Role of Regulatory T cells in immunomodulation: A review

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Abstract

Regulatory T (Treg) cells can be defined as a T-cell population that functionally suppresses an immune response by influencing the activity of another cell type (Shevach, 2004) and thereby maintain immune system homeostasis and tolerance to self. However, recent advances in the molecular characterization of this cell population have firmly established their existence and their critical role in the vertebrate immune system (Sakaguchi et al., 1995). Interest in Treg cells has been heightened by evidence from experimental mouse models demonstrating that the immunosuppressive potential of these cells can be harnessed therapeutically to treat autoimmune diseases and facilitate transplantation tolerance. If Treg cells are specifically eliminated that potentiate cancer immunotherapy (Sakaguchi, 2005). Treg cells are thought to dampen T-cell immunity to tumour-associated antigens and to be the main obstacle tempering successful immunotherapy and active vaccination (Zou, 2006).

History

Treg cells were initially described in the early 1970s and were called suppressive T cells (Gershon *et al.*, 1970, 1971). The existence of a dedicated population of "suppressor" or "regulatory" T cells was the subject of significant controversy among immunologists for many years. Five years later, it was suggested that these Treg cells negatively regulate tumour immunity and contributed to tumour growth in mice (Fujimoto *et al.*, 1975). After another five years, a series of experiments provided evidence that CD4+CD25+ T cells from tumour bearing mice inhibited tumour rejection, indicating the existence of tumour-suppressor T cells (Bursuker *et al.*, 1984, North *et al.*, 1984). These pioneering studies established the field of Treg cells in tumour immunology. In 1995, Sakaguchi and colleagues showed that the interleukin-2 (IL-2) receptor α-chain, CD25, could serve as a phenotypic marker for CD4+ suppressor T cells or CD4+ Treg cells (Sakaguchi et al., *1995*). More recent studies have shown that the transcription factor forkhead box P3 (FOXP3) is not only a key intracellular marker but is also a crucial developmental and functional factor for CD4+CD25+ Tregcells (Hori *et al.*, 2003, Fontenot *et al.*, 2003).

Development

Thymus plays the most critical role in the production of Treg cells that were proved by the initial experiments of Sakaguchi et al (Sakaguchi et al, 1995). Neonatal thymectomy at three days of age in mice results in autoimmunity, which provided the data which reignited interest in suppressor/Treg cells. All T cells come from progenitor cells from the bone marrow which become committed to their linage in the thymus. All T cells begin as CD4-CD8-TCR- cells at the double-negative stage, where an individual cell will combine its T cell receptor genes to form a functional molecule which they in turn test against cells in the thymic cortex for a minimal level of interaction with self-MHC. If they receive these signals they proliferate and express both CD4 and CD8, becoming double-positive cells. The selection of Tregs occurs on radio-resistant haemopoetically-derived MHC class II expressing cells in the medulla or Hassal's corpuscles in the thymus. It seems that at the double-positive stage they are selected by their interaction with the cells within the thymus begin the transcription of Foxp3 and become Treg cells, although they may not begin to express Foxp3 until the single-positive

stage, at which point they are functional Tregs. Treg do not have the limited TCR expression of NKT or γδ T cells; Treg have a larger TCR diversity than effector T cells, biased towards self-peptides.

The exact process of Treg selection is still unknown, but appears to be a process determined by the affinity of interaction with the self-peptide MHC complex. Selection to become a Treg is a "Goldilocks" process. After rearrangement of ab chain of TCR T cells undergo two selection procedures, a positive selection and a negative selection. T cells, whose receptor bind to the self MHC molecules, receive signals that inhibit them to go in apoptotic pathway and stimulate to proliferate further (Scott et al, 1989). During positive selection, the RAG-1, RAG-2, and TdT proteins required for gene rearrangement and modification continue to be expressed. Thus each of the immature thymocytes in a clone expressing a given b chain have an opportunity to rearrange different TCR a-chain genes, and the resulting TCRs are then selected for self-MHC recognition. Only those cells whose abTCR heterodimer recognizes a self-MHC molecule are selected for survival. During negative selection, dendritic cells and macrophages bearing class I and class II MHC molecules interact with thymocytes bearing high-affinity receptors for self-antigen plus self-MHC molecules or for self-MHC molecules alone (Blackman et al, 1990; Alam et al, 1996). This interaction initiates a signalling cascade that lead to apoptotic pathway (Figure Positive and negative seletion). If a T cell receives an intermediate signal, it would then have the chance to become a regulatory cell. On the basis of several data (Sakaguchi et al, 1995, 2001; Maloy and Powrie 2001; Bensinger et al, 2001) it is hypothesis that thymocytes that develop into Tregs would have to express a TCR with relatively high affinity to self-peptides, whereas lower-affinity interactions between TCR and self-peptides expressed on thymic antigen-presenting cells (APCs) would promote the development of conventional CD4+CD25– T cells. Single positive CD4+ T cells that detect Foxp3 become Treg cells. Evidence suggests that interaction between TCR and thymic stroma expressing class II MHCself peptide complex is crucial for Treg development signal. CD25 in this mean time play crucial role to drive the cells to Treg cell lineage.

