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Naturally Arising $CD25^+CD4^+$ Regulatory T Cells in Tumor Immunity

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Abstract Naturally arising regulatory T (T_R) cells, represented by $CD25^+CD4^+$ T_R cells, play an essential role in maintaining immunological self-tolerance. This T cell-mediated dominant control of the immune response not only inhibits the development of autoimmune disease, but also impedes effective immunosurveillance against autologous tumor cells. Attenuation of T_R cell-mediated immune suppression can therefore evoke effective tumor immunity in otherwise nonresponsive animals. This common regulatory mechanism for autoimmunity and tumor immunity can be exploited when devising a novel immunotherapy for cancer.

1 Introduction

There is substantial evidence from both animals and humans that the immune system controls cancer development; that is, a cancer immunosurveillance

mechanism exists in normal individuals (Dunn et al. 2004). Recent studies have also shown that many cancer patients develop cytotoxic T lymphocytes (CTL) that can recognize tumor-associated antigens of autologous tumor cells, although they are not sufficiently strong to eradicate tumors in the majority of patients (Boon et al. 1994; Houghton 1994). A key issue in tumor immunology is therefore to understand the cellular and molecular basis of immunosurveillance against cancer, why cancer immunosurveillance is apparently not so effective in preventing cancer, and how it can be strengthened to prevent or treat cancer. A clue to these issues is the finding that many tumor antigens recognized by autologous CTLs are antigenically normal self-constituents. This indicates that a normal individual bears a T cell repertoire for tumor antigens as well as self antigens; i.e., tumor immunity is, in part, an autoimmunity. It also implies that the mechanisms that maintain immunologic tolerance to self-constituents may impede immunity against autologous tumor cells, and that manipulation of the immune system to break immunologic self-tolerance may provoke effective immune responses to autologous tumor cells.

T cells play key roles in mediating autoimmune disease as well as destroying tumor cells. It is now well established that, in addition to clonal deletion in the thymus or anergy induction in the periphery, there exists a T cell-mediated dominant mechanism of controlling self-reactive T cells; that is, a population of T cells actively suppresses the activation and expansion of self-reactive T cells (Sakaguchi 2004). Indeed, there are accumulating demonstrations that various autoimmune diseases can be produced in normal rodents by simply removing a particular $CD4^+$ T cell subpopulation defined by expression levels of certain cell surface molecules, and that reconstitution of the eliminated population can prevent autoimmune disease. CD25 is to date the most specific cell surface marker for such naturally occurring T_R cells that engage in the maintenance of natural self-tolerance. $CD25^+CD4^+$ T_R cells are unique in that the majority of them are naturally produced by the normal thymus as a functionally distinct and mature T cell subpopulation and persist in the periphery with stable regulatory functions (Asano et al. 1996; Itoh et al. 1999; Suri-Payer et al. 1998). Furthermore, recent studies have shown that the *Foxp3* gene, which encodes a transcription factor, specifically controls their development and function (Fontenot et al. 2003; Hori et al. 2003; Khattri et al. 2003).

T_R cells are heterogeneous in phenotype, function, and the way of generation. Some develop in the thymus as endogenous or natural T_R cells, which are represented by $CD25^+CD4^+$ T_R cells. Others are adaptive T_R cells that are induced in the periphery from mature T cells under particular in vivo or in vitro conditions for antigenic stimulation (Bluestone and Abbas 2003).

In this review, we focus on naturally arising $CD25^+CD4^+$ T_R cells because there is now substantial evidence that they play key roles in the control

of autoimmunity and tumor immunity. We shall review the immunological properties of natural $CD25^+CD4^+$ T_R cells and their roles in immunologic self-tolerance and immunosurveillance against tumor cells. We also discuss how they can be exploited to provoke effective tumor immunity by breaching natural self-tolerance.

2 Immunological Characteristics of Natural $CD25^+CD4^+$ T_R Cells

2.1 Suppressive Activity of $CD25^+CD4^+$ T_R Cells

Upon in vitro T cell receptor (TCR) stimulation, $CD25^+CD4^+$ T_R cells from normal naive mice exert potent suppression on the activation/proliferation of other T cells (both $CD4^+$ T cells and $CD8^+$ T cells) in an antigen-nonspecific manner; i.e., once they are activated by a specific antigen, they suppress the proliferation of not only T cells with the same antigen specificity as the T_R cells, but also those specific for irrelevant antigens presented by the same antigen-presenting cells (APCs) (Takahashi et al. 1998; Thornton and Shevach 1998). This suppression, directly or indirectly, results in inhibition of IL-2 production by the T cells under suppression. In contrast with other regulatory T cells secreting immunoregulatory cytokines such as IL-10 and TGF-β, the $CD25^+CD4^+$ T_R cell-mediated suppression is not mediated by far-reaching or long-lasting humoral factors, such as IL-4, IL-10, or TGF-β, but is dependent, at least in vitro, on a cell-to-cell interaction among T_R cells, effector T cells, and APCs. This suppression is highly sensitive to antigenic stimulation. For example, when $CD25^+CD4^+$ T_R cells and $CD25^-CD4^+$ T cells are prepared from a TCR-transgenic mouse and stimulated with a specific peptide, the antigen concentration required for stimulating the former to exert suppression is much lower than that required for triggering the latter to proliferate. This high antigen sensitivity of $CD25^+CD4^+$ T_R cells is suited for the maintenance of self-tolerance but potentially a hindrance to provoking effective tumor immunity.

