Therapeutic Approach of T Regulatory Cells of Immune System

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***Abstract*—Immune system is a group of biological structures that protects against diseases by identifying and killing pathogens and tumor cells. Immune system discriminates between self and non-self but when this discrimination fails, immune system starts attacking self-tissue that causes autoimmune diseases whereas T regulatory (Tregs) cells prevent this self-reactivity, i.e. autoimmunity.**

Keywords—T regulatory cells, autoimmunity, Foxp3

Introduction

T lymphocytes originate from progenitor cells in bone marrow and once committed to their lineage, they undergo thymic maturation. T cells begin as $CD4^-CD8^-TCR^-$, where they rearrange its receptor genes to form a unique, functional molecule which is tested in thymic cortex for its interaction with self-MHC. At this stage T cells are selected by their interaction with epithelial cells which is a "Goldilocks" process i.e. T cell that receives strong signals, undergoes apoptotic death and T cell that receives weak signal survives and become a T effecter cell whereas T cell that receives an intermediate signal becomes Treg cell.

Treg cells are a subpopulation of CD4 T cells that maintains homeostasis and tolerance to self-antigens by suppressing the activation of immune system therefore also known as suppressor T cells. Foxp3; a member of Fox protein family, is the master regulator gene for development and function of Treg cells. Expression of Foxp3 gene in naïve T cells can convert them in to Tregs which exhibit suppressive function both *in vivo* and *in vitro.* $CD4^+Foxp3^+$ T cells are referred as "naturally-occurring" Treg cells which distinguish them from "suppressor" T cell population that is generated *in vitro.*[1]

Foxp3 expression must be maintained in peripheral Treg cells to maintain activity.[2] Majority of Foxp3-expressing Treg cells are major histocompatibility complex (MHC) class II restricted $CD4^+$ which express high levels of IL-2 receptor alpha chain (CD25) but, there is a small population of MHC class I restricted $CD8^+$ $Foxp3^-$expressing Treg cells as well. Activated $CD4^+CD25^-$ and $CD8^+$ T cells expressed Foxp3 which suggested that this transcription factor is linked to suppressor function.[3]

In humans, mutations of Foxp3 lead to immune dysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX)[4] which is characterized by overwhelming systemic autoimmunity in the first year of life. It provided striking evidence that Treg cells are critical in normal immune system. An analogous disease has been observed in a spontaneous Foxp3 mutant mouse known as "scurfy".[2]

Regulatory T cells represent 5-10% in both mice and humans while their number can be 1-2% of peripheral $CD4^+$ T cells in whole blood. In addition to surface expression of CD25, cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and glucocorticoid-induced TNFR-related protein (GITR) expression are also features of Treg cells. It was suggested that for self-tolerance CD25+ CD4+ Tregs may require CTLA-4 but not CD28 as a costimulatory molecule for its functional activation.[5]

Removal of CD25+CD4+ Treg cells triggers excessive or misdirected immune responses to microbial antigens e.g. inflammatory bowel disease (IBD) where hyper-reaction of remaining T cells to commensal bacteria occur in intestine.[6] Treg cells suppress CD4 T cells in part through competition for IL-2, render CD8 T cells inactive via cell contact and TGF-β and suppress inflammation in part through secretion of IL-10.[7]

Saoudi et al. described for the first time that potent Treg cells were present in thymus of adult rat[8] but unlike conventional T cells, they did not produce IL-2. Field of Tregs cells was reborn by Sakaguchi *et al* when he induced autoimmune disease by transfer of $CD4^+$ $CD25^-$ T cells to an immunocompromised recipient whereas transfer of $CD4^+CD25^+$ cells prevented autoimmunity. These experiments indicated direct involvement of Tregs in suppression of autoimmunity [8, 9] and they also documented Tregs as 8% of CD4 cells.

Molecular mechanisms by which Treg cells exert its suppressor activity have not been defined but they produce cytokines such as IL-10, IL-13, and IL-4.[10] Immunosuppressive cytokines; TGF-β and IL-10 have been implicated in Treg cell function because Tregs shift the balance from proinflammatory to antiinflammatory cytokine profile.[10] Activated $CD4^+$ $CD25^+$ Treg cells can suppress activated $CD8^+$ T cells by concealing production of IFN-γ.[11] Treg cells also express some chemokines receptors i.e. CCR2, CCR4, CCR5, CCR7, CCR8 and CXCR4 therefore Treg cells migrate in response to variety of chemokines such as CCL1 (I-309), CCL4 (MIP-1β), CCL17 (TARC) and CCL22 (MDC).[12]

Treg cells and TH-17 cells originate from CD4 cells but they constitute opposing immune responses. Both the cells are key mediators of inflammation in autoimmunity and bacterial infection but the decision of naive $CD4^+$ T cells to become TH17 or Treg cell under different cytokines, has important consequences in the success of immune response and progression of disease.[12] In mice, TGF-β plus IL-6 drives differentiation of $CD4^+$ T cells into TH17 cells, whereas presence of TGF- β alone promotes differentiation of Treg cells. Unlike mice, in humans IL-1 β instead of TGF- β plus IL-6 drives differentiation of TH17 cells which provides an explanation that elevated number of Treg cells in many cancers are because tumors and tumor-stimulated myeloid cells are rich source of TGF-β.[12] In a study it was proposed that Th17 cells may play a protective role in tumor immunity. [13] Therefore Treg cells and TH-17 cells are two distinct lymphocytes with opposing actions.[14]

Four subsets of Treg cells have been described. First, natural Tregs (nTregs) were recognized by its constitutive expression of CD4, CD25, Foxp3 and CD152. Second, anergic CD4+ cells generated by antigen stimulation in the absence of costimulation were recognized. Third, $CD4^+$ cells dependent on IL-10 for their differentiation and regulatory properties were identified; were labeled as Tr1 cells. They do not express Foxp3 but may express markers of Th2 cells. Fourth, CD8+CD28-suppressor T cells were characterized and like Tr1 cells, they are induced in the presence of IL-10.

