-cell proliferation in mixed lymphocyte cultures in a manner that depends on cell-to-cell contact (Koch et al. 2008). Even though they also secreted IL-10 and TGF-β, this inhibitory effect is not mediated through these cytokines. This makes them to be different from Tr1 cells. However, in other related studies, it was demonstrated that IFN-γ binding to CD8 Tregs is required to mediate its TGF-β-based suppression (Myers et al. 2005). Meanwhile, they identified a murine peptide-specific CD8+ T regulatory cell population that inhibited the response of CD4+ T cells. However, TGF-β expression in situ tightly associates with CD103 expression (Robertson et al. 2001). Chronic ileitis in TNF-driven model as well as liver transplantation provides compelling evidence that CD8+CD103+ Treg cells that secreted TGF-β have a protective effect (Ho et al. 2008; Lu et al. 2009).

Recent studies have provided multiple pathways for the induction of antigen-specific CD8+ Treg with inhibitory functions. The latest research has successfully established a method using TGF-β plus RAPA in addition to CD3/CD28 and IL-2 stimulation to effectively induce human CD8+ Tregs in vitro. These newly induced hCD8+ Tregs express stable and high levels of Foxp3, CD103, and PD-1 but have no IL-17A secretion, and survive in vivo after adoptive transfer in a CIA animal model (Sun et al. 2019). Moreover, these cells have a strong inhibitory ability and can alleviate collagen-induced arthritis. Our group has reported that CD8+CD103+ iTreg cells can be induced ex vivo in the presence of TGF-β and IL-2 (Liu et al. 2014; Zhong et al. 2018; Zhang et al. 2019). Interestingly, the functional activity of these Treg populations does not require Foxp3 expression (Liu et al. 2014; Zhong et al. 2018). Moreover, we further found that CD39 expression participated in the functional activity of these Treg population (Zhang et al. 2019). Further studies are warranted to determine the differences among these Treg populations.

1.6.2 CD8+CD122+ Treg Cells

CD8+CD122+ Treg cells are another natural CD8+ Treg subtype, which has been shown to have inhibitory capacity in transplantation and autoimmunity (Suciu-Foca et al. 2003; Rifa'i et al. 2004). Previous studies have shown that CD8+CD122+ Treg cells can control immune homeostasis, inhibit traditional T-cell responses (Rifa'i et al. 2004; Endharti et al. 2005; Chen et al. 2008; Shi et al. 2008;Molloy et al. 2011;Endharti et al. 2011), and regulate autoimmune responses (Kim et al. 2011; Mangalam et al. 2012), including experiments autoimmune encephalomyelitis (Mangalam et al. 2012; Lee et al. 2008; Seifert et al. 2017), Graves' disease (Saitoh et al. 2007), and colitis (Endharti et al. 2011). Earlier studies described CD8+CD122+ T cells as antigen-specific memory T cells (Zhang et al. 1998; Ku et al. 2000; Judge et al. 2002). CD122 expression was initially described in nonregulatory memory lymphocytes (Zhang et al. 1998; Judge et al. 2002). Subsequently, it was reported that central memory CD8+CD122+ T cells ($CD44^{high}CD62L^{high}$) also play a role in regulating T-cell homeostasis and act as regulatory T cells (Rifa'i et al. 2004). A series of studies in recent years have further shown that CD8+CD122+ T cells indeed inhibit conventional T-cell responses (Rifa'i et al. 2004; Endharti et al. 2005; Chen et al. 2008; Shi et al. 2008; Molloy et al. 2011; Endharti et al. 2011; Wang et al. 2010) and control autoimmune diseases (Kim et al. 2011; Mangalam et al. 2012). Moreover, some studies have found that memory CD8+CD122+ T cells and bystander central memory CD8+ T cells also belong to Treg that inhibit mouse allograft rejection (Wan et al. 2008; Dai et al. 2010). Furthermore, other studies have suggested that central memory CD8

+ T cells can modulate the acceptance of allogeneic lungs (Krupnick et al. 2014).

CD122 is β subunit of IL-2 receptor, while CD25 is an α subunit of the same receptor on T cells (Sakaguchi et al. 1995). Murine CD8+CD122+ Tregs express CD122 (IL-2Rβ) and CXCR3 but do not express CD25, while their CD4+CD25+ counterpart does not express CD122. CD8+CD122+ Tregs are also $CD44^{high}$, $CD62L^{high}$ CCR7+, and most CD127− (Suzuki et al. 2008; Dai et al. 2010). However, CD8+CD122+ Tregs are Foxp3 negative (Dai et al. 2010), suggesting that they are a different subset of induced CD8+Foxp3+ Treg cells (Lerret et al. 2012).