Foxp3+ Treg generation in the thymus is delayed by several days compared to Teff cells and does not reach adult levels either in the thymus or periphery until around three weeks postpartum. Treg cells require CD28 co-stimulation and B7-2 expression is largely restricted to the medulla, the development of which seems to parallel the development of Foxp3+ cells. It has been suggested that the two are linked, but no definitive link between the processes has yet been shown. TGF-β is not required for Treg development in the thymus, as thymic Treg from TGF-β insensitive TGFβRII-double-negative mice are functional.

Classification

Treg cells are categorized in four major groups. (a) The "natural" Treg cells originally recognised by their constitutive expression of CD4 and CD25 are further defined by expression of the transcription factor foxP3 and surface CD152. Their generation and some of their suppressive activity are dependent on TGF-beta, and it has been shown that they can induce IDO in appropriate DCs by CD152 mediated ligation of CD80/86. (b) Anergic CD4+ T cells generated by antigen stimulation in the absence of co-stimulation seem to be characterised by an intrinsic rising of their threshold for antigen stimulation that may be maintained by expression of E3 ubiquitin ligases such as GRAIL, c-cbl and Itch. Anergic cells can act as Treg cells by competing at the sites of antigen presentation and adsorbing out stimulatory cytokines such as IL-2. (c) Tr1 cells represent an induced subset of CD4 helper T cells that are dependent on IL-10 for their differentiation and for some of their regulatory properties. They do not express foxP3 but may express markers associated with Th2 cells and repressor of GATA (ROG). Like natural Tregs, they express high levels of surface CD152 and can induce IDO and trypophan catabolism in appropriate DCs. (d) CD8+CD28- suppressor T (Ts) cells were first characterised in human, but have recently also been demonstrated in rodents. Like Tr1 cells, they are induced in the presence of IL-10, and IL-10 may

be involved in the downregulation of dendritic cell costimulation and the upregulation of ILT-3 and ILT-4 (in human DC) that seem to play an important role in presenting antigen to tolerize further cohorts of T cells.

Molecular Characterization

Similar to other T cells, Treg cells develop in the thymus. The latest research suggests that Treg cells are defined by expression of the forkhead family transcription factor FOXP3 (forkhead box p3). Expression of FOXP3 is required for Treg cell development and appears to control a genetic program specifying this cell fate. The large majority of Foxp3-expressing Treg cells are found within the major histocompatibility complex (MHC) class II restricted CD4-expressing (CD4+) helper T cell population and express high levels of the interleukin-2 receptor alpha chain (CD25). In addition to the Foxp3-expressing CD4+CD25+, there also appears to be a minor population of MHC class I restricted CD8+ Foxp3-expressing Treg cells.

Prior to the identification of Foxp3, expression of these two cell surface molecules (CD4 and CD25) was used to define the population and thus these cells are often referred to as CD4+CD25+ Treg cells (TR or Treg). However, the use of CD25 as a marker for Treg cells is problematic as CD25 is also expressed on non-Treg cells in settings of immune activation such as during an immune response to a pathogen. As defined by CD4 and CD25 expression, Treg cells comprise about 5-10% of the mature CD4+ helper T cell subpopulation in mice and about 1-2% CD4+ helper T cells in humans. Foxp3 is not expressed on activated T cells and the Treg cell population as more accurately defined by Foxp3 expression extends beyond the CD4+CD25+ operational definition. Typically, high levels of CTLA-4 (cytotoxic T-lymphocyte associated molecule-4) and GITR (glucocorticoid-induced TNF receptor) are also expressed on Treg cells however the functional significance of this expression remains to be defined. There is a great interest in identifying cell surface markers that are uniquely and specifically expressed on all Foxp3-expressing Treg cells. However, to date no such molecule has been identified.

