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Interleukin-2 treatment of tumor patients can expand regulatory T cells

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Augmented numbers of regulatory T cells contribute to the overall immunosuppression in tumor patients. Interleukin-2 has been widely used in the clinics in anticancer therapy, yet evidence has accumulated that the major drawback, limiting clinical efficacy, is the expansion of regulatory T cells, which aggravates immunosuppression.

Interleukin-2 (IL-2) has been identified more than 30 years ago and primarily been described as a factor acting on conventional T cells to promote their activation and proliferation.[1] Due to its T-cell activating and expanding properties, IL-2 has been introduced early into the immunotherapy of cancer patients, either as a single agent or in combination with other cytokines or chemotherapy.[1] However, over the last several years it has become clear that IL-2 not only has beneficial properties, but also can expand regulatory T (T_{reg}) cells.[1]

T_{reg} cells are involved in self-tolerance, immune homeostasis, prevention of autoimmunity and suppression of immunity to pathogens.[2] The forkhead transcription factor FOXP3 is essential for T_{reg}-cell development and function, as mutations in *FOXP3* cause autoimmunity in mice and the IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome in humans.[2]

In tumor-bearing individuals, T_{reg} cells are increased in numbers both in the local tumor microenvironment and in the peripheral blood, and can contribute to the overall immunosuppression.[3] In several human tumors, T_{reg} cells even have prognostic significance and their abundance can be correlated with the stage of disease.[3] Depletion of T_{reg} cells has been suggested as a therapeutic option, with early clinical trials using an IL-2 immunotoxin showing promising results.[3]

One important and yet unresolved aspect in tumor immunology is how and where T_{reg} cells in tumor patients develop. Peripheral induction is one possibility, whereby tumor antigen-specific T cells might be converted into FOXP3-expressing T_{reg} cells with suppressive functions within the tumor microenvironment. An alternative scenario would be the accumulation of T_{reg} cells generated in the thymus, which would be attracted to the tumor site by specific factors such as chemokines.

Several studies have assessed the abundance and function of human T_{reg} cells after IL-2 administration, and the overall consensus was that IL-2 augments their frequency.[4-6] Possible explanations for such an increase were peripheral expansion of T_{reg} cells but also altered migratory activity.[7] However, none of these studies assessed how IL-2 influenced the thymic output of T_{reg} cells and whether this would interfere with efficient antitumor immune responses.

In a recent study,[8] we investigated how IL-2 treatment influences T_{reg}-cell numbers and function in colorectal cancer patients undergoing a combined immunochemotherapy. We observed increased levels of T_{reg} cells, as determined by a combined staining for CD4, FOXP3 and CD25 in the peripheral blood of these patients at the start of therapy, confirming previously published observations.[3] These cells expressed typical T_{reg}-cell markers including CTLA-4 and GITR and had normal immunosuppressive functions. Next, we assessed the influence of IL-2 on the number of total T_{reg} cells after completion of therapy, finding an expansion of the pool of T_{reg} cells in IL-2 treated patients. This is in line with previously published studies, which also reported elevated numbers of T_{reg} cells after treatment with IL-2.[4-6]

Similar to conventional T cells, T_{reg} cells can be distinguished into memory and naïve subsets, according to the surface expression of CD45RA. Valmori et al. were the first to report that a subset of naïve T_{reg} cells exists that is anergic following stimulation in the absence of IL-2, exerts ex vivo cell-cell contact-mediated suppressor functions yet proliferates in response to stimulation with autologous antigen-presenting cells.[9] These observations indicate that a high proportion of these cells have self-reactive T-cell receptors and hence that they are derived from the thymic T_{reg}-cell compartment.[9] The relationship between memory and naïve T_{reg} cells was further delineated in humans using genomic and functional approaches by Miyara et al. who clearly established that naïve T_{reg} cells are an important subpopulation of human FOXP3$^+$ T_{reg} cells.[10] Using a similar gating strategy, which included the assessment of CCR7 expression to distinguish between central- and effector-memory T_{reg} cells, we could detect

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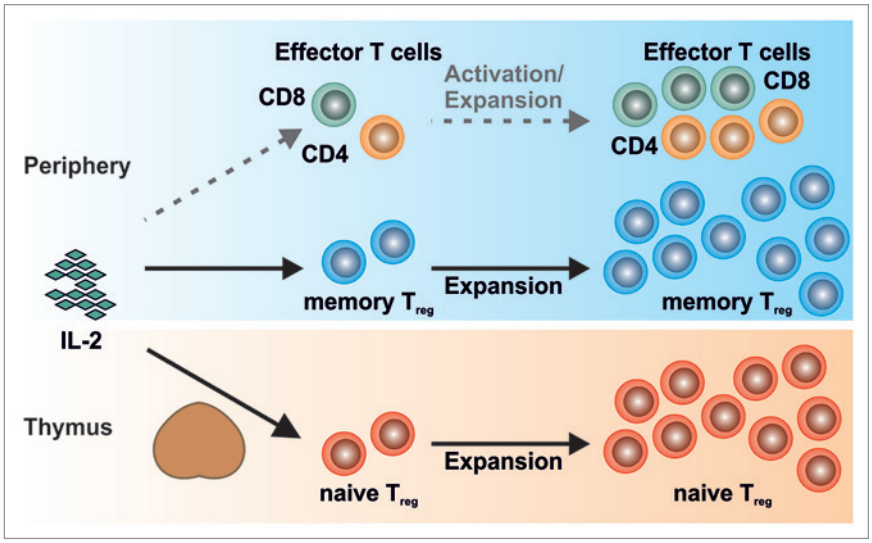

Figure 1. Interleukin 2 administration results in a preferential expansion of regulatory T (T_{reg}) cells. Administration of interleukin-2 to tumor patients only results in a weak activation and expansion of potentially tumor-counteracting effector T cells, while memory T_{reg} cells in the periphery as well as the naïve thymic T_{reg}-cell pool are consistently expanded.

an increase in naïve T_{reg} cells before the initiation of immunotherapy. This increase in naïve T_{reg} cells was even more pronounced after IL-2 administration and assessment of their suppressive function showed immunosuppressive activity comparable to that of memory T_{reg} cells. One approach to determine the vicinity of T cells to the thymus is to determine the number of T-cell receptor excision circles (TRECs). Assessing TRECs in sorted naïve T_{reg} cells from healthy donors and patients, before and after therapy, indicated that naïve T_{reg} cells are enriched in thymus-derived T_{reg} cells even before therapy, but particularly after the administration of IL-2, suggesting that IL-2 primarily acts on the thymus to produce additional T_{reg} cells that join those already present in the tumor microenvironment and the peripheral blood of these patients.

To substantiate this observation, we administered IL-2 in a murine model system and could show that the IL-2 treatment results in an expansion of naïve T_{reg} cells in all immunological cell compartments. This was mainly due to an increased thymic output, as assessed by analyzing TRECs in the sorted naïve T_{reg} cells from these animals.

Taken together, our data supports an overall increase in T_{reg} cells in tumor patients with an expansion of newly generated naïve T_{reg} cells post IL-2 therapy as a major mechanism of the T_{reg}-cell expansion in IL-2 treated tumor patients (**Fig. 1**). This finding has implications for the future direction on how to target T_{reg} cells in tumor patients. Depletion of T_{reg} cells, e.g., by the administration of T_{reg}-cell targeting antibodies or immunotoxins, will only result in a short-term depletion of peripheral T_{reg} cells. Long-term reduction of T_{reg} cells will warrant therapeutic strategies reducing the thymic output of T_{reg} cells, thus properly circumventing their immunosuppressive functions in tumor patients.

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