$CD25^+CD4^+$ T_R cells constitutively express CTLA-4 as an essential co-stimulatory molecule for their activation (Read et al. 2000; Takahashi et al. 2000). In an in vitro proliferation assay, blocking CTLA-4 with a Fab fragment of anti-CTLA-4 monoclonal antibody (mAb) abrogated the suppression by $CD25^+CD4^+$ T_R cells. In addition, $CD25^+CD4^+$ T_R cells from normal mice suppressed the activation of $CD25^-CD4^+$ T cells from CTLA-4 deficient mice in vitro, and Fab anti-CTLA-4 mAb abrogated this suppression. These data

suggest that engagement of CTLA-4 on $CD25^+CD4^+$ T_R cells transduces a costimulatory signal for activating them; failure to activate $CD25^+CD4^+$ T_R cells by blocking CTLA-4 expressed on them, therefore, results in attenuated suppression on self-reactive T cells. Indeed, administration of anti-CTLA-4 mAb in normal mice elicited autoimmune disease similar to the one produced by depleting natural T_R cells. Interestingly, $CD25^+CD4^+$ T cells from CTLA-4-deficient mice can also suppress other T cells in vitro. This is presumably because CTLA-4-deficient $CD25^+CD4^+$ T_R cells are somehow intrinsically activated already, and hence can exert suppression. It is also well substantiated that activated T cells in general express CTLA-4 and interaction with B7 molecules transduces a negative signal to activated T cells. Blockade of CTLA-4 on activated T cells therefore sustains their activated state and effector activity. Taken together, it is likely that CTLA-4 possesses two roles in immunoregulation: one is to transduce a braking signal to activated effector T cells, the other to activate $CD25^+CD4^+$ T_R cells. The outcome of these two effects is the same, i.e., attenuation of immune responses. Blockade of the two events simultaneously therefore enhances immune responses synergistically.

$CD25^+CD4^+$ T_R cells also predominantly express glucocorticoid-induced tumor necrosis factor-related gene (GITR) (McHugh et al. 2002; Shimizu et al. 2002), which encodes a member of the tumor necrosis factor (TNF) receptor superfamily. Every T cell also expresses GITR at a low level, and T-cell activation upregulates the expression. Interestingly, addition of a monoclonal or polyclonal antibody to GITR abrogates $CD25^+CD4^+$ T_R cell-mediated suppression in vitro. Whole Ig molecules of agonistic anti-GITR mAb abrogated in vitro $CD25^+CD4^+$ T_R cell-mediated suppression, whereas Fab anti-GITR did not. Furthermore, administration of anti-GITR mAb produced autoimmune disease similar to the one produced by anti-CTLA-4 treatment (Shimizu et al. 2002). These results, taken together, indicate that ligation of the GITR molecule expressed on $CD25^+CD4^+$ T_R cells transduces a signal that attenuates their suppressive activity. Alternatively, ligation of GITR on activated T cells and not T_R cells renders them resistant to suppression (Stephens et al. 2004). It remains to be determined how GITR ligand physiologically transduces a signal to GITR-expressing T_R cells or non-T_R cells, or both.

Thus, the attenuation of the suppressive function of natural T_R cells with anti-CTLA-4 or anti-GITR mAb not only induces autoimmunity but also may enhance immune responses to autologous tumor cells in otherwise unresponsive individuals.

2.2 Functional and Phenotypic Stability of Natural $CD25^+CD4^+$ T_R Cells

Another unique property of naturally arising $CD25^+CD4^+$ T_R cells is their anergy to TCR stimulation. Purified $CD25^+CD4^+$ T_R cells from normal mice hardly proliferate in response to in vitro antigenic stimulation, and fail to transcribe the IL-2 gene, which is the hallmark of T cell anergy. Importantly, this anergic state is tightly coupled with their suppressive activity. For example, when $CD25^+CD4^+$ T_R cells are stimulated by antigen in the presence of high-dose exogenous IL-2 or agonistic anti-CD28 mAb, they can proliferate and at the same time lose their suppressive activity; upon removal of IL-2 or anti-CD28 mAb, they spontaneously revert to the original anergic state and reacquire suppressive activity (Kuniyasu et al. 2000; Takahashi et al. 1998). This indicates that the anergic and suppressive state is the basal and default condition for $CD25^+CD4^+$ T_R cells, at least in vitro. It also suggests that a functional breach of their anergic and suppressive state may elicit autoimmunity and also enhance tumor immunity.

