Minireview

Naturally Occurring Self-Reactive $CD4^+CD25^+$ Regulatory T Cells: Universal Immune Code

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Naturally occurring thymus-arisen $CD4^+CD25^+$ regulatory T (Treg) cells are considered to play a central role in self-tolerance. Precise signals that promote the development of Treg cells remain elusive, but considerable evidence suggests that costimulatory molecules, cytokines, the nature of the TCR and the niche or the context in which the T cell encounters antigen in the thymus play important roles. Analysis of TCR from Treg cells has demonstrated that a large proportion of this population has a higher avidity to self-antigen in comparison with TCR from $CD4^+CD25^-$ cells and that peripheral antigen is required for their development, maintenance, or expansion. Treg cells have been shown to undergo expansion in the periphery, likely regulated by the presence of self-antigen. Many studies have shown that the involvement of Treg cells in the tolerance induction is antigen-specific, even with MHC-mismatched, in transplantation/graft versus host disease (GVHD), autoimmunity, cancer, and pregnancy. Theses studies concluded a vital role for self-reactive Treg cells in maintenance of the body integrity. Based on those studies, we hypothesize that self-reactive Treg cells are shared among all healthy individuals and recognize same self-antigens and their TCR encodes for few dominant antigens of each organ which defines the healthy self. These dominant self antigens can be regarded as "universal immune code". *Cellular & Molecular Immunology*. 2007;4(3):197-201.

Key Words: regulatory T cell, pregnancy, GVHD, autoimmunity, dominant self-antigen

Introduction

Naturally occurring regulatory T (Treg) cells arise during the normal process of maturation in the thymus (1, 2) and preferentially express high levels of CD25, the transcription factor forkhead box P3 (FoxP3) and a considerable number of other surface markers which have limited specificity to identify Treg cells (3). FoxP3 is currently considered as the most specific marker of Treg cells (4-6) and a mutation of this transcription factor is strongly linked to immune dysregulation. Precise signals that promote the development of Treg cells remain elusive, but considerable evidence suggests that costimulatory molecules and cytokines play important roles (7-10). Depending on these additional signals, thymocytes are then either negatively selected or induce a genetic program for Treg cells, including up-regulation of FoxP3 and CD25 (5). Other mechanisms suggested that something about the nature of the TCR, including its affinity for ligand and/or the level of expression, instructs the cells to become Treg cells (5, 11-14). On the other hand van Santen et al. (15) suggested that perhaps the niche or the context in which the T cell encounters antigen in the thymus is more important than affinity in determining cell fate.

Treg cells constitute approximately 5-10% of peripheral $CD4^+$ T cells in nonimmunized naïve mice and exhibit a vast spectrum of autoimmunity-preventive activity (16-19). Treg cells are naturally anergic and, upon TCR activation, potently suppress the proliferation of $CD4^+CD25^-$ T cells by an antigen-nonspecific mechanism (20-22). Molecular mechanisms by which Treg cells mediate suppression are unclear but seem to be independent of cytokine production and dependent on cell contact (20, 21). In addition, Treg cell-mediated suppression did not seem to be a variation of Th1/Th2 immune deviation, as antibodies against IL-4, IL-10, and TGF-β had no effect on the suppression (23, 24).

Analysis of TCR from Treg cells has demonstrated that a large proportion of this population has a higher avidity to self-antigen in comparison with TCR from $CD4^+CD25^-$ cells (13) and that peripheral antigen is required for their development, maintenance, or expansion. Treg cells have been shown to undergo expansion in the periphery, likely regulated by the presence of self-antigen (25, 26). Neonatally thymectomized mice, which are deficient in Treg cells,

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Received Feb 21, 2007. Accepted Apr.10, 2007.

develop multiorgan autoimmune disease, which can be overcome by the adoptive transfer of this population of T cells from normal mice. Several clinical observations in humans have supported a link between reduced thymic function, with impaired Treg cell generation, and the induction of autoimmune diseases, suggesting a central role for these cells in self-tolerance (1, 27, 28).

