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A review on prevention of graft-versus-host disease by the donor T regulatory cells with conventional T cells

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ABSTRACT

Graft-versus host disease is the major risk after stem cell transplantation. Stem cells of the donor, after transplantation attacks the immune system of the recipient's body and causes graft-versus host disease. Regulatory T cells represent a novel cell based approach for potentially reducing the risk of graft versus host disease (GVHD). Regulatory T cells (Tregs) are a subpopulation of $CD4^+$ T cells by the suppressive action on immune responses. These cells are also responsible for limiting tissue damage during ongoing and resolving immune responses. Before haemopoeitic stem cell transplantation, infusion of donor T regulatory cells with conventional T cells prevent graft versus host disease and promotes immune system recovery. After infusion of activated donor regulatory T cells the release of interleukin-10 and repress the activation of conventional T cells and thereby blocks rejection. Regulatory T cells and conventional T cells control adaptive immunity against pathogens and cancer by activating other effector immune cells.

Keywords: Regulatory T cells; Graft versus host disease; Haemopoeitic stem cell transplantation; Antigen presenting cells; Mixed lymphocyte reaction.

INTRODUCTION

Regulatory T cells maintain tolerance against antigens of its own and suppress autoimmune diseases. Mouse models suggest that modulation of Tregs can treat autoimmune disease and cancer and facilitate organ transplantation. Regulatory T cells of natural or induced type suppress T cells, especially naturally arising $CD25^+$ and $CD4^+$ Tregs, in which expression of the transcription factor forkhead box p3 (Foxp3) occurs in the thymusgical immune responses. Regulatory T cells suppress the activation of the immune system and prevent autoimmune diseases. The role of regulatory T cells play within the immune system is evidenced by the severe autoimmune diseases that results from a genetic deficiency in regulatory T cells. T regulatory cells were classified into two types, they are natural (derived in the thymus) or induced (derived in the periphery).

Thymus-derived regulatory T cells are homogeneous in population until they migrate into periphery from thymus gland, where a subpopulation of these cells can develop similar to conventional cells, memory cells and effector T cells. This change of regulatory T cells enables their migration to lymphoid and non-lymphoid tissues to maintain immune homeostasis. In

the periphery, T regulatory cells develop from conventional T cells.

Depending on the experimental model system studied, all induced Tregs not express $Foxp3^+$or $CD25^+$. Reports also demonstrate that, unlike thymically-derived Tregs, induced Tregs do not express high levels of $Foxp3^+$ and $CD25^+$. Contrary to conventional T cells, T regulatory cells express both glycoprotein A repetitions predominant (GARP), a membrane protein and tissue growth factor-beta transiently on their surface upon T cell receptor activation. Additional T regulatory cells subsets can be defined based on the expression of chemokine receptors and adhesion molecules.

Suppressive mechanisms of regulatory T cell

Secretion of IL-10

- The secretion of Interleukin (IL)-10 serves directly or indirectly to inhibit effector T cell responses. Treg cells also secret IL-35 and TGF-β to induce conventional $CD4^+$ T cells to differentiate into Treg cells, thereby skewing the ratio of Tregs to T helper cells during an immune response.

CTLA-4 and cell surface molecules

- Equally as important as IL-10 secretion, cell surface molecules such as cytotoxic T lymphocyte antigen 4 (CTLA-4) also participate in Treg cell-mediated suppression.
- Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibits dendritic cell (DC) mediated T cell stimulation by binding to CD80 and CD86, which leads to down regulation of these co-stimulatory molecules on the DC and induction of indoleamine 2,3-dioxygenase (IDO), an enzyme that depletes tryptophan from the microenvironment.

Master regulators

- Tregs use master regulators typically associated with specific T helper subsets also regulate the immune response customarily performed by those subsets.

Thus, understanding the mechanisms by which Tregs exert their suppressive function has broad implications for drug development strategies aimed at treating cancer, diabetes and other autoimmune diseases.