In a comparison study, investigators demonstrated that CD8+CD122+ Tregs were more effective than CD4+Foxp3+ Tregs in inhibiting allograft rejection (Dai et al. 2014). In fact, PD-1 is a key marker in identifying whether CD8+CD122+ T cells are Treg cells. The CD8+CD122+PD-1+ group in the CD8+CD122+ population is mainly responsible for CD8+CD122+ Treg-mediated inhibition (Dai et al. 2010). We previously also reported that PD1 and TNFRII expression contributes to CD8+ Treg functional capacity (Horwitz et al. 2013; Jacob et al. 2009; Yang et al. 2018, 2019a, b). Nonetheless, PD1 is also a marker of T-cell exhaustion, contributing to functional decline of these cells (Simon and Labarriere 2017). The expression of PD1 in Treg function and vitality needs more in-depth studies.

The effects of combined CD8+CD122+PD-1+ Treg and conventional co-stimulatory blockade on allograft rejection and allogeneic immunity were examined recently. A synergistic response was demonstrated with CD8+CD122+PD-1+ Treg and CD40/CD154, but no synergism was seen with B7/CD28 blockade on prolonging the survival time of skin allografts in wild-type mice (Liu et al. 2019). The B7/CD28 co-stimulatory block, but not CD40/CD154, had a negative impact on the in vivo expansion of adoptively transferred Treg and its IL-10 production in vitro (Liu et al. 2019). The increased effects of CD8+CD122+PD-1+ Treg cells on the survival rate of allografts depend to a considerable extent on the expression of IL-10 by Treg cells (Dai et al. 2010; Liu et al. 2017). CD8+CD122+ Tregs may regulate the immune response by producing IL-10, TGF-β1 and IFNγ, but the exact mechanism of their inhibitory effect is still unclear.

1.6.3 CD8+CD28− Cells

CD8+CD28− T cells are also classified as natural CD8+ Treg population. Among different CD8+ Treg subgroups, non-antigen-specific CD8+CD28− Tregs have been associated with a variety of clinical conditions such as pregnancy, cancer, organ transplants, and infectious diseases. Different reports showed that CD8+CD28−Tregs inhibit T-cell proliferation in an IL-10-dependent or TGF-β-dependent manner (Fenoglio et al. 2008; Miller et al. 2010).

CD8+ T cells play a key role in the recognition and clearance of intracellular pathogen-infected cells (Nagata and Koide 2010) and anti-tumor response (Mempel and Bauer 2009). Binding of CD8+ T-cell surface receptor TCR and MHC-I-binding antigen expressed on the surface of professional antigen-presenting cells (pAPC) led to the activation of CD8+ T cells (Strioga et al. 2011). However, the optimal activation of CD8+ T cells cannot be maintained by TCR stimulation alone, and a second co-stimulation signal is required for the complete activation and survival of these cells (Boesteanu and Katsikis 2009). The interaction between CD28 molecule on T lymphocytes and CD86 and CD80 molecules expressed on the surface of pAPC provides a co-stimulating signal that has been well described (Strioga et al. 2011). When a sufficient signal is delivered to naive CD8+ T cells, they will proliferate and differentiate into two different cell types. One is cytotoxic T lymphocytes (CTLS), which undergo apoptosis upon maturation and effector function. The other is CD8+ memory T cells, which are both central and effector cells (Zheng et al. 2006). Their continuous presence in the circulation is important for controlling another potential exposure to the same antigen in a faster and more efficient manner (Kaech