Phenotypically, natural $CD25^+CD4^+$ T_R cells show an "activated" or "antigen-primed" phenotype already in the thymus and this phenotype is stably maintained in the periphery; e.g., they are $CD25^+$, $CD45RB^{low}$, $CD44^{high}$, and $CD5^{high}$. This suggests that the majority of $CD25^+CD4^+$ T_R cells could be inherently reactive with self-antigens and continuously activated by them in the normal internal environment.

2.3 Control of $CD25^+CD4^+$ T_R Cell Development by *Foxp3*

Another feature of natural $CD25^+CD4^+$ T_R cells is that their generation is in part developmentally and genetically controlled. Recent studies showed that *Foxp3*, a gene encoding a transcription factor of the forkhead/winged-helix family, plays a key role in their development and function. *Foxp3* was originally identified as the disease gene of fatal autoimmune/inflammatory disease of scurfy mice (Brunkow et al. 2001). Subsequently, mutations in *FOXP3* (the human ortholog of murine *Foxp3*) were found in patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, which phenotypically and pathologically resembles autoimmune/inflammatory diseases that develop in rodents following the removal of natural $CD25^+CD4^+$ T_R cells (Bennett et al. 2001; Chatila et al. 2000; Wildin et al. 2001). It was indeed shown that $CD25^+CD4^+$ T_R cells in the thymus and periphery predominantly expressed *Foxp3* mRNA, whereas B cells, $CD8^+$ T cells, $CD4^+CD8^+$, or $CD4^-CD8^+$ thymocytes did not (Hori et al. 2003; Khattri et al. 2003). The *Foxp3* expression levels in $CD25^+CD4^+$ T_R cells were approximately 100-fold

higher than that in $CD25^{-}CD4^{+}$ T cells. Activation of $CD25^{-}CD4^{+}$ T cells, Th1, or Th2 cells failed to induce *Foxp3* expression, in contrast with their expression of CD25, CTLA-4, and GITR, which are generally expressed on any activated T cells (Hori et al. 2003). Importantly, retroviral transduction of *Foxp3/FOXP3* into $CD25^{-}CD4^{+}$ T cells converted them to $CD25^{+}CD4^{+}$ T_R-like cells (Hori et al. 2003, Yagi et al. 2004). Such *Foxp3*-transduced T cells showed hypoproliferation and low production of cytokines in response to in vitro antigenic stimulation, and suppressed the activation of co-cultured $CD25^{-}CD4^{+}$ naive T cells in a similar manner to natural T_R cells. *Foxp3*-transduced T cells were also able to negatively control self-reactive T cells in vivo; for example, co-transfer of *Foxp3*-transduced T cells inhibited the development of inflammatory bowel disease and autoimmune gastritis that can be induced in SCID mice by the transfer of $CD25^{-}CD45RB^{high}CD4^{+}$ T cells from normal mice. *Foxp3* is also indispensable for the development of $CD25^{+}CD4^{+}$ T_R cells, as bone marrow cells from *Foxp3*-deficient mice failed to give rise to $CD25^{+}CD4^{+}$ T_R cells (Fontenot et al. 2003).

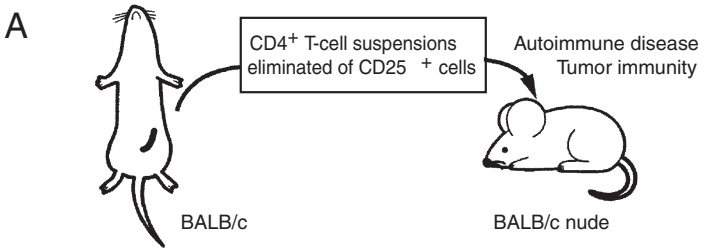
These results taken together indicate that *FOXP3/Foxp3* is a master control gene for the development and function of $CD25^{+}CD4^{+}$ T_R cells. Furthermore, *FOXP3/Foxp3* is a highly specific marker for natural T_R cells.

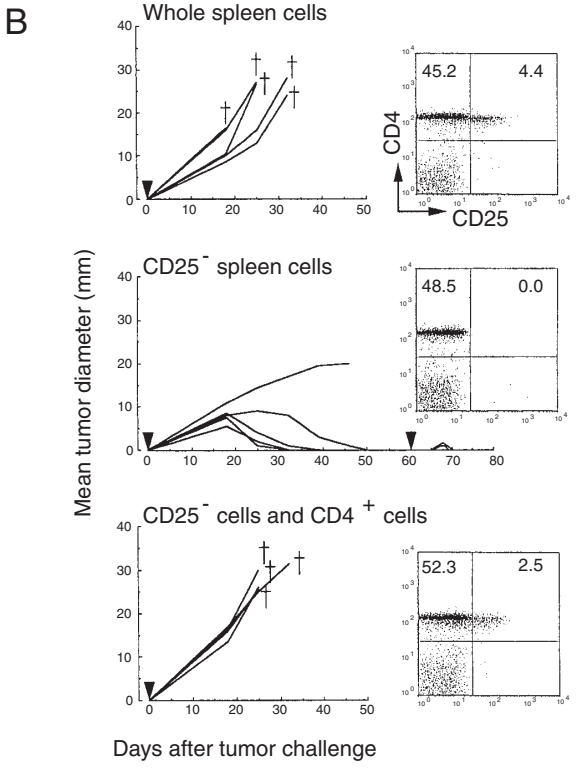
3
$CD25^{+}CD4^{+}$ T_R Cells in Tumor Immunity