Cell type	phenotype	Suggested immunosuppressive mechanism
$CD4^+$ Natural regulatory T cells(nTreg)	$CD25^+Foxp3^+45RO^+CTLA\text{-}4^+GITR^+CD134^+CD62L^+CD103^+$lymphocyte activation gene-$3^+CD12710CD26^+$	Cell-to-Cell contact-depend in vitro (CTLA-4) Cell-to-Cell contact, cytokine-mediated(IL-10>>TGF-β,IL-5,IFN Production) Cytokine-mediated(IL-10 and TGF-β Production)
Inducible regulatory T cells (iTreg)	$CD25^+Foxp3^+45RO^+CTLA\text{-}4^+$ $CD25^+Foxp3^+45RO^+CTLA\text{-}4$-	Cytokine-mediated(TGF-β Production>>IL-10) Cell-to-Cell contact, Cytokine-mediated (IL-10>>TGF-β,IL-5,IFN production)
Th3 Tr1 TGF-β/IL-10 Double positive Treg	CD25-Foxp3 cells	Cytokine-mediated(IL-10 and TGF-β Production)
$CD8^+$ T suppressor cells Naturally occuring	Fox $p3^+45RO^+CD25^+CTLA\text{-}4^+GITR^+$	Cell-to-Cell contact-depend(CTLA-4) Cytokine-mediated(TGF-β Production) Cytokine-mediated(IL-10 production)
Non-antigen specific inducible	$CD28^+Foxp3^+CD56^+$ $CD25^+Foxp3^+CD28^+GIRT^+CTLA\text{-}4$	Cell-to-Cell contact Cell-to-Cell contact, Cytokine-mediated(IL-2,IL-10,IL-7,IFN-,TGF-β production)

PHENOTYPE OR MARKER MOLECULES OF TREGS

Tregs were originally defined on the basis of constitutive expression of surface CD4 antigen and surface CD25 antigen (IL-2 receptor-chain) at high density.[1] Early studies lacked the incorporation of $FoxP3^+$, which has been recognized as a master regulation and lineage-specification factor for Tregs.[1] More recently, studies have shown that reciprocal expression of the IL-7 receptor (CD127) on $FoxP3^+$ Tregs is a more specific way to quantify Tregs. nTregs constitute approximately 5%–10% of the peripheral $CD4^+$ T cell population in normal naive mice and healthy humans and are characterized by the constitutive expression of CD25 (IL-2 receptor chain) and low expression levels of CD45RB. The $CD4^+CD25^+$ phenotype of Tregs has been insufficient to define them as CD25, is not T cell restricted and cannot be used to distinguish Tregs from effector T cells (Teffs). While in murine models, $CD4^+CD25^+$ population is highly enriched in Tregs, but in humans $CD25^+$ cells contain both T regulatory cells and effector T cells populations.

To obtain enriched Tregs with little T effector cell contamination, it is necessary to gate on the $CD4^+CD25^+$ high population that has regulatory activity.[2] This population accounts for only 1%–3% of human $CD4^+CD25^+$Tcells. The $CD4^+CD25^+CD127$ low population contains approximately 80% of the $FoxP3^+$ cells and is significantly larger than the $CD4^+CD25^+$ high population. Overall, the available data indicates that $FoxP3^+$ identifies a broader Treg population than that defined by $CD4^+CD25^+$ or $CD4^+CD25^+CD127$ low expression alone.

A definition of Tregs by combining $CD127^+$ and $FoxP3^+$ has the advantage of including not only Tregs expressing high levels of $CD25^+$ but also Tregs with low $CD25^+$ expression and excluding at the same time activated conventional T cells.[3] While a strong correlation between $FoxP3^+$ and $CD25^+$ expression in the resting $CD4^+$ T cell population has been reported, low levels of $FoxP3^+$ are detectable in $CD25^+CD4^+$ T cells. Thus, it seems that $FoxP3^+$ expression too, in humans, might not be confined to Tregs. Other cell-surface markers associated with the phenotype and function of Tregs are CTLA-4, CD62 ligand (CD62L), TGF, IL-10, lymphocyte activation gene-3 (LAG-3), integrin E7 (CD103), neuropilin-1 (Nrp1).

