

# Depletion of T<sub>reg</sub> Cells Augments the Therapeutic Effect of Cancer Vaccines

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Immune responses are controlled by both positive (upregulatory) and negative (downregulatory) signals. T cells generating the upregulatory signals, T helper (T<sub>H</sub>) cells, were intensively studied in a variety of experimental systems and their crucial role for immune surveillance can be demonstrated during human immunodeficiency virus (HIV) infection. HIV utilizes for its entry into human organisms surface receptors on T<sub>H</sub> cells. The cytopathogenic and other effects of HIV lead to the T<sub>H</sub> cell depletion, breakdown of immune responses, including breakdown of the resistance to various pathogens and some neoplasms. Therefore, the infection of the AIDS patients with pathogens that are harmless for HIV-uninfected individuals may be lethal for the patients.

The T cells generating downregulatory signals were, in analogy with CD4<sup>+</sup> T<sub>H</sub> cells, designated as CD4<sup>+</sup> T suppressor (T<sub>S</sub>) cells. For a long time their particular phenotype and function were not known in detail. The pioneer work of Gershon and Kondo (1970, 1971) helped in this sense. Fujimoto et al. (1975) suggested that T<sub>S</sub> cells can negatively regulate tumour immunity and contribute to tumour growth in tumour-bearing mice. In 1980 Berendt and North provided first definitive evidence that the T<sub>S</sub> cells that can be found in tumour-bearing individuals can inhibit tumour rejection (see also Bursucker and North, 1984, North and Bursucker, 1984). In 1995, Sakaguchi et al. demonstrated that the CD4<sup>+</sup> T<sub>S</sub> cells, which constitutively express interleukin-2 receptor  $\alpha$  chain (CD25), can maintain immunologic self-tolerance and that the CD25 molecules can serve as a phenotypic marker for CD4<sup>+</sup> suppressors designated here as CD4<sup>+</sup> regulatory T cells

(T<sub>regs</sub>). These authors have shown that breakdown of the single mechanism of self tolerance causes various autoimmune diseases. Transfer of CD4<sup>+</sup> T cells depleted of CD25<sup>+</sup> cells into nu/nu recipients induced organ-specific autoimmune diseases, and co-transfer of CD25<sup>+</sup>CD4<sup>+</sup> cells with the CD25<sup>-</sup>CD4<sup>+</sup> cells prevented development of the diseases. Additional studies (Fontenot et al., 2003; Hori et al. 2003) have demonstrated that the transcription factor forkhead box P3 (Foxp3) is a key internal cellular marker of T<sub>regs</sub> and a primary developmental and functional factor for CD4<sup>+</sup>CD25<sup>+</sup> T<sub>regs</sub>. At least two subpopulations of T<sub>regs</sub> exist: naturally occurring T<sub>regs</sub> (natural T<sub>regs</sub>) and adaptively induced T<sub>regs</sub> (adaptive T<sub>regs</sub>, Bluestone and Abbas, 2003). Natural T<sub>regs</sub> play a crucial role in the normal immune system by suppressing autoreactive T cells and maintaining immune tolerance. These cells are generated in thymus and constitute 5–10 % of peripheral CD4<sup>+</sup> T cells in mice. Adaptive T<sub>regs</sub> specific for foreign (infectious, tumour) antigens are probably of extrathymic origin and are generated in periphery from the peripheral T-cell repertoire.

How can we, by depletion of T<sub>reg</sub> cells, avoid autoimmunity and enhance the tumour immunity? The solution to this problem has not yet been definitively reached. It is supposed that normal animals harbour tumour-reactive T cells that are potentially capable of eradicating autologous tumours, but their activation and expansion is being suppressed by the presence of natural T<sub>regs</sub> that maintain self-tolerance. In some models it has been shown that the intensity and spectrum of autoimmune responses elicited by removal of T<sub>regs</sub> depend on the degree and duration of depletion, the age of the animals, their genetic background and route of the depleting antibody administration. Thus, tumour immunity could perhaps be evoked without serious autoimmunity by limiting the duration, the degree, and the mode of T<sub>reg</sub> depletion, and by taking into consideration the age and genetic makeup of the tumour host (for review and discussion, see Mahic et al., 2006; Yamaguchi and Sakaguchi, 2006).

*In vivo* depletion of CD25<sup>+</sup> T cells was capable of inhibiting, reducing, or preventing growth of a variety of murine neoplasms, such as melanoma B16, spontaneous leukaemias ASL1 and AKSL2, EL-4 leukaemia, radiation-induced leukaemias RL1 and RL8, plasmacytoma X5563, mastocytoma P815, mineral oil-induced myeloma MOPC70-A, methylcholanthrene-induced

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Abbreviations: CEA – human carcinoembryonic antigen, DC – dendritic cells, HIV – human immunodeficiency virus, HPV16 – human papillomavirus type 16, IL – interleukin, T<sub>H</sub> – T helper cells, T<sub>regs</sub> – regulatory T cells, T<sub>S</sub> – T suppressor cells.

fibrosarcoma CMS17, colorectal tumour CT26 (for a review, see Zhou, 2006), and HPV16-induced carcinomas TC-1 and TC-1/A9 (Šimová et al., 2006).