The findings on self-tolerance and autoimmunity controlled by $CD25^{+}CD4^{+}$ T_R cells indicate that elimination of this regulatory population may provoke specific immune responses to syngeneic tumors as a "quasi-autoimmune" response. To examine this possibility, we transferred to BALB/c athymic nude mice splenic cell suspensions depleted of $CD25^{+}$ cells and subsequently

Fig. 1A, B Induction of autoimmune disease and tumor immunity in T cell-deficient mice. **A** Transfer of T cell suspensions depleted of $CD25^{+}$ cells induces autoimmune diseases and also tumor immunity in the recipient a nude mice, without deliberate immunization. **B** Tumor growth was monitored for BALB/c nude mice subcutaneously transplanted with 1.5×10^{5} RLmale1 cells (*arrow*) immediately after intravenous transfer of 3×10^{7} whole spleen cells (*upper panel*), or 3×10^{7} $CD25^{-}$ spleen cells (*middle panel*), or mixture of $CD25^{-}$ spleen cells (3×10^{7}) and $CD4^{+}$ spleen cells (1×10^{7}) (*lower panel*). The $CD25^{-}$ spleen cell-transferred nude mice having rejected the tumors were re-challenged on day 60 (*arrow*) with a ten times larger dose (1.5×10^{6}) of RLmale1 cells (*middle panel*). *Insets* show staining of each cell inoculum with CD4 (*ordinate*) and CD25 (*abscissa*), and percentages of cells in each quadrant

transplanted BALB/c-derived RLmale1 leukemia cells (Shimizu et al. 1999) (Fig. 1A). In the majority of mice, the tumors first grew and then regressed within a month, allowing the hosts to survive more than 80 days after tumor inoculation (Fig. 1B, middle panel), whereas all the nude mice transferred with nondepleted spleen cells (Fig. 1B, upper panel) or the mixture of an





equal number of $CD25^-$ cells and $CD4^+$ T cells died of tumor progression (Fig. 1B, lower panel). Upon re-challenge with a larger dose of RLmale1, the $CD25^-$ cell-transferred nude mice rejected the tumor cells more rapidly and vigorously than the primary rejection, indicating that they had become immune to the tumor cells (Fig. 1B, middle panel). The results indicate that tumor immunity can be evoked by reducing the number of natural T_R cells or by attenuating their suppressive activity.

3.1
Induction of Tumor Immunity by Reducing Natural $CD25^+CD4^+$ T_R Cells

Transient elimination of $CD25^+CD4^+$ T_R cells from normal mice by administering anti-CD25 mAb can also elicit immunity to syngeneic tumors (Shimizu et al. 1999). When anti-CD25 mAb (PC61) were administered twice (on 4 and 2 days before tumor inoculation) to BALB/c or C57BL/6 mice, the number of

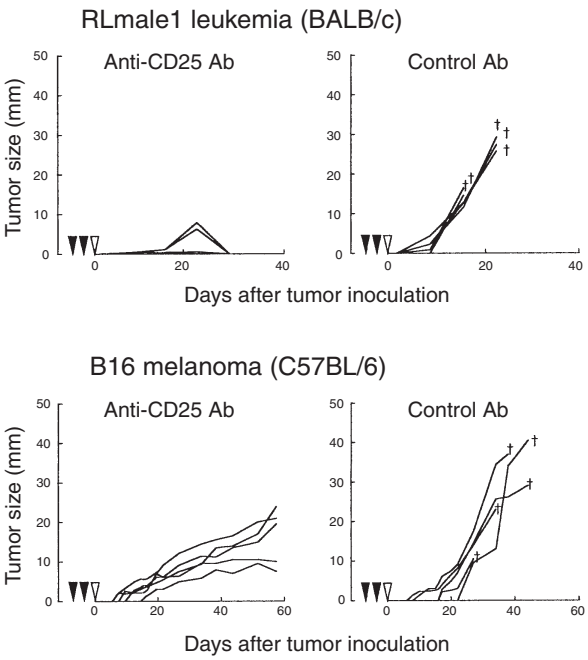


Fig. 2 Induction of tumor immunity by depleting $CD25^+$ cells in normal mice in vivo. Eight-week-old BALB/c or C57BL6 mice were each injected with 1 mg of purified PC61 (anti-CD25 depleting mAb) intravenously on 4 and 2 days (*filled arrows*) before subcutaneous inoculation of 1×10^5 Rlmale1 or B16 cells (*open arrow*), respectively. Tumor growth was monitored for individual mice