et al. 2002). Chronic antigenic stimulation leads to repeated activation cycles, which progressively loses CD28 molecule expression with each cycle of activation. This leads to the accumulation of "highly antigenic experienced" T cells with the CD8+CD28− phenotype, characterized by extremely short telomeres (Vallejo 2005). There is a close relationship between CD28 and telomerase denaturation. Telomerase activity is essential for cell proliferation, production of cytokines and chemokines, and antiviral activity. However, lack of CD28 leads to loss of the ability to activate telomerase activity in the cells. Maintaining the presence of CD28 through in vitro gene transduction slows down the rate of "immune aging" and improves the efficiency of the immune system (Cohen et al. 2013). Telomeres build on the ends of chromosomes and ensure their stability. Unprotected ends of chromosomes are at high risk of degradation, which leads to loss of genetic information and cell death (Kim Sh et al. 2002). Studies have found an association between a shortened telomere length in peripheral blood cells and autoimmune diseases, such as SLE (Haque et al. 2013; Honda et al. 2001), rheumatoid arthritis (Colmegna et al. 2008), systemic sclerosis (SSC) (Artlett et al. 1996), ANCA-related vasculitis (AAV) (Vogt et al. 2003), psoriasis, and atopic dermatitis (Wu et al. 2000). It is believed now that one of the main causes of abnormal immune response is abnormal telomeres in autoimmunity (Montoya-Ortiz 2013). Moreover, the loss of CD28 has been observed to be associated with increased surface expression of CD57 molecules. CD8+CD28− (CD8+CD57+) T cells are known to be antigen-specific, terminally differentiated, but are also known as functional memory or effector T cells that undergo multiple cell division cycles. These cells are characterized by reduced or even loss of telomerase five activity and low levels of expression of genes involved in cell cycle regulation. CD8+CD28− (CD8+CD57+) T cells are generally limited in their ability to proliferate after stimulation and are thought to have reached a state of "replicative senescence" or "clonal failure" (Strioga et al. 2011; Focosi et al. 2010).

The relationship between CD8+CD28− (CD8+CD57+) lymphocytes and apoptotic sensitivity is controversial. Some researchers (Borthwick et al. 2000; Wood et al. 2009) reported that these cells are highly sensitive to activation-induced apoptosis because they have observed an increased expression of Fas and caspase-3 and a decreased expression of anti-apoptotic molecules such as survivin or heat shock protein 27 (HSP27). Others suggested that CD8+CD28− (CD8+CD57+) T lymphocytes are highly resistant to apoptosis and thus accumulate gradually throughout life (Spaulding et al. 1999; Effros 2011).

Most autoimmune diseases are associated with an increase in CD8+CD28− (CD8+CD57+) T cells, which exhibit high cytotoxic activity, and their presence may correlate with more severe manifestations of the disease. Such changes in CD8+CD57+ population has been observed in multiple sclerosis (Mikulkova et al. 2010), type 1 diabetes (Mikulkova et al. 2010), Graves' disease (Sun et al. 2008), and rheumatoid arthritis (Wang et al. 1997). Moreover, regulatory properties have been attributed to lymphocytes with a CD8+CD28− phenotype. Further analysis confirmed the expression of Foxp3 in these cells (Frisullo et al. 2010; Manavalan et al. 2004). However, this finding was not confirmed by other groups (Korecka-Polak et al. 2011; Scotto et al. 2004). In addition to the lack of Foxp3 expression, characteristic markers of cytotoxic cells, such as granzyme A or perforin, were detected on the surface of CD8+CD28− population (Baeten et al. 2006).

1.6.4 CD8+ Qa-1-Restricted CD8αα +TCRαβ+ Regulatory T Cell

Major histocompatibility complex (MHC) class Ib molecules consist of rodent QA-1, QA-2, H2-M3, and CD1d and human leukocyte antigen (HLA)-E, HLA-G, and CD1(Braud et al. 1999). Qa-1-restricted CD8αα+TCRαβ+ T cells have been recognized as another CD8+ Treg subset (referred to as CD8αα+ Treg) that recognizes an antigenic determinant in the conserved CDR2

region of the TCRβ.82 chain (Smith et al. 2010). CD8αα+ Tregs can control experimental autoimmune encephalomyelitis (EAE), a prototype for multiple sclerosis. They control EAE by inducing apoptosis in activated and pathogenic CD4+ cells after recognition of the TCR–peptide/Qa-1 complex on their cell surface (Smith et al. 2009, 2010). There were data showing that a unique phenotype of CD8αα+TCRαβ+ Treg cells is enriched in a number of molecules expressed by NK cells, members of TNF-superfamily as well as in negative signaling molecules, including CD200 (Fanchiang et al. 2012). In general, peripheral class Ib-responsive CD8αα+TCRαβ+ T cells represent a unique type of regulatory T cells that are different from class Ia MHC-restricted conventional T cells. These findings have important significance for understanding the regulatory mechanism mediated by the CD8+ Treg cell population, and much work needs to be done to clarify their relationship to other CD8+ Treg populations.