CONTROL OF T CELL RESPONSES BY REGULATORY T CELLS

There are two categories of $CD4^+CD25^+$ Tregs, which differ in their origin, antigen specificity and effector mechanism. One subset of Tregs develops during the normal process of T cell maturation in the thymus, resulting in the generation of a naturally occurring population of $CD4^+CD25^+$ Tregs (nTregs) that survive in the periphery and poised to prevent potential autoimmune responses. The second subset of induced $CD4^+CD25^+$ Tregs (iTregs) whose precursor is also thymically derived, develops as a consequence of ex vivo peripheral activation of classical naive $CD4^+CD25^+$ T cell populations under particular conditions of suboptimal antigen exposure and/or co-stimulation. This figure depicts a model of peripheral T cell immunoregulation where the subset of nTregs can work in synchrony with iTregs to control the activation and function of adaptive immune responses. These iTregs can be generated exvivo from mature $CD4^+CD25^+$ T cell populations under different stimulatory conditions including antigen in the presence of immunosuppressive cytokines, such as IL-10 and TGF, vitamin D_3 and dexamethasone, CD40-CD40L blockade or immature dendritic cell populations. iTregs function in vitro and in vivo generally in a cytokine-dependent manner. In vitro, $CD4^+CD25^+$ nTregs are anergic to T cell receptor (TCR) stimulation, but require activation via the TCR to exert their regulatory functions. Once activated, they suppress both $CD4^+$ and $CD8^+$ T cell responses in an antigen non-specific manner.

GRAFT -VERSUS- HOST DISEASE

Graft-versus-host disease (GVHD), a major complication following allogeneic hematopoietic stem cell transplantation, is mediated by donor-derived T cells. On activation with alloantigens expressed on host antigen-presenting cells, naive $CD4^+$ T cells differentiate into T-helper cell subsets of effector T cells expressing distinct sets of transcriptional factors and cytokines. Classically, acute GVHD was suggested to be predominantly related to Th1 responses. However, a different and complex process involving possible roles of newly identified Th17 cells as well as Tregs in GVHD. Accumulating data from experimental and clinical studies suggest that the fine balance between Th1, Th2, Th17 and Tregs after

transplantation may be an important determinant of the severity, manifestation and tissue distribution of GVHD.[5] Understanding the dynamic process of reciprocal differentiation of regulatory and T-helper cell subsets as well as their interactions will be important in establishing novel strategies for preventing and treating GVHD.

HEMATOPOIETIC STEM CELL TRANSPLANTATION

Allogeneic hematopoietic stem cell transplantation (HCT) is a curative therapy for many hematologic, some epithelial malignancies, and a variety of non-malignant diseases. Hematopoietic stem cell transplantation represents the most effective treatment for patients with high risk and relapsed hematologic malignancies. However, donor T cells included in the graft react with recipient alloantigens present on APCs (antigen presenting cells) and produce a syndrome consisting of diarrhoea, weight loss, skin changes, and liver abnormalities called GVHD. Despite the enormous potential of hematopoietic stem cell transplantation, the risks associated with GVHD limit its extensive application.[6]

Billingham, an early pioneer in the field of bone marrow transplantation (BMT), described three requirements for the development of GVHD. First, the donor graft must contain immunologically competent cells (mature T cells). It was seen in both experimental and clinical allogeneic BMT that the severity of GVHD correlates with the number of donor T cells transfused. Second, the recipient must be immune-compromised and incapable of rejecting the transplanted cells. And, finally the recipient must express tissue antigens that are not present in the transplant donor.

DIRECT AND INDIRECT PRESENTATION

After allogeneic HCT transplants, both host- and donor-derived APCs are present in secondary lymphoid organs.[7] The donor T cells that are included in the graft recognize host alloantigens that are presented by either host APCs (direct presentation) or donor APCs (indirect presentation).