Treg cells in pregnancy

Recent studies have demonstrated the key role of Treg cells in pregnancy. During pregnancy, the mother's immune system has to tolerate the persistence of paternal alloantigens without influencing immune response to infections. Since normally the maternal immune system does not reject its semiallogeneic concept, pregnancy has been thought as a state of immunological tolerance (29, 30). It has been shown that normal pregnancy is associated with an elevation in the number of Treg cells which may be important in maintaining materno-fetal tolerance (31-33). Increased expression of FoxP3 gene and increased functional regulator activity confirmed that the numbers of functional Treg cells present in maternal circulation during pregnancy were increased (33). As pointed out by the authors an intriguing possibility is that the increase in regulatory cells provides an explanation for the observation that a number of autoimmune conditions tend to remit during pregnancy (34-36). On the other hand the acceptance of paternally derived tumor cells during pregnancy (30) supports the presence of systemic regulatory processes in pregnancy. Aluvihare et al. (37) observed an expansion of Treg cells in almost all tissues of pregnant compared to non-pregnant female mice independent of the paternal major histocompatibility complex (MHC) difference. During another study Zenclussen et al. (38) reported that the accumulation of paternal alloantigen-specific Th1 cells at the decidua of the well-established murine abortion model, CBA/J-DBA/2J (H-2^k - H-2^d), seems to be due to insufficient generation of pregnancy-induced Treg cells. BALB/c-mated CBA/J females (H-2^k - H-2^d) showed on the contrary augmented number of Treg cells during pregnancy. Moreover, fetal rejection could be completely prevented by adoptive transfer of Treg cells exclusively from normal pregnant mice (39). The result presented by Zenclussen et al. (38, 39) is an indication of the key role of Treg cells in normal pregnancy. Other authors supported the important role of Treg cells in human pregnancy (32, 33). Interestingly, Zenclussen et al. (39) also showed that Treg cells action may be antigen-specific during pregnancy, since the transfer of Treg cells from BALB/c (H-2^d)-pregnant CBA/J (H-2^k) females could prevent fetal rejection while the transfer from Treg cells obtained from virgin naïve CBA/J females could not. They suggested that alloantigen stimulation of Treg cells was required for priming of Treg cells, and optimal Treg cells activity in protecting allogeneic fetuses from rejection, as described for allografts. Accordingly, another study on syngeneic pregnancies showed that expansion of Treg cells also occurred during syngeneic pregnancy. Importantly, it was also shown that Treg cells transferred from pregnant females mated with syngeneic or allogeneic males equally protected allogeneic fetuses from rejection (37, 40).

Treg cells in GVHD and autoimmunity

Yamazaki and colleagues (41) showed that allogeneic DCs-expanded Treg cells suppressed mixed leukocyte reaction (MLR). Many reports have found that Treg cells could expand with spleen cells, as APCs or with DCs, and that these suppressed GVHD (41-46), skin graft rejection in nude mice (47), and allogeneic bone marrow transplants (48). For example, in the latter study B6 $CD4^+CD25^+$ host splenic T cells were cultured *in vitro* with B6D2F1 (H-2^{bd}) donor APCs. When such *in vitro*-cultured regulatory T cells were coinjected with B6 $CD4^+$ T lympho- cytes, rejection of semiallogeneic bone marrow was inhibited very efficiently (48). A murine fully MHC-mismatched (B6 : H-2^b → BALB/c : H-2^d) bone marrow transplantation (BMT) model is another example in which cotransplantation of donor B6 Treg cells into sublethally conditioned BALB/c recipients supported significantly greater lineage-committed and multipotential donor progenitors in recipient (49). Studies in murine models of hematopoietic stem cell transplantation (HSCT) have shown a significant reduction in GVHD by co-transplantation of purified or expanded polyclonal Treg cells, while potent graft-versus leukemia responses are retained (42-44, 46). On the other hand, based on the fact that like all other T-cell-receptor αβ (TCR αβ)-expressing T lymphocytes, thymus-derived $CD4^+CD25^+$ regulatory T lymphocytes are antigen specific. These cells have demonstrated an antigen-specific proliferation *in vivo* (26, 50, 51). When Treg cells specific for a pancreatic islet cell antigen are stimulated by DCs together with IL-2, the expanded antigen-specific Treg cells regulate the development of autoimmune diabetes in nonobese diabetic (NOD) mice and do so much more effectively than polyclonal populations (52). Studies in mice have also shown that Treg cells expanded with alloantigen, in order to increase the frequency of allo-reactive $CD4^+CD25^+$ Treg cells, were more effective in preventing some of the graft-versus-host pathologies (53). A comparison of alloantigen-expanded Treg cells to polyclonal Treg cells by Masteller et al. (54) demonstrated that both populations prevented clinical signs of GVHD. However, mice receiving polyclonal Treg cells showed histological signs of GVHD in the spleen, lung, and liver, whereas mice receiving alloantigen-expanded Treg cells showed no or only residual signs of GVHD. The authors concluded that antigen-specific Treg cells may also improve efficacy in the setting of GVHD and efficacy of Treg cell-based therapy should be increased by using organ-specific Treg cells rather than polyclonal Treg cells. This was also illustrated by experiments done by Albert et al. (55) indicating that antigen-specific Treg cells suppress allo-responses more effectively than polyclonal Treg cells. In addition, it seems likely that antigen-specific Treg cell therapy has the most potential to treat autoimmune diseases