1.7 Similarity and Differences Between tTreg and iTreg Cells

As we discussed before, CD4+Foxp3+ Tregs can be classified into three subtypes, thymus-derived Treg (tTreg) originates from the thymocytes in response to self-antigen stimulation during T-cell development in vivo, induced Treg in the periphery (pTreg) and induced Treg (iTreg) from naïve T cells by means of T-cell receptor (TCR) stimulation through exposure to the IL-2 and TGF-β ex vivo (iTreg) (Zheng et al. 2002; Sakaguchi 2004). The different Treg subtypes share significant similarities, such as their high levels of IL-2 receptor alpha chain (CD25) expression and dependence on the forkhead box P3 transcription factor (Foxp3). As pTregs are developed in vivo, it is hard to distinguish them from tTregs, and Foxp3+CD4+ cells in the periphery actually could be a mixture population of both tTreg and pTreg cells. We will focus here on similarities and differences between tTreg and iTreg subpopulations.

First, developmentally, iTreg requires TGF-β signaling since lack of this signal fails to induce iTreg (Zheng et al. 2002, 2004; Lu et al. 2010). However, tTreg development is independent upon TGF-β signaling (Piccirillo et al. 2002; Jordan et al. 2001). Second, CTLA-4 has an essential role in the generation of Foxp3 and acquisition of suppressor activity by naïve cells activated with TGF-β in vitro while it is not necessary for the development of tTreg cells in the thymus (Zheng et al. 2006; Liang et al. 2005). Third, phenotypically, both tTreg and iTreg have negative immune regulation function in vitro and in vivo through the secretion of inhibitory cytokines, such as TGF-β, IL-10, IL-35, and cAMP, expression of ectoenzymes CD39 and CD73 to degrade extracellular ATP, and both express similar levels of Foxp3. In contrast, phenotypic distinguishable features include tTregs overexpress Helios (a member of the Ikaros family of transcription factors) and Nrp1(a type 1 transmembrane protein), which are the two immunosuppressive protein molecules, while iTregs frequently express less of those proteins (Atif et al. 2020; Thornton et al. 2010; Weiss et al. 2012). However, there is controversy surrounding these observations, and others suggested that iTregs also express similar levels of Helios and Nrp1 (Akimova et al. 2011; Verhagen and Wraith 2010; Szurek et al. 2015). Therefore, there is a need to identify specific markers that reliably distinguish these two populations (Wing et al. 2019). Fourth, the two Treg populations have functional differences in inflammation and high salt diet. tTreg population tends to convert from regulatory cells to effector cells with loss of immune regulation ability in inflammatory and high salt microenvironment (Wu et al. 2013a, b; Kleinewietfeld et al. 2013; Sakaguchi et al. 2013). However, we and others have demonstrated that iTreg sustained their function even under inflammation and high salt conditions (Zheng et al. 2008; O'Connor et al. 2010; Zhou et al. 2010; Kong et al. 2012a, b), demonstrating potential advantages of iTreg versus tTreg in treating these diseases. Interestingly, *all-trans* retinoic acid could overcome tTreg instability in the presence of inflammatory milieu in mice and humans

Table 1.1 Similarity and differences between tTreg and iTreg cells

		Thymus-derived (tTreg)	Induced in vitro (iTreg)
Similarities	High levels of CD25 and Foxp3 secreting TGF-β, IL-10, IL-35 expression CD39 and CD73 have suppressive activities in vitro and in vivo		
Differences	**Development** TGF-β CLTA-4	Not Required Not dependent	Required Dependent
	Phenotype Helios/Nrp1	Overexpression	Less expression
	Stability (inflammatory and high salt environment)	Not stable (convert to effector cells)	Stable
	Function of B cells	Killing ability	Cytokine secretion
	Antigen-specificity	Polyclonal	Antigen-specific
	Epigenetic	Hypomethylation	Hypermethylation
	Treatment on established CIA	Not effective	Effective

(Zhou et al. 2010; Lu et al. 2014; Luo et al. 2019). Fifth, iTreg and tTreg cells have a unique mechanism in targeting B cells. It has been previously reported that tTregs suppressed B-cell function mainly through killing target cells (Zhao et al. 2006; Iikuni et al. 2009), while iTregs suppressed B cells via their cytokine secretion rather than killing (Xu et al. 2016). The latter mechanism may be more optimal. Sixth, tTregs are polyclonal while iTregs can be developed into antigen-specific iTregs (Zheng et al. 2006; Kong et al. 2012a, b). In addition, tTreg cells are rare cell population while iTreg cells can be manipulated in a large scale, demonstrating their translational value. Seventh, there are also some similarities and differences between the two Treg subpopulations in the epigenetic mechanisms such as specific DNA methylation, histone modification which show the important role for the differentiation and stabilization of cells. tTreg cells exhibit the CpG hypomethylation pattern of the Treg cell representative regions such as Foxp3, Tnfrsf18, Ctla4, Ikzf4, and Il2ra even after anti-CD3/CD28 stimulation. The methylation status of those regions is stably high in iTreg except Il2ra which is also gradually demethylated with TCR stimulation. Interestingly, histone modifications are less specific for tTreg because of the similar histone such as H3K4me3 and H3K27me3 modification in the Treg associated genes of tTreg and iTreg cells (Ohkura et al. 2012). The combination of hypomethylation establishment and Foxp3 expression are essential for regulating and stabilizing the expression of the molecules required for tTreg cell development and function. In fact, many studies demonstrated that iTregs are superior to tTreg in treating the established autoimmune and inflammatory diseases (Kong et al. 2012a, b; Su et al. 2012). The studies are needed to delineate the significance of these Treg populations. The difference between these two Treg populations were compared in detail in Table 1.1.