In the case of direct presentation, the donor T cells recognize either peptide bound to allogeneic (MHC) molecules or allogeneic MHC molecules without peptide, whereas in indirect presentation, T cells respond to the peptide generated by degradation of the allogeneic MHC molecules which are presented on self-MHC.[8] It was previously reported that host APCs, rather than donor APCs, are important for GVHD induction in MHC mismatch.[9]

Studies indicate that presentation of distinct target antigens by the host and donor type APCs might play a differential role in mediating damage to target organs.[10] Additionally, recent findings indicate that alloreactive Tregs specific for both directly and indirectly presented alloantigens are required for the induction of tolerance in organ transplantation.[11]

REGULATORY T CELLS: CELLULAR THERAPEUTIC FOR GVHD

Studies conducted with mixed lymphocyte reaction (MLR) experiments with both mouse and human cells demonstrate the ability of regulatory T cells to suppress the proliferative responses of alloreactive $CD4^+$ T cells.[12] It was reported that Tregs are effective in suppressing autoimmune diseases as well as solid organ transplantation.[13]

These findings lead researchers to investigate the role of Tregs in GVHD.[14] It was initially reported that depletion of $CD4^+CD25^+$ T cells from the donor graft accelerated GVHD and increased lethality.[15] Additionally; Tregs have been reported to be effective in preventing the development of GVHD across major and minor MHC barriers in various HCT models.[16] These studies demonstrate an important role of Tregs in the development of GVHD.[17]

However, even though physiological levels of endogenous $CD4^+CD25^+$ T cells may contribute to the development and course of GVHD, their small number is likely insufficient to control the overwhelming alloreactive T cell responses involved in major MHC (major histocompatability) mismatched BMT settings.[17]

Furthermore, the use of Tregs in allogeneic HCT is very promising since it was reported that the Tregs can suppress GVHD while preserving the GVL activity. However, three major issues still hinder the implementation of Tregs as immunotherapy in the clinic. These include the low circulating numbers of Tregs in the peripheral blood, the loss of suppressor activity following ex vivo expansion and the lack of Treg-specific markers to purify ex vivo expanded Tregs.[17]

However, the in vivo dynamics of Tregs trafficking and survival predict effective strategies to control GVHD after allogeneic transplantation.[18] Despite these considerations, several clinical trials are ongoing that adoptively transfer Tregs as immunotherapy to prevent the development of GVHD. One group has transplanted freshly isolated donor Tregs while a second group has expanded Tregs from cord blood with anti-CD3 and anti-CD28 coated micro beads and utilized them in double umbilical cord blood transplantation.[19]

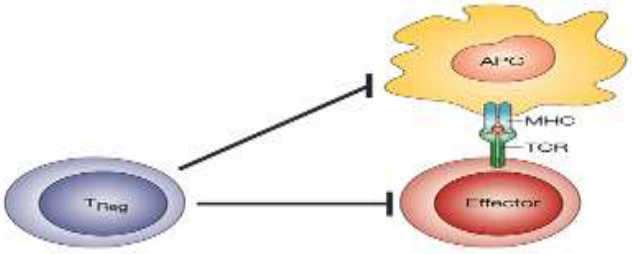

Figure 1 .Regulatory T cells can control immune responsiveness in vivo.

$CD4^+CD25^+$ T cells have been shown to regulate immune responses in vivo. This might be the result of the suppressive effects of regulatory T cells on effector T cells directly or on antigen-presenting cells (APCs). This can be beneficial to the host by preventing autoimmunity and enabling tolerance to organ, tissue and cell transplants to develop. However, it can also be detrimental as T regulatory cells can prevent effective immune responses to tumors and infectious agents.

