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FOXP3-specific immunity

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Abbreviations: CTCL, cutaneous T-cell lymphoma; CTL, cytotoxic T lymphocyte; DC, dendritic cell; FOXP3, Forkhead box P3; GRZB, granzyme B; PBMC, peripheral blood mononuclear cell; Treg, regulatory T cell

Forkhead box P3 (FOXP3)-specific cytotoxic CD8$^+$ T cells are present among human peripheral blood mononuclear cells (PBMCs), especially in cancer patients. Such T lymphocytes are able not only to specifically recognize dendritic cells (DCs) that have been exposed to recombinant FOXP3 and regulatory T cells, but also to kill FOXP3$^+$ malignant T cells. The natural occurrence of FOXP3-specific cytotoxic T lymphocytes among human PBMCs suggests a general role for these cells in the complex network of immune regulation.

Forkhead box P3 (FOXP3)$^+$ regulatory T cells (Tregs) play a critical role in the regulation of immunity, for instance as they modulate the responses of the host to neoplasms and infections as well as they prevent autoimmunity. Tregs have received much attention in the field of tumor immunology during recent years. Cancer patients have been reported to harbor increased numbers of FOXP3$^+$ Tregs, which inhibit the development of efficient antitumor immune responses. In particular, several studies have reported elevated numbers of Tregs within the peripheral blood, lymphoid tissues and neoplastic lesions, a finding that has often been associated with poor prognosis. Recently, the levels of peripheral CD4$^+$FOXP3$^+$ Tregs have been negatively associated with the clinical response of malignant melanoma patients to adoptive T-cell transfer.[1]

Owing to the critical role that Tregs play in the regulation of immune responses, many immunotherapeutic anticancer regimens involve their depletion or modulation, although agents that specifically target Tregs are currently unavailable. Cyclophosphamide has emerged as a clinically compatible agent that can suppress Tregs, hence allowing for the induction of effective antitumor immune responses. Recently, in a randomized trial, single-dose cyclophosphamide has been associated with improvements in the survival of renal cell cancer patients treated with a therapeutic anticancer vaccine.[2] Likewise, clinical trials harnessing a variant of the diphtheria toxin specifically redirected to CD25$^+$ cells (Ontak) to eliminate Tregs have been performed in patients affected by renal cell carcinoma or melanoma.[3]

Immunotherapy might represent an unconventional method of targeting FOXP3$^+$ Tregs. In this context, Nair et al. employed a vaccine consisting of dendritic cells (DCs) electroporated with FOXP3-encoding mRNA in an animal model.[4] This intervention induced strong FOXP3-specific cytotoxic T lymphocyte (CTL) responses, leading to a significant reduction in the FOXP3$^+$ cell population. FOXP3-specific CTLs were able to kill FOXP3$^+$ Tregs in vitro. Interestingly, the simultaneous vaccination of mice against the well-described tumor-associated antigen dopachrome tautomerase (DCT, best known as TRP2) and FOXP3 enhanced the vaccine-induced protection against highly metastatic B16/F10.9 melanomas.

We have recently reported that FOXP3 is a natural CTL target in humans[5] (**Fig. 1**). Hence, also in humans, CD8$^+$ T cells are naturally reactive against FOXP3. In particular, we detected the reactivity of peripheral blood mononuclear cells (PBMCs) from HLA-A2$^+$ cancer patients against a FOXP3-derived peptide with high affinity toward HLA-A2 by means of an ELISPOT assay. A few individuals exhibited high numbers of FOXP3-targeting T cells, as in these subjects we were able to measure specific T-cell responses directly ex vivo. Of note, FOXP3-specific CD8$^+$ T cells released both interferon γ (IFNγ) and tumor necrosis factor α (TNF9). We confirmed FOXP3-specific T cells to be cytolytic effector cells by means of granzyme B (GRZB)-specific ELISPOT assays. Furthermore, we generated FOXP3-specific T-cell lines by re-stimulating PBMCs with FOXP3-derived peptides in vitro. These FOXP3-specific CTLs were able to directly recognize CD4$^+$CD25$^+$CD127$^-$FOXP3$^+$ cells ex vivo. Hence, intracellular FOXP3 is degraded and FOXP3-derived epitopes are processed and presented on the cell surface in complex with HLA-A2 molecules. Finally, we found that full-length recombinant FOXP3 is taken up and cross-presented by autologous DCs. This observation may be of importance, since it suggests that DCs presenting FOXP3-derived peptides may be able to activate T cells in vivo.