1.8 Treg in Diseases

1.8.1 Treg in Autoimmune Diseases

Autoimmune diseases are characterized by a breakdown in immune tolerance. Regulatory T cells regulate peripheral immune tolerance and

homeostasis and play an important role in the development of autoimmune diseases. Functional defects and reduced numbers of Treg cells have been observed in a variety of autoimmune diseases (Scheinecker et al. 2019) including type 1 diabetes, multiple sclerosis, systemic lupus erythematosus (SLE), myasthenia gravis, and rheumatoid arthritis (RA) (Chen et al. 2013; Goschl et al. 2019). Patients with CD25 (IL-2R) deficiency suffer from autoimmune phenomena, lymphadenopathy, and persistent viral infections similar to the mentioned IPEX syndrome. However, in spite of the presence of Foxp3+CD4+ T cells, impaired production of IL-10 was demonstrated following T-cell activation (Malek 2008). RA is a systemic autoimmune disease that causes chronic inflammation and tissue destruction of joints (Zou et al. 2018). The role of Treg cells in pathogenesis is not fully understood, as changes in the number of Treg cells in synovium and peripheral blood have been described in the past few years, but these observations have not been uniform (Han et al. 2008; Cao et al. 2004; Jiao et al. 2007; Sempere-Ortells et al. 2009; Kawashiri et al. 2011; Niu et al. 2012; Samson et al. 2012; Lina et al. 2011; Yang et al. 2019a, b). In addition to the quantitative defects, there have been reports of functional defects in patients with RA (Rapetti et al. 2015; Flores-Borja et al. 2008; Nadkarni et al. 2007). Anti-TNF and anti-IL-6 therapies can restore the balance of Treg and Th17 cells in RA patients and affect Treg function (Samson et al. 2012, Nadkarni et al. 2007; Ehrenstein et al. 2004). Over the past two decades, improved anti-rheumatic drugs (bDMARD) have revolutionized the treatment of RA patients. Among them, TNF blockade, IL-6R blockade, and inhibition of T-cell activation through targeting co-stimulation signals are second-line drugs for treating patients with refractory RA with methotrexate (Smolen et al. 2016). The latest data showed that anti-TNF therapy in RA patients increases the frequency of TNFR2+ Treg cells, and the lack of TNFR2 leads to an increase in the methylation of Foxp3 TSDR, suggesting that TNFR2 expression has an effect on Treg function and stability in RA patients (Santinon et al. 2019). This is consistent with our recent reports that TNFRII favors Tregs while TNFRI promotes T effector cells (Yang et al. 2018; 2019a, b). IL-6 is another driver of inflammation in RA patients and is involved in the plasticity of Treg cells (Xu et al. 2007; Zheng et al. 2008; Luo and Zheng 2016). After treatment with tocilizumab, the number of Treg cells in RA patients increased in patients with good clinical response (Thiolat et al. 2014; Kikuchi et al. 2015).

Similarly, in the study of systemic lupus erythematosus (SLE), it was found that helper T cells (Th17) can induce tissue inflammation and immune responses. In contrast, Treg cells can mediate immune tolerance and inhibit inflammatory responses. The imbalance of Th17 cells and Treg cells affects the occurrence and pathogenesis of SLE (Ma et al. 2010; Szmyrka-Kaczmarek et al. 2014). Therefore, inhibiting the differentiation of Th17 cells and promoting the differentiation of Treg cells can help restore the balance of Th17/Treg cells and provide a potential new therapeutic approach in SLE. Low doses of IL-2 can increase the number of Treg cells, maintain immune homeostasis, and temporarily relieve clinical symptoms in patients with SLE (Goudy et al. 2013; Hartemann et al. 2013; Saadoun et al. 2011; He et al. 2016; von Spee-Mayer et al. 2016; Ye et al. 2018). However, most of these studies focus on short-term effects (von Spee-Mayer et al. 2016) and are not feasible long-term treatment (Humrich et al. 2015). The decrease in Treg and the imbalance of Th17/Treg cells are related to the occurrence and development of refractory SLE. It was proposed that the use of low-dose IL-2 combined with rapamycin can restore the Treg number and the balance of Th17/Treg cells. This method can induce immune tolerance, promote clinical response, reduce the dosage of prednisone or cytotoxic drugs, reduce drug side effects, and collectively benefit patients (Zhao et al. 2019).

