Research Highlights

Highlights from the latest articles in immunotherapy

Role of cytotoxic CD4⁺ T cells in cancer immunotherapy

Evaluation of: Quezada SA, Simpson TR, Peggs KS *et al.*: Tumor-reactive CD4⁺ T cells develop cytotoxic activity and eradicate large established melanoma after transfer into lymphopenic hosts. *J. Exp. Med.* 207, 637–650 (2010).

Adoptive transfer of tumor-reactive lymphocytes into lymphoablated patients is a promising therapy for the treatment of metastatic solid tumors, and it has shown objective clinical responses in a significant numbers of patients [1]. Clinical investigations of adoptive therapy have primarily focused on CD8⁺ T cells, given their capacity to directly engage and kill transformed cells [2]. However, a growing amount of evidence has accumulated emphasizing the use of CD4⁺ T cells for cancer immunotherapy [3]. CD4⁺ cytotoxic T lymphocytes (CTLs) have been found to exhibit anti-tumor activity in several malignancies.

The study by Hunder *et al.* was the first to report that the adoptive transfer on CD4⁺ CTLs expanded and differentiated *in vitro* from a single T-cell clone was able to generate an impressive persistence of adoptively transferred cells together with durability of clinical response in metastatic melanoma [4]. However, the generation of anti-tumor-specific CD4⁺ cells *ex vivo* for adoptive T-cell therapy required expansion and manipulations *in vitro* to achieve a large number of tumor-reactive T cells, which limited the development of clinic applications.

In a recent study, Quezada *et al.* demonstrated that the transfer of a small number of naive self-reactive CD4⁺ T cells into lymphopenic mice induced high levels of T-cell expansion and differentiation with the rejection of large

vascularized melanoma tumor, avoiding *in vitro* manipulations [5]. Naive CD4⁺ T cells specific for melanoma differentiation antigen (tyrosinase-related protein 1 [Trp1]) were isolated from Trp1-transgenic mice and transferred into irradiated recipients, which received anti-CTL antigen 4 (CTLA4) monoclonal antibodies. The authors highlighted that CD4⁺Trp1⁺-transferred cells developed cytotoxic activity, and the combination with the blockade of CTLA4 greatly enhanced therapeutic efficacy of CD4⁺Trp1⁺ adoptive transfer, resulting in increased expansion of effector T cells, a reduced number of regulatory T cells and superior anti-tumor immunity. These data underline the importance of cytotoxic CD4⁺ T cells in cancer immunotherapy and provide an advantageous alternative to current approaches using *in vitro* differentiation and expansion. Furthermore, the data promote the rational development of combinatorial strategies, which include CD4⁺ adoptive transfer and CTLA4 blockade, that elicit a potent anti-tumor response causing rejection of advanced-stage tumors.

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Margherita Gigante¹
& **Elena Ranieri^{†1}**

¹Department of Biomedical Sciences, Faculty of Medicine, University of Foggia, Ospedali Riuniti di Foggia, Viale Luigi Pinto, 1-71100 Foggia, Italy

[†]Author for correspondence:

Tel.: +39 088 158 7119

Fax: +39 088 158 7123

e.ranieri@unifg.it

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TGF- β promotes immune suppression by inhibiting Treg cell apoptosis

Evaluation of: Ouyang W, Beckett O, Ma Q, Li MO: Transforming growth factor- β signaling curbs thymic negative selection promoting regulatory T cell development. *Immunity* 32, 642–653 (2010).

Foxp3⁺CD25⁺CD4⁺ Tregs play an important role in the maintenance of immune tolerance and may be one of the obstacles preventing successful tumor immunotherapy [1]. Several clinical trials are currently being conducted to examine various reagents that may deplete levels of CD25⁺CD4⁺Tregs or attenuate their functions [2].

Naturally occurring Tregs (nTregs) are produced in the thymus as a functionally mature T-cell subpopulation, the development of which is controlled by the affinity of T-cell receptors for MHC-II self-peptide complexes and by costimulatory and cytokine signaling pathway [3]. In addition to the nTregs that are produced in the thymus, naive T cells can acquire Foxp3 expression and differentiate into induced Treg cells in peripheral tissues under certain conditions; for example, by antigenic stimulation in the presence of high-dose TGF- β [4]. TGF- β has pleiotropic functions in regulating T-cell responses and plays an important role in tumor initiation and progression, functioning as both a suppressor and a promoter [5]. TGF- β is also crucial for the development of Tregs but the mechanism by which controls nTreg cell differentiation and maintains peripheral T-cell tolerance has been not well demonstrated.

Recently, Ouyang *et al.* demonstrated that TGF- β signaling protected thymocytes from negative selection and inhibited nTreg apoptosis by using a T cell-specific TGF- β receptor II (TGF- β RII)-deficient mouse [6]. The inhibition of nTreg apoptosis was associated with imbalanced expression

of anti- and pro-apoptotic Bcl-2 family proteins: the proapoptotic proteins Bim, Bax and Bak were highly expressed whereas the antiapoptotic protein Bcl-2 was down-regulated in TGF- β RII-deficient nTregs. The ablation of Bim in mice restored Tregs and inhibited T-cell activation and differentiation, highlighting the fact that TGF- β signaling was essential for the survival of peripheral Tregs and for the inhibition of autoreactive T cells.

These observations uncover previously undefined functions for TGF- β signaling in the control of Treg-mediated immunosuppression *in vivo* and facilitate the harnessing of TGF- β to treat a variety of immune-related disorders.

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TLR7 and TLR8 as targets for tumor immunotherapy

Evaluation of: Cherfils-Vicini J, Platonova S, Gillard M *et al.*: Triggering of TLR7 and TLR8 expressed by human lung cancer cells induces cell survival and chemoresistance. *J. Clin. Invest.* 120, 1285–1297 (2010).

There is a growing interest in the targeting of Toll-like receptors (TLRs) for the prevention and treatment of cancer, rheumatoid arthritis, inflammatory bowel disease and systemic lupus erythematosus [1]. TLR signaling pathways could promote cancer initiation and progression through the activation of NF- κ B, which play a prominent role in inflammation-promoted cancer and tumor-cell survival [2]. The expression of TLRs in several cancer cell types has been reported; however, the exact roles they play in cancers are still unclear.

In this study, Cherfils-Vicini *et al.* investigated the expression and signaling of TLRs in human lung cancer, and they reported that TLR7 and TLR8 were highly expressed in human lung tumors *in situ* and in lung cancer cell lines [3]. The triggering of TLR7 and TLR8 with specific agonists resulted in the activation of NF- κ B and the modulation of a set of genes implicated in immune escape, such as *Bcl-2*, which conferred increased tumor-cell survival and resistance to apoptosis induced by chemotherapeutic agents currently used to treat patients. Interestingly,

the authors revealed that human lung cancer cells had a gene-expression profile similar to that of TLR7- or TLR8-stimulated cell lines, indicative of chronic tumor stimulation by TLR ligands.

These findings provide evidence that an improper activation of TLR pathways can lead to massive inflammation and directly favor tumor development. Thus, the characterization of TLR7 and TLR8 ligands present in the tumor microenvironment *in vivo* as well as TLR7 or TLR8 expression by lung tumor cells in patients should be taken in account in order to adapt the chemotherapeutic protocols. The regulation of these pathways is essential, and the targeting of proteins involved in the localization and movement of TLRs [4], such as specific TLR inhibitors, may provide a novel method for the treatment of inflammatory diseases.

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