

INTERVIEW about

Immature myeloid dendritic cells, TGF-beta and tumor with

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How can regulatory T cells accumulate in lymphoid organs of tumor-bearing hosts?

Francois Ghiringhelli: Regulatory T cells constitute a small subpopulation (<10%) of CD4⁺ T lymphocytes with the capability to inhibit the effects of CD4⁺ and CD8⁺ effector lymphocytes and NK cells. They constitutively express CD25, the α receptor to IL-2, and a specific transcription factor, Foxp3. We previously demonstrated that these regulatory T cells

accumulated inside the tumor and the related lymphoid organ of tumor-bearing hosts, and that this accumulation was proportional to the tumor volume (1). In the present paper, we demonstrate that this accumulation is due to a selective proliferation of the regulatory CD4⁺ CD25⁺ T lymphocytes in the tumor and its lymphoid environment.

How important is TGF- for the tumor-associated regulatory T cell proliferation?

Francois Ghiringhelli: We provide compelling evidence indicating that regulatory T cell proliferation induced by tumors depends on at least three factors: 1: IL-2, likely produced by tumor-reactive effector T cells, and acting on the CD25 IL-2 receptor of regulatory T cells that do not produce IL-2 by themselves; 2: presentation of antigenic peptides (probably self antigens) by immature dendritic cells; 3: expression of TGF- β by the same dendritic cells. This was demonstrated by stimulation

experiments *in vitro*, but also *in vivo*, by using transgenic mice which express a dominant negative form of the TGF- β receptor II on their T cells. Both TGF- β receptors I and II are required for TGF- β -induced signal transduction. No tumor-induced Treg proliferation was observed in these mice. Interestingly, we had previously demonstrated that TGF- β was also directly required for the suppressive effect of regulatory T cells on its effector targets (1).

What is role of dendritic cells during this process?

Francois Ghiringhelli: Myeloid dendritic cells are the main antigen presenting cells for the T lymphocytes. Differentiated with monocytes and macrophages from myeloid precursors, they are first immature sentinel cells widespread in the tissues where they collect antigenic information. In the presence of a signal danger, like that delivered by infectious microorganisms, they mature and present the hazardous antigens to

the effector T lymphocytes. Tumor beds and the tumor-draining lymphoid organs are infiltrated by immature myeloid dendritic cells. These cells have not the capacity to present correctly tumor antigens to effector T lymphocytes, as they are deficient in costimulatory signals of the B7 family. On the contrary, we show here that tumor-infiltrating immature myeloid cells inhibit the tumor immune response by stimulating the

proliferation of regulatory T lymphocytes. They achieve this aim by presenting self and tumor antigens to the regulatory T cells

in association with TGF- β that they express and secrete in the tumor microenvironment.

Through which mechanism can tumor cells manipulate Treg and dendritic cells?

Francois Ghiringhelli: Regulatory T cell accumulation in tumor-bearing hosts is directly correlated with the presence and the abundance of the cancer cells. Interestingly, subtotal resection of a tumor is sufficient by itself to significantly reduce the accumulation of regulatory T cells in the lymphoid organs. This strongly suggests a direct effect of the tumor cells on the mechanisms leading to regulatory T cell proliferation. We found that culture supernatant of tumor cells, but not of noncancerous cells was able to modify maturation of dendritic cells, inducing them to produce TGF- β and enhance proliferation of regulatory T cells. We did not yet identify the soluble tumor cell

factor(s) which could be similar with previously reported inhibitory cytokines produced by tumor cells, such as vascular growth factor (VEGF), macrophage- or granulocyte-macrophage colony stimulating factors (M-CSF, GM-CSF), IL-6 or IL-10, which are known to maintain immaturity of tumor-infiltrating dendritic cells or promote their macrophage differentiation, for instance through activation of STAT-3 and inactivation of the NF- κ B signaling pathway (2). A proteomic approach will help us to characterise the soluble factors produced by the tumor cells that induce regulatory T cell proliferation *via* their effect on tumor-infiltrating T lymphocytes.

Is there any hope of a pharmacological intervention of this mechanism?

Francois Ghiringhelli: The accumulation of regulatory T cells in the tumor microenvironment prevents the natural immune response that could identify the tumor antigens and potentially eliminate the tumor cells. It is also a major obstacle for any attempt of tumor immunotherapy. Several reports showed that reduction of regulatory T cells through injection of an anti-CD25 antibody could prevent tumor growth in several models of experimental cancers, but did not succeed in eliminating established tumors. This failure could result from the effect of anti-CD25 antibody, that eliminates not only the regulatory T cells, but also the activated CD4⁺ CD25⁺ T lymphocytes that are required for an efficient anti-tumor immune response. We previously demonstrated that a single injection of cyclophosphamide in tumor-bearing animals had a selective depleting effect of regulatory T cells. This treatment

allowed to cure subcutaneous or intraperitoneal established (15-day-old) tumors, when associated with a tumor specific immunotherapy (tumor cells + BCG) which had no curative effect when given alone at this step (1). Even more advanced, larger subcutaneous tumors could be cured when this treatment sequence was preceded by an incomplete tumor resection. The continuous proliferation of regulatory T cells in tumor-bearing hosts could be a factor explaining their particular sensitivity to cyclophosphamide. Our present work opens new pathways that could be investigated for eliminating regulatory T cell proliferation in cancer, for instance biochemical modifications of TGF- β and STAT-3 pathways, or induction of differentiation and maturation in tumor-infiltrating dendritic cells.

REFERENCES

- Ghiringhelli et al. Eur J Immunol 34, 336, 2004
- Wang et al. Nat Med 10, 48, 2004