in which target antigens and epitopes have been identified. Antigens involved in various autoimmune diseases have been identified, such as insulin and glutamic acid decarboxylase in type 1 diabetes (T1D), myelin basic protein in multiple sclerosis (MS), and type II collagen in rheumatoid arthritis (RA). Since transferred antigen-specific Treg cells are able to suppress T cells with other specificities through bystander suppression, it is not necessary that the initiating antigen for disease be identified or targeted by the Treg cells, although tissue-specific antigens, capable of activating Treg cells, are necessary. Treg cells with a single specificity against an islet antigen specicifc are able to suppress diabetes, which is believed to involve multiple antigens (54). This is best exemplified by the data mentioned previously where $CD4^+CD25^+$ regulatory T cells specific for myelin basic protein protected better against experimental autoimmune encephalomyelitis than regulatory T cells with a restricted (but non-myelin basic protein-specific) TCR repertoire (56). Also in NOD mice, Treg cells with a single specificity against an islet antigen are able to suppress diabetes, which is believed to involve multiple antigens (57). The use of antigen-specific Treg cells may also avoid deleterious effects of transferring large numbers of polyclonal Treg cells as they are involved in dampening the immune response to tumors and infectious agents.

Treg cells at the tumor site

Reports directly on the role of Treg cells in cancer were documented over two decades ago by Berendt and North (58). In 1986, Mukherji and colleagues (59) directly isolated suppressor T cells from a patient tumor sample and demonstrated that the suppression was specific to the autologous melanoma. Other studies also showed the presence of antigen-specific $CD4^+$ Treg cells in tumor-infiltrating lymphocytes (TILs) derived from fresh tumor samples (60, 61). Further studies demonstrated that the removal of $CD4^+CD25^+$ T cells by an anti-CD25 antibody in animal models enhanced antitumor responses (28, 62-64). In addition, elevated proportions of $CD4^+CD25^+$ Treg cells in the total $CD4^+$ T cell populations were found in different types of cancers, including lung, breast and ovarian tumors (65-68). Nicholl et al. (69) found that Treg cells with suppressive function *in vitro* comprised 48% of tumor-infiltrating $CD4^+$ T cells. More importantly, these Treg cells suppressed the proliferation of naïve $CD4^+$ T cells and inhibited IL-2 secretion by $CD4^+$ effector cells upon activation by tumor specific ligands. Newly identified LAGE1 and ARTC1 antigens, as natural ligands for Treg cells, established from cancer patients provides compelling evidence that antigen-specific Treg cells are present at tumor sites and mediate antigen specific and local immune suppression of antitumor immunity (60). All these studies suggest that in contrast to the role of antigen specific Treg cells in autoimmune diseases, which often have compromised function (70), tumor-infiltrating Treg cells provide an enriched source of natural Treg cells for establishing tumor (71). Thus it is conceivable that tumor antigen(s) or auto-antigen(s) of the affected tissue, which stimulate Treg cells for immune suppression, determine the fate of tumor progression or rejection. It emphasizes the need to revise in tumor vaccines, as a tumor vaccine may preferentially activate self-reactive Treg cells. An example of antigens which promote elicitation of naturally occurring Treg cells are SEREX-defined self-antigens (72-74).

Considering all the above-mentioned results, notably results on syngeneic pregnancies which showed expansion of Treg cells during syngeneic pregnancy and importantly the point that Treg cells transferred from pregnant females mated with syngeneic or allogeneic males equally protected allogeneic fetuses from rejection (37, 39) we suggest that self-reactive Treg cells are shared among all healthy individuals and recognize same self-antigens. In pregnancy, it is the similarities, but not the difference, between the mother and the fetus, which helps the maintenance of pregnancy. In other words we interpret the results in the way that activation of maternal Treg cells by antigens in common between the mother and the fetus causes the maintenance of systemic regulatory scene in normal pregnancy. Other mentioned results showed allogenic DC-expanded Treg cells have suppressive effect on GVHD, leading us to say that allogenic DC is capable of inducing Treg cells in suppression of GVHD. Moreover results of Masteller et al. (54) and Albert et al. (55) indicated that antigen-specific Treg cells suppressed alloresponses more effectively than polyclonal Treg cells. It was also confirmed by antigen-specific Treg cell therapy of autoimmune diseases (54, 56, 57). Finally, considering the similarities between cancer and pregnancy (reviewed by 75) results on pregnancy led us to hypothesize that Treg cells expanded at the different sample of a specific tumor recognize same self-antigen. This raises the potential of therapeutic purpose of Treg cells in cancer.

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