In human lung cancer and pleural mesothelioma patients, a low but significant increase in the number of T<sub>regs</sub> was observed as compared to normal healthy controls. Total CD4<sup>+</sup> T cells from these patients proliferated less than those from controls, suggesting that the increase of T<sub>regs</sub> had functional importance (Meloni et al., 2006). *In vivo* depletion of CD25<sup>+</sup> lymphocytes performed with the Demileukin Diftitox preparation was capable of increasing T-cell activation and inducing regression of human ovary, breast and lung cancer (Barnett et al., 2005), as well as of improving the dendritic cell-mediated, tumour antigen-specific immunity in human renal cell carcinoma patients (Dannull et al., 2005). However, the same pharmaceutical product, Denileukin Diftitox, the fusion protein of IL-2 and diphtheria toxin, was unable to eliminate T<sub>regs</sub> and had no clinical efficacy in the Rosenberg's melanoma patients (Attia et al., 2005).

In experimental tumour model systems a series of papers have definitely shown that depletion of T<sub>reg</sub> cells is capable of augmenting the therapeutic effect of different cancer vaccines. In 2004 it was demonstrated that *in vivo* depletion of T<sub>regs</sub> combined with local IL-12 production can substantially inhibit growth of B16 melanoma IL-12 transfectants in syngeneic mice (Nagai et al., 2004). Interestingly, such treatment also induced an autoimmune vitiligo-like coat colour alteration. In another study, the effect of CEA-coding recombinant vaccinia virus vaccine against human CEA-transduced murine MC38 colon carcinoma was optimally augmented by anti-CD25 monoclonal antibody depleting the CD25<sup>+</sup> T<sub>regs</sub> and local tumour irradiation (Kudo-Saito et al., 2005). Similarly, the effect of recombinant adenovirus vaccine coding for CEA directed against human CEA-expressing murine tumour transplants was augmented by T<sub>reg</sub> inactivation (Elia et al., in press). Also, augmentation of the prophylactic effects of DC-based anti-tumour vaccines by T<sub>reg</sub> depletion was demonstrated in the murine experimental melanoma B16 model system (Prasad et al., 2005). The *in vivo* elimination of T<sub>regs</sub> was capable of enhancing the immunostimulatory efficacy of tumour RNA-transfected DC vaccines in human metastatic renal cell carcinoma patients; specifically, the T<sub>reg</sub> depletion resulted in enhanced stimulation of proliferative and cytotoxic human T-cell responses *in vitro* (Dannull et al., 2005). Immunotherapy of murine breast adenocarcinoma micrometastases with irradiated vaccine composed of IL-21-producing breast carcinoma IL-21-transfected cells was substantially augmented by depletion of T<sub>regs</sub>. More than 70 % of mice bearing micrometastases were cured, whereas the anti-CD25 monoclonal antibody treatment alone had no effect (Comes et al., 2006). Depletion of T<sub>regs</sub> in combination with irradiated

homologous tumours cell vaccine promoted tumour-specific immune responses against murine pancreas cancer and resulted in longer survival of tumour-transplanted mice, whereas the vaccination or T<sub>reg</sub> depletion alone was without any effect with regard to the survival of tumour bearers (Viehl et al., 2006).

In conclusion, CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> T<sub>regs</sub> are an important cell population that plays a crucial role in the maintenance of homeostasis of the peripheral T-lymphocyte pool as well as peripheral self-tolerance, provides protection from autoimmune diseases, protection from graft-versus-host disease in patients after bone marrow transplantation, protection from transplant rejection, protection from overwhelming tissue destruction during infections and, last but not least, from the immune destruction of autologous tumours, since tumour antigens are often self antigens. The tumour-induced T<sub>regs</sub> are activated and expanded in some of experimental and human cancer bearers. They suppress naive and T<sub>H1</sub> effector cell proliferation and function. Currently, a variety of strategies for the induction and enhancement of specific anti-tumour immune responses based on T<sub>reg</sub> depletion are being tested in pre-clinical and clinical settings. Whereas in most experimental tumour models these strategies provided satisfactory results, in cancer patients their success was rather episodic and many basic as well as technical problems have still to be solved, before the therapeutic protocols for the depletion of specific adaptive T<sub>regs</sub> in cancer patients can be proposed. Nevertheless, the depletion of T<sub>regs</sub> belongs at present to the highlights of prospective cancer immunotherapy, particularly as a potent adjuvant to cancer vaccines.

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