peripheral $CD25^+CD4^+$ T_R cells reduced to a quarter of control mice for nearly 1 month. In the majority of PC61-treated BALB/c mice, the subsequently inoculated RLmale1 cells first grew and then regressed within 1 month, whereas all the BALB/c mice treated with normal rat immunoglobulin as a control died of tumor progression within 1 month (Fig. 2, upper panels). Likewise, PC61 treatment of C57BL/6 mice significantly suppressed the growth of B16 melanoma cells when compared with control C57BL/6 mice treated with normal rat IgG, allowing the former to survive longer (>60 days) compared with the latter (<40 days) (Fig. 2, lower panels). This anti-CD25 treatment was also effective in eradicating a variety of tumors in other mouse strains (Onizuka et al. 1999).

Tumor effector cells can also be generated in vitro from normal spleen cells by simply eliminating $CD25^+CD4^+$ T_R cells. During this in vitro induction of tumor immunity, $CD25^-CD4^+$ T cells responding to self-peptides/class II MHC molecules expressed on syngeneic APCs spontaneously proliferated following removal of $CD25^+CD4^+$ T_R cells. A large amount of IL-2 produced by such $CD4^+$ self-reactive T cells generated natural killer-like tumor effector cells as lymphokine-activated killer (LAK) cells that were capable of indiscriminately killing various tumor cells (Shimizu et al. 1999).

3.2 Attenuation of Immune Suppressive Activity of $CD25^+CD4^+$ T_R Cells Can Evoke Tumor Immunity

The finding that blockade of CTLA-4 or signaling through GITR can attenuate $CD25^+CD4^+$ T_R cell-mediated suppression indicates that these treatments may also enhance tumor immunity (Shimizu et al. 2002). When DTA-1 anti-GITR mAb, which is nondepleting, was administered after the inoculation of Meth-A, a methylcholanthrene-induced sarcoma of BALB/c origin, the growth of tumor cells was significantly inhibited even when the treatment was commenced after tumor grew to a visible mass (Ko et al., manuscript in preparation). Interestingly, examination of *Foxp3* expression in tumor masses revealed that the number of *Foxp3*-expressing cells was decreased to a larger degree compared with other tumor infiltrating T cells in the DTA-1 treated mice. This indicates that DTA-1 treatment enhanced the activation and proliferation of tumor effector cells by abrogating T_R cell-mediated suppression, inhibited infiltration of T_R cells to the tumor mass, or both.

In vivo administration of anti-CTLA-4 antibody also enhances tumor immunity (Leach et al. 1996). This effect has been attributed to the possible hindrance of CTLA-4-induced negative signals to activated effector T cells mediating anti-tumor immune responses (for a recent review see Chen 2004).

Another possibility, which is not mutually exclusive, is the blockade of CTLA-4 molecules expressed on $CD25^+CD4^+$ T_R cells and consequent interference with T cell-mediated immunoregulation, as in the case of induction of autoimmunity by anti-CTLA-4 antibody treatment (Luhder et al. 1998; Perrin et al. 1996).

Taken together, reduction of natural $CD25^+CD4^+$ T_R cells or attenuation of immunosuppressive function of $CD25^+CD4^+$ T_R cells can break immunological unresponsiveness to syngeneic tumors both in vivo and in vitro, leading to spontaneous development of tumor-specific as well as tumor-nonspecific effector cells.

3.3 $CD25^+CD4^+$ T_R Cells and Tumor Immunity in Humans

T cells reactive with normal self-constituents or tumor-associated antigens are present in the peripheral blood of normal individuals. For example, peripheral blood $CD4^+$ T cells show in vitro proliferative responses to self-antigens such as human heat shock protein-60 (hHSP60) and myelin oligodendrocyte glycoprotein (MOG) when $CD25^+CD4^+$ T_R cells are removed before culture (Taams et al. 2002; Wing et al. 2003). Direct visualization of self-reactive T cells in healthy individuals was achieved by using class II tetramers loaded with the diabetes-associated antigen glutamic acid decarboxylase (GAD) 65, the vitiligo and melanoma-associated antigen tyrosinase, or the cancer/testis antigen NY-ESO-1 (Danke et al. 2004). Following removal of $CD25^+CD4^+$ T_R cells and stimulation with antigens, tetramer positive T cells became easily detected in vitro. T cells specific for tumor-associated antigens, most of which are normal self-constituents, can also be detected in the peripheral blood, within tumors, and in draining lymph nodes of cancer patients (Boon et al. 1994; Houghton, 1994). Despite the presence of such tumor-reactive T cells, it is rare to observe spontaneous regression of cancers. Although it remains to be determined whether cancer cells may somehow escape immune attack, or the immune system protects cancers from the attack, it is likely that naturally present $CD25^+CD4^+$ T_R cells play a role in suppressing the development of effective tumor immunity. A recent clinical study indeed showed that ovarian or gastric carcinomas with intra-tumor accumulation of $CD25^+CD4^+$*FOXP3*$^+$ T cells, supposedly $CD25^+CD4^+$ T_R cells, were associated with poor prognosis (Curiel et al. 2004; Sasada et al. 2003). Further study is needed to determine the role of natural T_R cells in tumor immunity in humans.