Ankylosing spondylitis (AS) is an inflammatory autoimmune disease related to HLA-B27. Similar to RA, Treg cells also have functional deficits in AS (Guo et al. 2016). Recent studies have found that treatment with anti-TNF may affect the Th17/Treg ratio (Dulic et al. 2018; Liao et al. 2015). While most have reported that

Treg cells are involved in the pathogenesis of AS (Cao et al. 2004; Wang et al. 2015; Ye et al. 2016), there are few reports that are inconsistent with that concept. Several studies have reported a significant decrease in circulating Treg cells in AS patients compared to healthy controls (Wu et al. 2011; Zhao et al. 2011). Other studies have found no change in the percentage of circulating Treg cells in patients with AS (Guo et al. 2016; Cao et al. 2004; Wang et al. 2015; Ye et al. 2016).

Systemic sclerosis (SSc) is a connective tissue disease characterized by immune dysfunction, microvascular damage, and fibrosis of the skin and various organs (Frantz et al. 2018). A growing body of evidence indicates that T-cell proliferation and cytokine secretion play a major role in SSc (Meloni et al. 2009; Kalogerou et al. 2005). There is also controversy regarding the role of T regs in SSC with some reports showing impaired function while others demonstrating normal function (Liu et al. 2013a, b; Radstake et al. 2009; Papp et al. 2011; Fenoglio et al. 2011; Mathian et al. 2012; Klein et al. 2011). Studies have shown that Treg cells in the blood of patients with SSc have a normal phenotype and do not produce T effector cytokines. In contrast, skin Treg cells affected by SSc produce large amounts of IL-4 and IL-13 (MacDonald et al. 2015). Mathian et al. found that in SSc patients, activated and resting Treg cells were not functionally matched. The number of activated Treg cells decreased early in the disease (Mathian et al. 2012). Some authors have found that Tregs are significantly upregulated in all SSc phenotypes (Radstake et al. 2009; Giovannetti et al. 2010), especially in active and severe diseases (Slobodin et al. 2010). These inconsistencies could be explained by disease stages, new onset and treated patients, and methods used to define Treg cells. However, most studies reported that their functional capacity is reduced or T effector cells have gained the increased resistant ability to Treg suppression in disease status. Thus, the increase in the number of Treg cells and the restoration of their functions are important ways to induce immune tolerance and treat autoimmune diseases. Although Treg cells have heterogeneity and stability issues in clinical application, this treatment strategy is an important direction in the prevention and treatment of patients with autoimmune diseases.

1.8.2 Tregs in Infectious Diseases

In the field of infectious diseases, immune checkpoints limit protective immunity in many chronic infections, but therapies based on immune checkpoint suppression have not been well developed. Clearly, most pathogens have developed mechanisms to evade and suppress the host's protective immune response. Although Treg cells must control the activation of T-effector cells to prevent autoimmunity, it is also known that enhanced activation of Treg cells may lead to suppression of host immunity against microorganisms (viruses, bacteria, protozoa, fungi, and worms), resulting in reduced antimicrobial immunity and the persistence of pathogens (Belkaid et al. 2002, 2006). Many animal models of bacterial infection (e.g., *Listeria monocytogenes*, *Salmonella enteritis*, and *Mycobacterium tuberculosis*) exhibit the proliferation of Foxp3+ Treg. The inhibitory function of Treg cells can lead to increased bacterial load and invasion of systemic tissues (Johanns et al. 2010; Scott-Browne et al. 2007; Rowe et al. 2011). In addition, higher Treg frequency was associated with increased titer of hepatitis C virus RNA and dengue virus (Cabrera et al. 2004; Lühn et al. 2007). Paradoxically, Tregs play an early protective role in local infection in animal models of herpes simplex virus 2 and west Nile virus (Lanteri et al. 2009; Lund et al. 2008).