Cutaneous T cell lymphoma (CTCL) is the most frequent primary lymphoma

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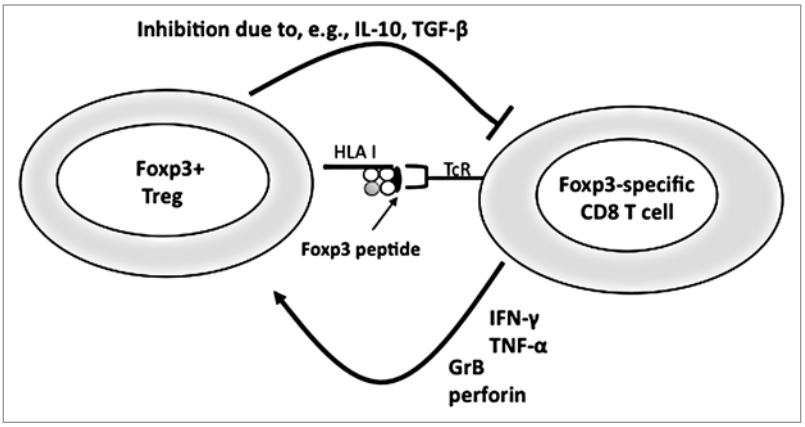

Figure 1. Role of FOXP3-targeting T cells in immune homeostasis. Regulatory T cells (Tregs) are characterized by the expression of Forkhead box P3 (FOXP3). As most other proteins, FOXP3 is degraded within the cell and FOXP3-derived peptides are presented on the cell surface in complex with HLA class I molecules, where they are recognized by $CD8^+$ T cells. Hence, cytotoxic FOXP3-specific T cells might be able to recognize HLA-restricted, FOXP3 epitopes on the surface of Tregs, thereby blocking and/or delaying local immunosuppression. In addition, FOXP3-specific T cells can eliminate cancer cells that express FOXP3. It must be assumed that the activity of cytotoxic FOXP3-specific T cells themselves is hampered by the immunosuppressive effects of their targets.

of the skin and is believed to involve the malignant proliferation of Tregs. Notably, FOXP3-specific CTLs killed malignant CTCL cells expressing high levels of FOXP3, suggesting that FOXP3-targeting vaccines might be useful in patients at risk of developing aggressive CTCL from malignant $FOXP3^+$ T cells.

FOXP3 is not the only protein expressed by immunoregulatory cells that is targeted by cytotoxic T lymphocytes, as recently this has been shown to concern also indoleamine 2,3-dioxygenase (IDO) isoforms and CD274 (best known as PD-1 ligand 1, PD-L1).[6–9] The expression of all these self proteins by normal cells of the immune system can be induced by different pathophysiological conditions including inflammation and other forms of stress. FOXP3 is expressed in the thymus, both in thymocytes destined to become Tregs and in thymic stromal cells.[10] Thus, the apparent lack of tolerance against FOXP3 is fascinating, as it could be indicative of a general role of FOXP3-specific cytotoxic T cells in the fine-tuning of immune responses. In particular, FOXP3-specific CTLs could play an important role by eliminating Tregs, thereby limiting and/or delaying local immunosuppression and contributing to immune homeostasis.[5]

Although it remains to be determined how and when FOXP3-specific T cells become activated as well as what, if any, potential role they play in immune regulation, such self-reactive CTLs may be immensely useful for anticancer immunotherapy, a setting in which Tregs generally antagonize clinical benefits. Targeting FOXP3-derived peptides presented by HLA molecules on the cell surface represents a different concept in immunotherapy, as in this case specificity is not limited to proteins expressed on the cell surface. Since Tregs might antagonize the desired effects of multiple immunotherapeutic approaches, the depletion of such cells by FOXP3-targeting vaccines might synergize with other vaccination strategies. Naturally, particular caution should be taken relative to the possible induction of autoimmunity when a self protein like FOXP3 is targeted. In this regard, Nair et al. found no effects of their FOXP3-targeting vaccine on the number of circulating Tregs, whereas the amount of tumor-infiltrating Tregs was significantly reduced.[4] This might be due to the homing of activated T cells to inflammation sites and neoplastic lesions, perhaps suggesting that this approach is associated with a limited risk of autoimmune disorders.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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