4
Autoimmunity and Tumor Immunity

Removal of $CD25^+CD4^+$ T_R cells may elicit autoimmunity in addition to provoking tumor immunity. This raises the issue of how tumor immunity can be evoked without autoimmunity by manipulating natural T_R cells. It is of note in this regard that the intensity and the range of autoimmune responses (i.e., the severity, the incidence, and the spectrum of autoimmune diseases) elicited by removal of T_R cells depend on the degree and duration of depleting $CD25^+CD4^+$ T_R cells, and, more importantly, the genetic background of the hosts (Sakaguchi et al. 1995, 1996). For example, in genetically autoimmune-prone BALB/c mice, generation of effective tumor immunity can be achieved without deleterious autoimmunity by limiting the period of depleting $CD25^+CD4^+$ T_R cells, whereas, in genetically autoimmune-resistant C57BL/6 mice, complete depletion of $CD25^+CD4^+$ T_R cells leads to tumor rejection without producing autoimmune disease (S. Yamazaki et al., unpublished results). Thus, autoimmunity and tumor immunity evoked by abrogation of the T_R-mediated immunoregulation can be differentiated by the duration or degree of T_R cell-depletion required for induction of autoimmunity or tumor immunity, and by host genetic factors that determine susceptibility or resistance to autoimmune disease. In addition, effector T cells involved in autoimmunity and tumor immunity may be different; for example, $CD8^+$ CTLs may play a more important role in tumor immunity than autoimmunity.

5
Conclusion and Perspective

It has been postulated since the 1970s that one of the elements that impedes the generation of effective tumor immunity in tumor-bearing hosts may be concomitant development of a T cell population suppressing the generation or action of tumor-killing effector cells. Although some of such suppressor T cells were previously shown to be $CD4^+$, they eluded further characterization and manipulation because of the lack of reliable markers specific for them (Awwad and North 1988). There is now accumulating evidence that such suppressive T cells, at least in part, can be naturally present $CD25^+CD4^+$ T_R cells (Fig. 3). The $CD25^+CD4^+$ T_R cells, however, bear several characteristics distinct from the suppressor T cells concomitantly induced by sensitization to tumor antigens. First, natural T_R cells are present before the appearance of tumor cells; that is, removal of $CD25^+CD4^+$ T_R cells before tumor development is effective in evoking specific tumor immunity. This means that they are physiologically