Tregs and GVHD

Preclinical murine transplantation models have convincingly established that Tregs have the capacity to prevent alloreactive T-cell responses and experimental GVHD.[19] Although the early data on human Tregs and allo-HCT were mixed, the majority of recent studies support a role for Tregs in the protection from GVHD.[20] For example, stem cell grafts with a higher content of Tregs have been correlated with less GVHD. Likewise, more rapid Tregs reconstitution is associated with less GVHD, whereas patients with delayed Treg recovery have a higher likelihood of GVHD.[21]

Regulatory T cells are considered as candidates for immunotherapy after BMT (bone marrow transplantation) as they may reduce GVHD while maintaining Graft versus leukemia effects. Regulatory T cells (Tregs) play a vital role in the homeostasis of the immune system and in the modulation of the immune response. Tregs have emerged as key players in the development and maintenance of peripheral immune tolerance. [22]

Naturally occurring thymus-derived $CD4^+CD25^+$ Tregs are a subset of T cells which have immunosuppressive properties and are 5%–10% of the total peripheral $CD4^+$ T cells. In normal conditions, Tregs regulate ongoing immune responses and prevent autoimmunity. Imbalanced function or number of these cells, either enhanced or decreased, might lead to tumor development and autoimmunity, respectively. These cells thus play a major role in autoimmune diseases, transplantation tolerance, infectious diseases, allergic disease and tumour immunity.[23]

These natural properties make Tregs attractive tools for novel immunotherapeutic approaches. The in vivo manipulation or depletion of Tregs may help devise effective immunotherapy for patients with cancer, autoimmunity, graft versus-host disease, infectious diseases and allergic diseases. It is crucial to understand the biology of Tregs before attempting therapies, including (i) the injection of expanded Tregs to cure autoimmune disease or prevent graft-versus-host disease or (ii) the depletion or inhibition of Tregs in cancer therapy. Recent findings in murine models and studies in humans have opened new avenues to study the biology of Tregs and their therapeutic potential. This overview provides a framework for integrating these concepts of basic and translational research.[23]

REGULATORY T CELL- MEDIATED SUPPRESSION

Tregs are able to suppress the proliferation, activation and cytokine production of conventional T cells.

Multiple mechanisms of suppression by Treg have been identified which can be divided into four categories: (1) cell-cell contact, (2) secretion of suppressive factors, (3) competition for IL-2, and (4) modulation of APC by Treg.

Cell-cell contact

Tregs can suppress effector T cells (Teff) directly via a cell-cell contact-dependent mechanism, as suppressive activity in vitro is abrogated when responder T cells and Tregs are physically separated by a Transwell membrane insert.[24] It has been suggested that contact-dependent suppression is mediated by TGF, as murine and human Tregs express membrane-bound TGF suppression is abolished in the presence of anti-TGF-beta.[25] However, Tregs isolated from neonatal TGF-beta knockout mice exhibit normal suppressive activity in vitro. Contact-dependent suppression may also be mediated by the modulation of the level of cyclic adenosine monophosphate (cAMP) in T cells.[26] Tregs can deliver cAMP directly to the T cells via gap-junctions and thereby inhibit their proliferation and differentiation and cause selective inhibition of cytokine gene expression. Activated Tregs can also kill activated T cells by perforin, granzyme, or Fas-dependent mechanisms.[27]

Secretion of suppressive factors

Neutralizing in vitro antibodies to IL-10 or TGF not block Tregs activity and Tregs from mice lacking IL-10 and TGF-b show similar suppressive activity. In contrast, in certain in vivo models, TGF-beta and IL-10 are active players in the effector function of Treg.[28] IL-35 may contribute to the suppressive function of murine Tregs in vitro and in vivo as Treg from IL-35 mice have significantly reduced regulatory activity in vitro and fail to control homeostatic proliferation and to cure inflammatory bowel disease in vivo.[29] Furthermore, ectopic expression of IL-35 confers regulatory activity on naive T cells, whereas recombinant IL-35 suppresses T cell proliferation. Finally, Tregs can also convert extracellular 5'-AMP (adenosine mono phosphate) to adenosine via the ectonuclidases $CD73^+$ and $CD39^+$ expressed on their cell surface.[30] Binding of adenosine to the adenosine A2A receptor on T cells increases intracellular cAMP levels. Tregs from $CD39^+$mice show impaired suppressive properties in vitro and fail to block allograft rejection in vivo.[31]