D4+CD25+ Treg cells have also been referred to as "naturally-occurring" Treg cells to distinguish them from "suppressor" T cell populations that are generated in vitro. In fact, the "naturally-occurring" CD4+CD25+ Treg cell population is a subset of the total Foxp3-expressing Treg cell population. The Treg cell field is further complicated by reports of additional "suppressor" T cell populations, including Tr1, CD8+CD28-, and Qa-1 restricted T cells. However the contribution of these populations to self-tolerance and immune homeostasis is less well defined.

Recent evidence suggests mast cells may be important mediators of Treg-dependent peripheral tolerance (Lu, et al, 2006).

Functions

To function properly, the immune system must discriminate between self and non-self. Though the immune system was evolved to protect organisms from a virtually infinite variety of disease-causing agents but it is also capable to avoid harmful responses to self. The potential capacity for the immune response to induce or activate disease was clearly recognized at the turn of the 20th century by Paul Ehrlich, who emphasized that the immune system must carefully distinguish between self and non-self in order to avoid autoimmunity. Ehrlich envisioned that during the ontogeny and outgrowth of the immunocompetent clones responsive to foreign antigens, there had to be mechanisms to control the outgrowth of clones reactive with self (Ehrlich, 1900). Moreover, the failure to control the outgrowth of autoreactive cells would lead to a state of “horror autotoxicus,” or autoimmunity. Because immune protective mechanisms include the elaboration of potent inflammatory molecules, antibodies, and killer cell activation — which together can not only destroy

invading microorganisms, pathogenic autoreactive cells, and tumours, but also mortally injure normal cells — the immune system is inherently a "double-edged sword" and must be tightly regulated. Immune response regulation includes homeostatic mechanisms intrinsic to the activation and differentiation of antigen-triggered immunocompetent cells and extrinsic mechanisms mediated by suppressor cells or Treg cells. When self/non-self discrimination fails, the immune system destroys cells and tissues of the body and as a result causes autoimmune diseases. Treg cells actively suppress activation of the immune system and prevent pathological self-reactivity, i.e. autoimmune disease (Zou, 2006). The critical role Treg cells play within the immune system is evidenced by the severe autoimmune syndrome that results from a genetic deficiency in Treg cells.

The molecular mechanism by which Treg cells exert their suppressor/regulatory activity has not been definitively characterized and is the subject of intense research. In vitro experiments suggest that suppressive mechanism requires cell-to-cell contact with the cell being suppressed (Zou, 2006). However, the immunosuppressive cytokines TGF-beta and Interleukin 10 (IL-10), produced by Th3 and Tr1 cells respectively, have also been implicated in regulatory T cell function.

An important question in the field of immunology is how the immunosuppressive activity of Treg cells is modulated during the course of an ongoing immune response. While the immunosuppressive function of Treg cells prevents the development of autoimmune disease, it is not desirable during immune responses to infectious microorganisms. Current hypotheses suggest that upon encounter with infectious microorganisms the activity of Treg cells may be down regulated, either directly or indirectly, by other cells to facilitate elimination of the infection. Experimental evidence from mouse models suggests that some pathogens may have evolved to manipulate Treg cells to immunosuppress the host and so potentiate their own survival. For example, regulatory T cell activity has been reported to increase in several infectious contexts, such as retroviral infections and various parasitic infections including Leishmania and malaria.

Genetic deficiency

Genetic mutations in the gene encoding Foxp3 have been identified in both humans and mice based on the heritable disease caused by these mutations. This disease provides the most striking evidence that Treg cells play a critical role in maintaining normal immune system function. Humans with mutations in Foxp3 suffer from a severe and rapidly fatal autoimmune disorder known as Immune dysregulation, Polyendocrinopathy, Enteropathy X-linked (IPEX) syndrome.

The IPEX syndrome is characterized by the development of overwhelming systemic autoimmunity in the first year of life resulting in the commonly observed triad of watery diarrhea, eczematous dermatitis, and endocrinopathy seen most commonly as insulin-dependent diabetes mellitus. Most individuals have other autoimmune phenomena including Coombs positive anemia, autoimmune thrombocytopenia, autoimmune neutropenia, and tubular nephropathy. The majority of affected males die within the first year of life of either metabolic derangements or sepsis. An analogous disease is also observed in a spontaneous Foxp3 mutant mouse known as "scurfy".