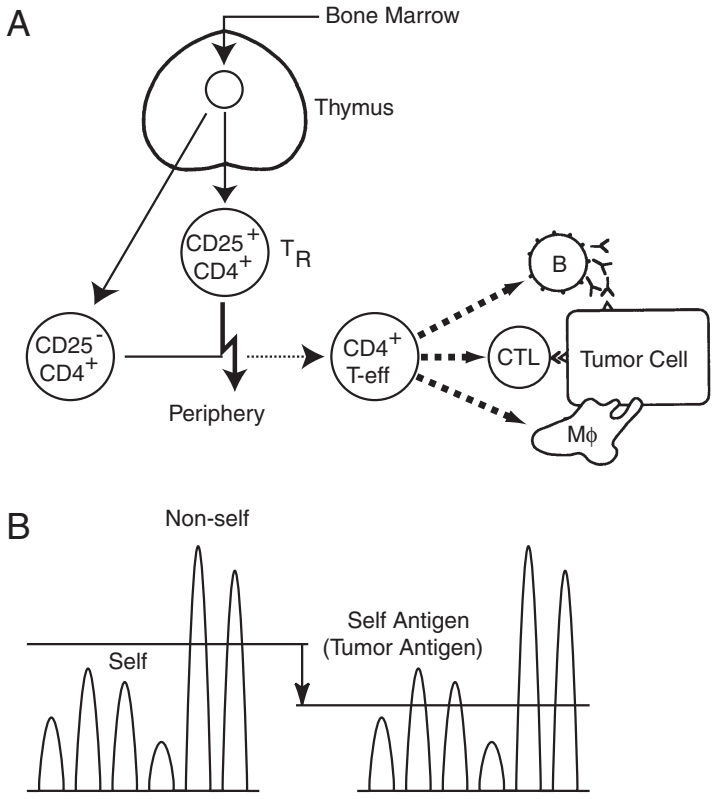


Fig. 3A, B Dominant suppression of tumor immunity by $CD25^+CD4^+$ T_R cells. **A** Although highly self-reactive T cells are eliminated during their thymic generation, the normal thymus continuously produces potentially pathogenic self-reactive $CD4^+$ T cells that persist in a $CD25^-$ quiescent state in the periphery. The normal thymus also continuously produces naturally anergic and suppressive $CD25^+CD4^+$ T_R cells that dominantly suppress the activation and expansion of $CD4^+$ self-reactive effector T cells from their $CD25^-$ dormant state. When $CD25^+CD4^+$ T_R cells are reduced in number or functionally impaired, $CD25^-$ self-reactive T cells become activated, expand, and differentiate to $CD25^+$-activated effector T cells (*dotted thin arrow*), which help B cells to form antibodies, conduct cell-mediated tumor immunity by recruiting inflammatory cells, including activated macrophages (*Mϕ*), and help activation and expansion of $CD8^+$ cytotoxic lymphocytes (*CTLs*) (*dotted thick arrows*). **B** Immune responsiveness to self and non-self depicted as a continuum. The *upper horizontal line* indicates the level of immunoregulation by T_R cells. The *peaks above the line* represent overt immune responses to non-self antigens. When the level goes down, immune responses to certain self-antigens (including tumor antigens) become apparent. The peaks representing immune responses to self-antigens are depicted as being lower than those to non-self-antigens, because T cells bearing high-avidity T cell receptors for the self antigens are supposed to be deleted in the thymus

impeding natural immunosurveillance and depletion/reduction of this population can augment immunosurveillance against cancer. Second, they are engaged in the maintenance of natural self-tolerance; therefore their removal can elicit not only tumor immunity but also autoimmunity. Third, natural $CD25^+CD4^+$ T_R cells are continuously produced by the normal thymus, constantly replenishing a fraction of the T-cell compartment (Itoh et al. 1999). This means that elimination of T_R cells for induction of tumor immunity may not impair immune function for a long period of time, and recovery of T_R cells may prevent the development of serious autoimmune disease.

Manipulation of natural $CD25^+CD4^+$ T_R cells is thus instrumental for cancer immunotherapy. For example, administration of anti-CD25, anti-CTLA-4, or anti-GITR antibody, or their combination, to cancer-bearing hosts for a limited period may evoke or enhance tumor immunity. Removal of T_R cells prior to in vitro culture of lymphocytes from cancer patients with high-dose IL-2 may lead to production of more potent or larger numbers of cytotoxic cells, including CTLs and NK cells (Rosenberg and Lotze 1986). Furthermore, monitoring FoxP3 expression in tumor tissue may be informative in assessing local tumor immunity.

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References

Asano M, Toda M, Sakaguchi N, Sakaguchi S (1996) Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. J Exp Med 184:387–396

Awwad M, North RJ (1988) Immunologically mediated regression of a murine lymphoma after treatment with anti-L3T4 antibody. A consequence of removing L3T4+ suppressor T cells from a host generating predominantly Lyt-2+ T cell-mediated immunity. J Exp Med 168:2193–2206

Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, Kelly TE, Saulsbury FT, Chance PF, Ochs HD (2001) The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet 27:20–21

Bluestone JA, Abbas AK (2003) Natural versus adaptive regulatory T cells. Nat Rev Immunol 3:253–257

Boon T, Cerottini JC, Van den Eynde B, van der Bruggen P, Van Pel A (1994) Tumor antigens recognized by T lymphocytes. Annu Rev Immunol 12:337–365

Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA, Wilkinson JE, Galas D, Ziegler SF, Ramsdell F (2001) Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet 27:68–73

Chatila TA, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C, Bowcock AM (2000) JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. J Clin Invest 106:R75–81

Chen L (2004) Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. Nat Rev Immunol 4:336–347

Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, Zhu Y, Wei S, Kryczek I, Daniel B, Gordon A, Myers L, Lackner A, Disis ML, Knutson KL, Chen L, Zou W (2004) Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med 10:942–949

Danke NA, Koelle DM, Yee C, Beheray S, Kwok WW (2004) Autoreactive T cells in healthy individuals. J Immunol 172:5967–5972

Dunn GP, Old LJ, Schreiber RD (2004) The immunobiology of cancer immunosurveillance and immunoediting. Immunity 21:137–148

Fontenot JD, Gavin MA, Rudensky AY (2003) Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 4:330–336

Hori S, Nomura T, Sakaguchi S (2003) Control of regulatory T cell development by the transcription factor Foxp3. Science 299:1057–1061

Houghton AN (1994) Cancer antigens: immune recognition of self and altered self. J Exp Med 180:1–4

Itoh M, Takahashi T, Sakaguchi N, Kuniyasu Y, Shimizu J, Otsuka F, Sakaguchi S (1999) Thymus and autoimmunity: production of CD25+CD4+ naturally anergic and suppressive T cells as a key function of the thymus in maintaining immunologic self-tolerance. J Immunol 162:5317–5326