In the early stages of HIV infection, Tregs were found to control viral replication in target CD4+ T cells (Moreno-Fernandez et al. 2011). Moreover, Tregs may play an important and beneficial role in preventing the vigorous inflammatory response in the course of infection by parasites such as *Pneumocystis carinii* (Hori et al. 2002) and *Schistosoma mansoni* (Layland et al. 2013). Similarly, Tregs protect the host from parasites such as plasmodium and toxoplasma as well as the fungus *Candida albicans* (Haque et al. 2010; Oldenhove et al. 2009; Pandiyan et al.

2011). These complicated roles of Treg cells in acute and chronic microbial infections require a delicate balance between Foxp3+ Treg and effector T cells to provide an effective immune response against pathogens without inducing destructive autoimmunity.

Immunosuppressive cytokines IL-10 and TGF-β have established roles in suppressing anti-pathogenic effector T-cell responses (Mills 2004), but there is also growing evidence that immune checkpoints and Treg cells play a role. The use of immune checkpoint inhibitors may help reverse chronic infections, especially the immunosuppressive state in parasitic infections, and has great potential to promote an immune response that cures parasites. However, the greatest potential may lie in combining immune checkpoint blockades with therapeutic vaccination. Indeed, one study has demonstrated that vaccine-induced immune responses can be enhanced by reducing Treg cells or blocking the production or function of immunosuppressive cytokines (Jarnicki et al. 2008; Moore et al. 2005). Although blocking Treg cells or immune checkpoints is unlikely to be used to enhance the efficacy of routine vaccination, it does have great potential to enhance the efficacy of therapeutic vaccines against many chronic infections such as malaria, tuberculosis, and HIV.

1.8.3 Treg in Cancer

The association between Treg and cancers has been widely studied for decades. Many studies have shown that Tregs infiltrate into various types of cancers, such as breast, lung, liver, and gastrointestinal tract (Liyanage et al. 2002; Anna et al. 2002; Lars et al. 2005; Fumiko Ichihara et al. 2003). Additionally, the immunosuppressive function of Treg cells in patients with cancers is also significantly increased compared with healthy controls (Ju et al. 2009). Furthermore, Tregs account for 10–50% of the CD4+ T cells in tumors sites and, therefore, contribute substantially to tumor development by impairing the activation, survival, and expansion of anti-tumor T cells. The high levels of Treg and decreased ratios of tumor-infiltrating CD8+ T cells to Foxp3 + Treg have been demonstrated to correlate with the poor prognosis (Sato et al. 2005; Bates et al. 2006). Investigations on tumor specimens are crucial to understanding the phenotypes and origins of tumor-infiltrating Tregs which is more complicated than what we expected. It is still unclear whether the large amounts of Treg in tumor tissues have migrated from the periphery, amplified tTreg, or iTreg differentiated from naïve or effector CD4+ T cells in the tumor microenvironment. It is noted that the increase in intra-tumoral expression of chemokines such as CCL17, CCL22, and CCL28 may facilitate the recruitment of Treg cells (Speiser et al. 2016). With the rapid development of single cell sequencing technologies and the adoption of novel deeper immunophenotyping, a more comprehensive understanding of Treg heterogeneity is within reach. This is critical to safely and effectively exploit their anti-tumor immune response and to enhance their targeting therapeutic potential. Considering the crucial role of Treg in promoting tumorigenesis, cancer immunotherapy targeting Treg got more attention and achieved great progress. Therefore, the functional molecules related to Treg such as CTLA-4, GITR, PD-1, OX-40, and LAG3 are potential candidates that can be used for Treg depletion or functional modulation (Tanaka and Sakaguchi 2017). Checkpoint blockade therapy such as the clinical use of anti-CTLA-4 antibody predominantly affected Treg cells, thereby enhancing the anti-tumor immune response (Simpson et al. 2013). Additionally, agonistic interference such as anti-GITR antibody could abrogate Treg suppressive function because Tregs express elevated level of GITR and upregulate it upon activation (Shimizu et al. 2002). In general, Tregs have the opposite effect in cancers and autoimmune diseases. Thus, an effective anti-tumor response such as systemic Tregs depletion is likely to result in autoimmunity. Therefore, selective targeting specific subpopulations of Treg cells such as effector Treg in tumor tissues may be a more effective approach to promote tumor immunity without causing serious autoimmune reaction.