Treg and human diseases

Naturally occurring thymic-derived CD4+CD25+ Tregs are characterized by constitutive expression of the transcription factor FOXP3, while antigen-induced or adaptive Tregs are mainly identified by expression of immunosuppressive cytokines (interleukin-10 (IL-10) and/or transforming growth factor-β (TGF-β)). Treg cells act as a double edged sword. While Tregs in normal conditions regulate ongoing immune responses and prevent autoimmunity, imbalanced function or number of these Tregs, either enhanced or decreased, might lead, respectively, to decreased

immunity (e.g., with tumour development or infections) or autoimmunity (e.g., multiple sclerosis). Treg cells are related with several human deseases:

Autoimmunity

Reduced functional activity of Tregs results in an increased susceptibility to autoimmune disease. Significant decrease in the suppressive function of CD4+CD25+ Tregs has been observed in patients with multiple sclerosis (MS) (Viglietta et al, 2004), polyglandular syndrome of type II , active rheumatoid arthritis (RA) (Ehrenstein et al, 2004), type-I diabetes (Lindley et al, 2005), psoriasis, and myasthenia graves (Balandina et al, 2005) as compared with cells from healthy donors. In addition, in some autoimmune diseases, reduced levels of CD4+CD25+ Tregs have been observed in the peripheral blood of patients (Boyer et al, 2004, Longhi et al, 2006). However, decreased number of Tregs in peripheral blood in such patients may be result of impaired recruitment or migration of Tregs from the blood to the inflammatory site. It is found that at the site of inflammation (i.e., in the synovial fluid) the percentage of CD4+CD25+ Tregs was significantly increased as compared with the percentage in peripheral blood in patients with RA or juvenile idiopathic arthritis (JIA) (Mottonen et al, 2005). There is strong evidence for a dysfunction of CD4+CD25+ Tregs in suppressing Th2 responses in allergic patients (Grindebacke et al, 2004). A decrease and/or dysfunction of IL-10 secreting Tr1 cells were observed in allergic or asthmatic disease as compared to healthy individuals (Hawrylowicz and O'Garra 2005).

Infectious diseases

Several studies have also reported involvement of Tregs in infectious diseases, as Tregs might affect the magnitude of the immune response and therefore the outcome of viral clearance (Rouse et al, 2006). In humans with chronic hepatitis B virus (HBV) and HCV infection, an increase in peripheral CD4+CD25+ Tregs, as compared to healthy individuals, has been described (Cabrera et al, 2004, Stoop et al, 2005). Moreover, these Tregs are able to suppress HCV-specific CD8+ T cell immune responses (Boettler et al, 2005, Rushbrook et al, 2005). Besides increased levels of Tregs in patients, IL-10 producing Tr1 cells could also be isolated and cloned from patients with chronic HCV infection, but not from patients who cleared the infection (MacDonald, 2002). On the one hand, the frequency of Tregs inversely correlates with the magnitude of SIV/HIV-specific CTL responses (Aandahl et al, 2004, Estes et al, 2004)

Treg and cancer

Although the central physiological function of Treg cells is to maintain self tolerance, this negative regulatory activity can also be counterproductive as tregs might also suppress bonafied immune responses against tumours. High numbers of CD4+CD25+ Tregs have been found in lung, pancreas , breast, liver, and skin cancer patients, either in the peripheral blood or around and within the tumour (Woo et al, 2001, Liyanage et al, 2002, Ormandy et al, 2005). Moreover, Tregs isolated from tumours of lung cancer patients demonstrated potent immune suppressive activity of autologous peripheral blood T cells stimulated by anti-CD3 or anti-CD3/anti-CD28 in vitro (Woo et al, 2002). Therefore, it can be postulated that Tregs can impair antitumour immune responses in cancer patients. In human ovarian tumours, it is demonstrated that plasmacytoid DCs induce IL-10 secreting CD8+ Treg cells capable of suppressing antitumour immunity through IL-10 (Wei et al, 2005). Tumour cells and surrounding macrophages produce the CCL22 chemokine, which mediates Treg-trafficking to the tumour through CCR4, thereby possibly contributing to the immune privileged features of these tumours (Curiel et al 2004). This observation was recently also confirmed in B cell non-Hodgkin lymphomas (Yang et al, 2006). Furthermore, it is now believed that increased frequencies of Tregs in cancer patients are associated with a high mortality and reduced disease-free survival (Kono et al, 2006, Gallimore and Sakaguchi, 2002, Zou 2005).