Khattri R, Cox T, Yasayko SA, Ramsdell F (2003) An essential role for Scurfin in CD4+CD25+ T regulatory cells. Nat Immunol 4:337–342

Kuniyasu Y, Takahashi T, Itoh M, Shimizu J, Toda G, Sakaguchi S (2000) Naturally anergic and suppressive CD25(+)CD4(+) T cells as a functionally and phenotypically distinct immunoregulatory T cell subpopulation. Int Immunol 12:1145–1155

Leach DR, Krummel MF, Allison JP (1996) Enhancement of antitumor immunity by CTLA-4 blockade. Science 271:1734–1736

Luhder F, Hoglund P, Allison JP, Benoist C, Mathis D (1998) Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) regulates the unfolding of autoimmune diabetes. J Exp Med 187:427–432

McHugh RS, Whitters MJ, Piccirillo CA, Young DA, Shevach EM, Collins M, Byrne MC (2002) CD4(+)CD25(+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. Immunity 16:311–323

Onizuka S, Tawara I, Shimizu J, Sakaguchi S, Fujita T, Nakayama E (1999) Tumor rejection by in vivo administration of anti-CD25 (interleukin-2 receptor alpha) monoclonal antibody. Cancer Res 59:3128–3133

Perrin PJ, Maldonado JH, Davis TA, June CH, Racke MK (1996) CTLA-4 blockade enhances clinical disease and cytokine production during experimental allergic encephalomyelitis. J Immunol 157:1333–1336

Read S, Malmstrom V, Powrie F (2000) Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. J Exp Med 192:295–302

Rosenberg SA, Lotze MT (1986) Cancer immunotherapy using interleukin-2 and interleukin-2-activated lymphocytes. Annu Rev Immunol 4:681–709

Sasada T, Kimura M, Yoshida Y, Kanai M, Takabayashi A (2003) CD4+CD25+ regulatory T cells in patients with gastrointestinal malignancies: possible involvement of regulatory T cells in disease progression. Cancer 98:1089–1099

Sakaguchi S (2004) Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol 22:531–562

Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. (1995) J Immunol 155:1151–1164

Sakaguchi S, Toda M, Asano M, Itoh M, Morse SS, Sakaguchi N (1996) T cell-mediated maintenance of natural self-tolerance: its breakdown as a possible cause of various autoimmune diseases. J Autoimmun 9:211–220

Shimizu J, Yamazaki S, Sakaguchi S (1999) Induction of tumor immunity by removing CD25+CD4+ T cells: a common basis between tumor immunity and autoimmunity. J Immunol 163:5211–5218

Shimizu J, Yamazaki S, Takahashi T, Ishida Y, Sakaguchi S (2002) Stimulation of CD25(+)CD4(+) regulatory T cells through GITR breaks immunological self-tolerance. Nat Immunol 3:135–142

Stephens GL, McHugh RS, Whitters MJ, Young DA, Luxenberg D, Carreno BM, Collins M, Shevach EM (2004) Engagement of glucocorticoid-induced TNFR family-related receptor on effector T cells by its ligand mediates resistance to suppression by CD4+CD25+ T cells. J Immunol 173:5008-5020

Suri-Payer E, Amar AZ, Thornton AM, Shevach EM (1998) CD4+CD25+ T cells inhibit both the induction and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells. J Immunol 160:1212–1218

Taams LS, Vukmanovic-Stejic M, Smith J, Dunne PJ, Fletcher JM, Plunkett FJ, Ebeling SB, Lombardi G, Rustin MH, Bijlsma JW, Lafeber FP, Salmon M, Akbar AN (2002) Antigen-specific T cell suppression by human CD4+CD25+ regulatory T cells. Eur J Immunol 32:1621–1630

Takahashi T, Kuniyasu Y, Toda M, Sakaguchi N, Itoh M, Iwata M, Shimizu J, Sakaguchi S (1998) Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. Int Immunol 10:1969–1980

Takahashi T, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N, Mak TW, Sakaguchi S (2000) Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. J Exp Med 192:303–310

Thornton AM, Shevach EM (1998) CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. J Exp Med 188:287–296

Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N, Levy-Lahad E, Mazzella M, Goulet O, Perroni L, Bricarelli FD, Byrne G, McEuen M, Proll S, Appleby M, Brunkow ME (2001) X-linked neonatal diabetes mellitus, enteropathy

and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet 27:18–20

Wing K, Lindgren S, Kollberg G, Lundgren A, Harris RA, Rudin A, Lundin S, Suri-Payer E (2003) CD4 T cell activation by myelin oligodendrocyte glycoprotein is suppressed by adult but not cord blood CD25+ T cells. Eur J Immunol 33:579–587

Yagi H, Nomura T, Nakamura K, Yamazaki S, Kitawaki T, Hori S, Maeda M, Onodera M, Uchiyama T, Fujii S, Sakaguchi S (2004) Crucial role of FOXP3 in the development and function of human CD25+CD4+ regulatory cells. Int Immunol 16:1643–1656