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Review

Making sense of regulatory T cell suppressive function

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ABSTRACT

Several types of regulatory T cells maintain self-tolerance and control excessive immune responses to foreign antigens. The major regulatory T subsets described over the past decade and novel function in transplantation will be covered in this review with a focus on $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells. Multiple mechanisms have been proposed to explain how Treg cells inhibit effector cells but none can completely explain the observed effects in toto. Proposed mechanisms to explain suppressive activity of Treg cells include the generation of inhibitory cytokines, induced death of effector cells by cytokine deprivation or cytolysis, local metabolic perturbation of target cells mediated by changes in extracellular nucleotide/nucleoside fluxes with alterations in intracellular signaling molecules such as cyclic AMP, and finally inhibition of dendritic cell functions. A better understanding of how Treg cells operate at the molecular level could result in novel and safer therapeutic approaches in transplantation and immune-mediated diseases.

Major roles in the maintenance of peripheral tolerance have been attributed to the activity of suppressor or regulatory T cells. Studies by Gershon et al. nearly 40 years ago first described the existence of suppressor T cells that could downregulate immune responses of antigen-specific T cells [1]. Around the same time, thymectomy studies suggested the generation of thymic-derived natural regulatory T cells that maintained immunological tolerance. In 1969, Nishizuka et al. noted that in mice, neonatal thymectomy induced an ovarian autoimmune disease [2]. Furthermore, Penhale et al. reported in 1973 that adult thymectomy of normal rats followed by sublethal X-irradiation also resulted in the development of autoimmune thyroiditis associated with tissue-specific autoantibodies [3]. Reconstitution of thymectomized animals with normal T cells inhibited autoimmunity [4].

There were major issues at that time which precluded continued investigation of putative regulatory T cells. These included the lack of reliable and validated markers for the identification of these cells together with great difficulty in isolating cell clones and defining peripheral cell effects [5]. The initial enthusiastic and intensive investigations of suppressor T cells therefore declined in the late 1980s.

However, in 1995 a seminal paper published by Sakaguchi et al. reinvigorated the field [6]. In recent years with the development of advanced molecular and cellular tools, several new cell markers were identified and suppressive functions of multiple cytokines were characterized resulting in the reappraisal of regulatory T cells as mediators of self-tolerance [5].

The existence of various regulatory T cell subsets has been demonstrated, which express distinct cytokines or receptors and function by diverse pathways at differing stages of the immune response [7]. The proposed suppressive functions of regulatory T cells include: generation of inhibitory cytokines; death of effector cells by cytokine deprivation or cytolysis; local metabolic perturbation of target cells mediated by changes in extracellular nucleotide/nucleoside fluxes with alterations in intracellular sig-

Abbreviations: APC, antigen presenting cells; BM, bone marrow; BMT, bone-marrow transplantation; cAMP, cyclic AMP; CTL, cytotoxic T lymphocytes; CTLA-4, cytotoxic T-lymphocyte antigen 4; DC, dendritic cells; DN, double negative Treg cells; EAE, experimental autoimmune encephalomyelitis; EC, endothelial cells; FcR, Fc receptor; FITC, fluorescein isothiocyanate; FGL2, fibrinogen-like protein 2; Foxp3, forkhead box P3; GITR, glucocorticoid-induced tumor necrosis factor receptor; GVHD, graft-versus-host-disease; H&E, hematoxylin and eosin; HLA, human leukocyte antigen; HSC, hematopoietic stem cells; IBD, inflammatory bowel disease; IDO, indoleamine 2,3-dioxygenase; IEL, intestinal intraepithelial lymphocyte; IFN, interferon; IHC, immunohistochemistry; IL, interleukin; LAG-3, lymphocyte activation gene-3; LPS, lipopolysaccharide; MBP, myelin basic protein; MHC, major histocompatibility complex; MS, multiple sclerosis; mTOR, mammalian target of rapamycin; NAD, nicotinamide adenine dinucleotide; NKT, natural killer T cells; PAMP, pathogen associated molecular patterns; SCID, severe combined immunodeficiency; TCR, T cell receptor; TGF-β, tumor growth factor; Th3, T helper type 3; TLR, Toll-like receptor; TNF, tumor necrosis factor; TRAIL-DR5, tumor-necrosis-factor-related apoptosis-inducing ligand-death receptor 5; Treg, $CD4^+CD25^+Foxp3^+$ regulatory T cells; Tr1, regulatory T cells type 1.

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naling molecules such as cyclic AMP (cAMP), and finally inhibition of dendritic cell functions [8].

It is generally accepted that regulatory T cells comprise two major subsets based on presumed ontogeny: adaptive regulatory T cells, which are induced in the periphery in response to antigen stimulation under tolerogenic conditions, and naturally occurring regulatory T cells, which are constantly produced by the thymus. Together these regulatory T cells maintain self-tolerance and control excessive immune responses to foreign antigens in the periphery. The major regulatory T cell subsets described over the past decade will be reviewed here (Fig. 1); with a focus on the most extensively studied regulatory T cell subset viz. $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells.