Table 1.2 Relationship between Treg cells and diseases

Disease	Circulating Treg frequency (compared to normal people)	Frequency of Tregs at focus	Function of Tregs	Changes in cytokines associated with Treg cells	Treg types most relevant to disease activity	Treg-related treatments
Autoimmune disease						
RA	↓	↑	↓	TNF-α IL-6 IL-1↑	$CD4^+CD25^{hi}CD127^{low/-}$ $Foxp3^+Helios^+$Treg	1. Treg adoptive transfer
SLE	↓	–	↓	IL-6 IL-17 IL-23↑ TGF-β IL-2↓	$Helios^+Foxp3^+$Treg	2. Pharmacological -based boosting Tregs
AS	↓	Unclear	↓	TNF-α IL-17 IL-6 IL-23↑	$CD4^+CD25^+Foxp3^+$ $CD127^-$Treg	
SSc	↓	Contradictory	↓	IL-4 IL-13↑ IL-10↓	$CD4^+CD25^+Foxp3^+$ Treg	3. Foxp3(+)Treg-induction in vivo
Type 1 diabetes	Unaltered	–	↓	IL-2 IL-10↓	$CD4^+CD25^+Foxp3^+$ Treg	4. "Tregitope" therapy
Infection disease	↑	↑	↑	IL-2 TGF-β↑	$Foxp3^+$ Treg	Blocking Treg cells
Cancer	↑	↑	↑	IL-2 TGF-β↑	$Foxp3^+$ Treg	1. Anti-CTLA-4 antibody 2. Anti-GITR antibody
Organ transplantation	↓	↓	↓	IL-6 IL-17↑ TGF-β IL-2↓	$Foxp3^+$ Treg	Treg adoptive transfer

1.8.4 Treg in Organ Transplantation

The achievement of graft tolerance and prevention of graft-versus-host disease (GvHD) have always been the important aims in the field of organ transplantation (Atif et al. 2020). Research has been focusing on preventing the chronic allograft dysfunction and reducing the side effects of long-term immunosuppression (Issa et al. 2013). In this regard, Treg within the peripheral circulating blood and graft microenvironment contribute to induce immune tolerance through employing multiple regulatory mechanisms (Liu et al. 2013a, b; Ferrer et al. 2014). Tregs limit graft damage by migrating to the organ and then retreating to the draining lymph nodes to maintain tolerance. Additionally, Tregs constitutively express cytotoxic T-lymphocyte antigen-4 (CTLA-4), which can interact with the costimulatory ligands CD80, CD86 of APCs, thereby inhibiting the activation of T cells (Tang and Vincenti 2017). Importantly, adoptive transfer of Treg has been shown to prevent transplant rejection and GVHD in mouse models which spur the development of experimental Treg therapies, particularly with antigen-specific Treg cells (Wu et al. 2013a, b; Brunstein et al. 2016; Liao et al. 2017; Gu et al. 2014). Currently, several Treg phase I clinical trials have shown encouraging safety and efficacy (Brunstein et al. 2011; Satoru et al. 2016). One trial was designed to identify the function of ex vivo expanded recipient polyclonal Treg in inducing immune tolerance in kidney transplant. The expanded Tregs amplified circulating Treg levels in a sustained manner without infusion-related side effects, infectious, or rejection events up to 2 years posttransplant (Mathew et al. 2018). Another trial was to determine the safety and feasibility of expanding polyclonal Treg ex vivo in kidney transplant recipients with subclinical graft inflammation noted on 6-month surveillance biopsy (Chandran et al. 2017). Treg infusion was safe and well tolerated without the impaired stability. In addition, compared with polyclonal Treg, alloantigen-specific Tregs have superior effects which potentially led to more targeted suppression, lower dose, and better safety (Esensten et al. 2018). There are still many challenges regarding Treg therapies in organ transplantation that require more basic and clinical studies. The above diseases are summarized in Table 1.2.

1.9 Conclusion

Treg cells play a vital role in regulating immune tolerance and balance, preventing autoimmunity, and have distinct roles in various disease environments. Restoring the frequency and function of Treg cells favor the therapy of autoimmune diseases and maintenance of allo-graft organ transplantation. Conversely, they probably are detrimental in patients with cancers and infection. Thus, they are a double-edged sword and selective manipulation of their frequency and function may be therapeutic in various immunological diseases. Indeed, it has been demonstrated to be effective in a series of clinical trials. However, it is still necessary to further explore the molecular mechanisms of how Treg cells regulate immune response. Treg surface molecular markers are still needed to be further identified since this will help to isolate live Treg cells in the clinical setting. In addition, the difference in phenotype and biological characteristics of various Treg subpopulations require to be further analyzed and compared. The epigenetic modification and mechanisms remain an in-depth study as well. Overall, understanding in-depth the role, mechanisms, and approaches in operating Treg cells helps to develop an innovative strategy for the treatment of many relevant diseases.

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