Competition for IL-2

Local competition for IL-2, because Tregs constitutively express the IL-2 receptor $CD25^+$, it was suggested that Tregs suppress T cell responses by competing for IL-2 produced by effector T cells. By consuming the available IL-2, Tregs would prevent Teff (T effector cells) proliferation and differentiation.[32]

In agreement with this, blocking of IL-2 uptake in Tregs by selective inhibition of their IL-2 receptor completely abrogates their suppressive function.[33] Furthermore, the effects of Tregs on T cells can be mimicked by anti-IL-2. However, Tregs suppression cannot be entirely explained by the competitive consumption of IL-2 as Tregs can efficiently suppress proliferation of IL-2-receptor deficient T cells in vitro.[34]

Modulation of APC by Treg

Apart from direct interactions with T cells, Tregs can inhibit immune responses through modulation of major subpopulations of APC, i.e., B cells, monocytes or macrophages and most importantly; dendritic cells(DC).[35] DC constitute a heterogeneous population of professional APC that have the potential to induce immunity or tolerance depending on the state of activation, activation signals and cytokine milieu.[36] DC exposed to Treg down regulate their antigen presenting function by reducing the expression of MHC class II and the co-stimulatory molecules $CD80^+$ and $CD86^+$. The interaction between CTLA-4 on Treg and CD80/86 on DC can induce the expression of the suppressive mediator indoleamine 2, 3-dioxygenase (IDO) by DC.[37] Indoleamine 2, 3-dioxygenase catalyzes the breakdown of tryptophan into kynurenine and other catabolites, which have potent immunosuppressive effects in the local microenvironment of DC. Furthermore, DC can up regulate immunosuppressive molecules like B7-H3 and B7-H4 after interaction with Tregs, which results in reduced T cell stimulatory capacity. Interaction between Tregs and DC can also result in the secretion of the immunosuppressive cytokine IL-10 by the latter, which exerts suppressive effects on T lymphocyte proliferation.[38] Altogether, these data demonstrate that

Tregs inhibit DC activation and induce inhibitory DC, which are ineffective in activating Teff cells.

However, DC are not absolutely required for Tregs suppressor function, at least in vitro, since Tregs keep their suppressive capacity in DC-free systems.[39] Although several mechanisms of suppression have been described, it is still unclear which mechanisms contribute to Tregs-mediated suppression in vivo.[40] Most likely, Tregs do not rely on just one mechanism, but use different mechanisms simultaneously depending on environmental factors and the site of action.

TREG-MEDIATED SUPPRESSION: A SITE OF ACTION

To modulate immune responses in vivo, appropriate trafficking and retention of Tregs to specific sites is required. Tregs have been identified in lymphoid tissues, including thymus, spleen and lymph nodes, in peripheral blood as well as within various peripheral sites, including inflamed organs, tumors and infectious sites.[41] In order to enter all these sites, Tregs must express a variety of chemokine receptors and tissue-specific homing receptors that guide their migration to specific tissues. Some Treg subsets appear to be specialized in inhibiting the initiation of the immune response within lymphoid tissues, like Treg expressing the lymph node homing receptor CD62L and chemokine receptor CCR7.[42]

Other Treg subsets may limit peripheral expansion, cytokine secretion or cytolytic function of Teff cells at the effector site, like in inflamed tissues.[43] These Treg might include subsets expressing tissue-specific adhesion molecules and chemokine receptors like the inflammatory chemokine receptor CCR2 or CCR5, or the aE-integrin CD103.[44] Recently demonstrated that Treg sequentially migrate from inflamed tissues to the draining lymph node and that this migration pattern is necessary for the optimal suppressive function of Treg and islet graft survival .Whether Treg also follow this migration pattern during the course of other immune responses remains to be determined.[45]

CONCLUSION

Based on this review, infusion of donor T regulatory cells with conventional T cells reduces the graft-versus-host disease and enhances the immune recovery in high-risk leukemia patients.

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