Tr1 cells are adaptive Tregs that differ from nTregs as they produce IL-10 and TGF- β in large amounts which suppresses naïve and memory $CD4^+$ T-cell function.[15] Tr3 cells are another adaptive Tregs which are vital for maintenance of oral tolerance, they achieve suppressive effect through IL-10, IL-4 and TGF-β while indirectly by promoting peripherally differentiation of antigen-specific Foxp3. Tr3 cells differ from Tr1 in their dependence on TGF-β for differentiation from $CD4^+CD25^-$T cells.[16]

Inducible Tregs (iTreg) are similar in function to nTregs but are derived from Foxp3 negative naive T cells from periphery. They express Foxp3, CTLA-4, and secrete IL-10 and TGF-β.2 In the presence of TGF-β, Foxp3+ expression on T cells is also increased (50–70%), and IL-27 is a potent inhibitor of iTreg.[17] The domain of Treg cell is further complicated by reports of additional suppressive T cell populations, including CD8+CD28-, and Qa-1 restricted T cells. Although Tr3 cells and iTreg are derived from $CD4^+CD25^-$T cells and they also function as nTregs yet

there is no documentation which shows these are the types of T regs.

Treg cells have been implicated in the immune responses against autoimmunity, allergy, microbial infection, tumor and transplantation.[1] Treg cell lines suppressed proliferation of dendritic cell–driven allo-mixed lymphocyte reaction (MLR) cultures by more than 90%.[18] Treg cells provide protection against inflammation in heart and its alteration by viral infection may contribute substantially towards myocarditis.[19]

Treg cells prevent autoimmune diseases but this immunosuppressive function is not required against infectious microorganisms hence in case of infection Treg cells are down regulated, directly or indirectly by other cells to facilitate elimination of infection. Experiments in mouse models suggested that some pathogens manipulated Treg cells suppression and potentiated their survival e.g. Treg cell activity had been increased in retroviral infection (HIV), mycobacterial infection (tuberculosis), and parasitic infections (leishmania and malaria).

Little is known about the molecular and cellular mechanisms responsible for the elevated levels of Treg cells in cancer.[20] Treg cells inhibit cytotoxic action of $CD3^-$ $CD56^+$ NK cells and steer monocyte differentiation toward alternatively activated macrophages (AAM); cells with immune regulatory properties that contribute in tumor progression.[21] *In Vitro* CD4+CD25+ T cells from tumor-bearing mice and cancer patients showed similar Foxp3 expression and suppressive activity when compared to naturally occurring Treg cells.[12]

A number of studies have reported $CD4^+CD25^+$ T cells and $CD4^+Foxp3^+$ T cells in tumor tissue i.e. tumor masses, ascites, draining lymph nodes, spleen and in peripheral blood of variety of cancers.[12] It is thought that tumor microenvironment is able to induce development of Treg cells by converting $CD4+CD25^-$ T cells into $CD4^+CD25^+$ T cells.[22]

Depletion of Tregs leads to decrease in tumor cell growth and eventual rejection of established tumor when used in combination with immunotherapeutic agent (PROb tumor cells mixed with BCG).[1] The suppressive effect of Treg cells is a major obstacle towards development of effective cancer immunotherapy. [1, 12] The spread of certain cancers could be due to excessive suppression of anti-tumor immunity. In an experiment, human T reg cells expressed membrane-bound TGF-β, which directly inhibited NK cell effector functions and therefore accelerated growth of tumors.[23] Accumulation of Tregs conferred poor prognosis in ovarian cancer.[24] However, some studies have shown benefits of high Tregs in patients of B-cell lymphoma.[25]

Inadequate number of Treg cells or their functional deficiency is linked with infertility, miscarriage and pre-eclampsia.[26] Medawar *et al* proposed the existence of regulatory mechanisms that suppressed maternal immune system. [13] Cytokines proposed to overrule Th1 cellular immune response which could lead to fetal abortion.[27]

In humans, a number of methods are employed to identify and monitor Treg cells. High expression of CD25 and CD4 surface markers were used but CD25 is also expressed on non-regulatory T cells e.g. some B lymphocytes. Sakaguchi *et al* studied FOXP3 as a marker for T regulatory cells.[4] Cellular expression of Foxp3 protein provides more specific analysis of Treg cells. Foxp3 is also transiently expressed on activated effecter T cells. Expression of CD127 in combination with CD4 and CD25 is another way. Additional markers used are high levels of CTLA-4 and GITR however functional significance of Tregs remains to be defined. Many studies concentrated on either number or percentage of $Foxp3^+$ cells for enumeration of Treg cells but many tumor cells also express Foxp3, lending the possibility that not all cells identified as Foxp3 positive were, in fact, Tregs.

Conclusion:

Lack of specific marker for Treg cells presents a serious challenge to researchers.[28] Adoptive transfer of Tregs

expressing FoxP3 and Bcl-xL demonstrated more effective suppression of rheumatoid arthritis (RA) than CD4+ T cells expressing Foxp3 alone. [29] Therefore, cultured Treg cells can be used as a novel form of immunosuppressive therapy in autoimmune patients[18] but Treg cells are an obstacle for immune therapy in cancers, however blockade of CD8+ suppressor–pathway, perhaps with antibodies specific for HLA-E–self-peptide complexes, could be useful.[3] The key to success will be to establish the correct balance between Treg cells and effector cells.[30]

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