1. Regulatory T cells type 1 (Tr1)

Tr1 cells are unusual as these regulatory T cells do not express high levels of either CD25 or Foxp3. These cells were first described in patients with severe combined immunodeficiency (SCID), who had been successfully transplanted with allogeneic hematopoietic stem cells (HSCs). $CD4^+$ host-reactive T cell clones that were isolated from these SCID patients produced high levels of IL-10 and low amounts of IL-2 after antigen stimulation *in vitro*. Immunologic reconstitution and induction of tolerance following HLA-mismatched transplantation of hematopoietic stem cells (HSC) were associated with the presence of IL-10-producing $CD4^+$ T cells [9]. Subsequent studies by Groux et al. demonstrated that *ex vivo* activation of human and murine $CD4^+$ T cells in the presence of high levels of IL-10 induced the generation of IL-10-producing $CD4^+$ T cells with low proliferative responses. These cells produced a unique set of cytokines, characterized by high levels of IL-10, TGF-β and IL-5 but low levels of IL-2 and IFN-γ, and no IL-4 [10]. Due to their ability to suppress T-cell immune responses *in vitro* and *in vivo*, IL-10-producing $CD4^+$ T cells were termed regulatory T cells type 1 (Tr1) and had the recognized potential to prevent colitis (8).

Tr1 arise in the periphery following activation of naïve T cells with antigen in the presence of IL-10, and act as important regulators of adaptive immune responses through the suppression of naïve and memory T cell-responses with associated inhibition of antigen presenting cell or dendritic cell (DC) stimulatory activities [11]. Although Tr1 are induced in response to antigenic-specific stimulation, they exert their suppressive function in an antigen non-specific manner through the production of IL-10 and TGF-β. The release of the anti-inflammatory cytokines IL-10 and TGF-β is likely the reason for the bystander suppression of Tr1 cells [11].

The regulatory activity of Tr1 cells has been implicated in various immunopathologies both in mice and humans, inclusive of intestinal inflammation [11]. The importance of Tr1 cells in the maintenance of self-tolerance has been demonstrated. Isolated Tr1 cells bearing self-MHC reactive TCR from the peripheral blood of apparently healthy individuals have been shown to suppress proliferative responses of autoreactive T cells in an antigen-specific manner via production of IL-10 and TGF-β [12]. Furthermore, the numbers of Tr1 cells isolated from peripheral blood and synovial tissues of patients with rheumatoid arthritis are significantly lower compared to control patients [13]. The presence of Tr1 cells also correlates with the absence of graft-versus-host-disease (GVHD) and long-term graft tolerance in SCID patients transplanted with allogeneic HSCs [14]. In addition, a high frequency of Tr1 cells was associated with the absence of acute GVHD following bone-marrow transplantation (BMT), while low proportions correlate with development of severe GVHD [15]. Moreover, acceptance of kidney and liver allografts has been associated with the presence of Tr1 cells, which suppress naïve T cell responses through the production of IL-10 and TGF-β [16].

Clinical trials are currently ongoing to evaluate the potential therapeutic effects of Tr1 cells in the prevention and treatment of GVHD post BMT [17]. The clinical protocol involves the transfer of *ex vivo*-generated Tr1 cells to patients with hematological cancers treated with HSC transplantation. Treatment of the host with the IL-10-anergized donor T cells has potential to reconstitute the immunity without GVHD while guarding against infection and recurrence of cancer [17]. Success in such clinical trials might pave the way for Tr1-based immunotherapy in other immune-mediated diseases, such as autoimmunity and allergy.

2. T helper 3 (Th3)

Early studies of oral tolerance led to the identification of a unique $CD4^+$ T cell subset, which was later referred to as T helper type 3 (Th3) cells which express TGF-β. Such Th3 cell subpopulations were induced in the gut-associated lymphoid tissues of SJL mice by oral administration of myelin basic protein (MBP). These cells were able to suppress the proliferation and cytokine production of MBP-specific Th1 cells, and inhibit the development of experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis [18]. Production of TGF-β by Th3 cells was shown to account for the inhibition of EAE, as antibodies to TGF-β blocked the suppressive activity of Th3 cells [18]. In subsequent studies, Th3 cells were also identified in multiple sclerosis patients who were orally treated with MBP and proteolipid protein (PLP), which increased the frequency of TGF-β-secreting Th3 cells specific for MBP and PLP [19].

The gut microenvironment with high levels of TGF-β and Th2 cytokines, as well as unique subsets of DC, promotes the development of Th3 cells upon encounter with oral antigens [20]. Generation of Th3 cells is thought to be important in the induction and maintenance of tolerance to non-pathogenic resident bacteria and potentially immunogenic food antigens. Th3 cells are activated in response to specific antigens but suppress in an antigen-nonspecific manner through the release of TGF-β, and therefore mediate bystander suppression. Th3-type cells down regulate both Th1 and Th2 cells and provide help for B cells in the production of IgA antibodies [20].

The regulatory activity of Th3 cells has been implicated in the control of various experimental autoimmune disease models other than EAE, including spontaneous autoimmune diabetes, experimental autoimmune myasthenia gravis and autoimmune glomerulonephritis [21]. The importance of Th3 cells was also reported in donor transfusion-induced allograft tolerance and in suppression of lung eosinophilic inflammation [20]. Recent studies have also demonstrated a role for Th3 cells in some cases of human autoimmunity and allergy, as reduction in the number of the TGF-β-producing T cells or production of TGF-β was found in the duodenal mucosa of children with food allergy and patients with active chronic idiopathic thrombocytopenic purpura, respectively [22,23].

3. $CD8^+$ regulatory T cells

In recent years several types of $CD8^+$ T cells with regulatory or suppressive function have been identified. These include antigen nonspecific cells that are naturally produced in the thymus and antigen specific cells that are generated in response to foreign or self-antigens during the course of the peripheral immune response. In addition, other antigen specific and nonspecific subsets of $CD8^+$ regulatory T cells have also been induced *in vitro* [24].

Recent studies defined $CD8^+CD25^+$ human thymocytes that share similar phenotypic and functional properties with naturally occurring $CD4^+CD25^+$ T cells produced by the thymus [25].