Discussion

As mentioned before, Treg cells act as double edged sword. Therefore strategies for modifying Treg functions in therapeutic applications in different human diseases focus to induce Treg functions in several autoimmune diseases in one hand and in other hand, Treg depleting strategies have been adopted to combat cancer.

Treg-inducing strategies

Various antibodies and anti-inflammatory cytokines have been experimentally used to modify Treg function. For example, the defective suppressive function of Tregs was able to restore by treatment with infliximab (anti-TNF-α) as well as it also increased the number of peripheral Tregs in RA (Ehrenstein et al, 2004). Also, administration of immunomodulatory cytokines, such as TGF-β (Chen, et al, 2003) and nonmitogenic anti-CD3 monoclonal antibodies (Belghith et al, 2003) are Treg-modulating strategies currently under investigation. It is also found that costimulation through CD28 promotes Treg proliferation in vitro (Yamazaki et al, 2003, Tang et al, 2003). Administrations of superagonistic anti-CD28 antibodies, which probably cause augmented CD28 signaling, are particularly effective at supporting Treg expansion in vivo (Lin and Hunig, 2003). Keeping in mind the clinical risks associated with the use of these antibodies, current research focuses on the development of novel antigen-specific Treg therapies in order to reduce or prevent immune-mediated pathologies by selective enhancement of antigen-specific Treg populations in vitro or in vivo. These expanded cells retained expression of CD25, FOXP3, and lymph node homing receptors. Moreover, such in vitro expanded Tregs appeared to be more efficient in in vitro suppression assays as compared to freshly isolated Tregs (Hoffmann et al, 2004, Godfrey et al, 2004). Jaeckel et al, in 2005 developed another strategy based on transduction of naive CD4+ T cells from non-obese diabetic (NOD) mice with FOXP3, the transcription factor associated with Treg development and function. These FOXP3-transduced CD4+ T cells produce IL-10 and they are able to suppress CD4+ T cell proliferation. However, a therapeutic effect was only observed when FOXP3 was transduced in T cells from TCR transgenic mice that recognize a pancreatic islet antigen.

Treg-depleting strategies

Suppressing Treg responses are some time desired to enhance insufficient immune responses especially against certain viral and tumour antigens. Several studies demonstrated that administration of ONTAK (denileukin diftitox) which is a ligand-toxin fusion protein that consists of full-length IL-2 fused to the translocating and enzymatically active domain of diphtheria toxin, in cancer patients results in reduced prevalence of peripheral Tregs and increased effector T cell activation (Foss, 2000, Dannull et al, 2005, Frankel et al, 2006, Foss 2006). Enhanced immune responses have been observed after addition of anti-GITR antibodies. Ligation of GITR on Tregs results in abrogation of their suppressive function (Shimizu et al, 2002). Moreover, ligation of GITR on effector T cells provides effector T cells with additional costimulation and makes them refractory to the suppressive effects of Tregs (Shevach and Stephens, 2006, Stephens et al, 2004). Alternatively, it has been demonstrated that anti-CTLA-4 antibody inhibits the suppressive activity of Tregs in patients with malignant melanoma. Effective reduction in tumour mass was shown in approximately 20% of patients. Interestingly, reduction of tumour size was linked to the development of severe, but manageable, autoimmune syndromes (Blansfield et al, 2005, Maker et al, 2005, Maker et al, 2006, Phan et al, 2003). However, in cancer patients treated with the anti-CTLA-4 antibody, no effect was observed on the number or the suppressive activity of peripheral blood Tregs. This indicates that CTLA-4 signaling might represent a regulatory mechanism independent, at least in part, of Tregs (Atria et al, 2005). Moreover, it is also demonstrated that both mechanisms, Treg depletion and CTLA-4 blockade, can work synergistically (Sutmuller et al, 2001) on enhancing antitumour immunity in experimental B16 melanoma. Finally,

another potential strategy to interfere with Treg function is to target molecules involved in Treg trafficking. Blocking CCL22 has been proposed to reduce Treg trafficking in ovarian cancer in order to prevent their inhibitory function on APCs and on tumour-specific T cells (Curiel et al, 2004).

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