Evaluation of Immuno-Oncology Related Treatment in Syngenic Mouse Models
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Introduction

Immuno-ontherapy based on mAbs targeting cancer cells is now developed as a valid approach to treat cancer. Suppressive mechanisms in immune responses normally play a critical role in maintaining immune homeostasis. However, these suppressive mechanisms are also considered as one of the main reasons for the failure of cancer immunotherapies because they induce peripheral tolerance of tumor-specific immune responses and allow tumor growth. COH, CD25+ Foxp3+ regulatory T cells have been revealed as the most important population of immune suppressors, and their depletion has been reported to enhance antitumor immune responses. CTLA-4 (CD152) was reported as a critical target for regulatory T cell function [1] and thus blockade of CTLA-4 mediated signals has been suggested as a possible strategy to treat cancers. The first anti-CTLA-4 human monoclonal antibody (mAb), ipilimumab, was approved in 2011 by the FDA for use in metastatic melanoma. Success for ipilimumab was reported in a large phase III clinical trial involving patients with metastatic melanoma, who had undergone previous failed treatment [2]. Moreover, phase I-III trials for PD-1 mediated signals was also reported as a critical inhibitory mechanism regulating antitumor immune responses [3]. BMK-93658, a fully human mAb that blocks the programmed death-1 (PD-1) protein showed responses lasting over 1 year in previously treated metastatic melanoma patients [4]. Combination therapy concurrently targeting PD-1 and CTLA-4 immune checkmarks leads to remarkable antitumor effects [4].

A comprehensive panel of tools was constructed and validated aimed at evaluating the modulation of the immune system by new therapies. In immunocompetent mice, immune cells were studied for the detection of their cell markers using FACS phenotyping. We report on a panel of syngenic tumor models (CT26, A20, A2B2, B16-F10, C130, C57, CT26, EMT6, Hepa-1, IL-12, IL-3, B48-2, IPR-1, PBLS, Renca and TC-1) our capacity to correlate subpopulation of immune infiltrating cells and the therapeutic effects of critical antibodies directed against Cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed death 1 membrane protein receptor (PD-1) and ligand (PD-L1).

In vivo experiments

Immunocompetent mice were obtained from Charles River (France). Animals were orthotopically or subcutaneously injected with syngenic cancer cell lines on D0. The animals received repeated injections of antibodies directed against CTLA-4, PD-1 and PDL-1. Isoflurane was used to anesthetize the animals before cells injection, IV treatments and termination. All logistical parameters of the study (biodos, cell harvesting, measurements, raw data, lethality, behavior and results of autopsy...) were managed using Viro-Manager software (Biostysmes, Dijon). During the course of the experiment, animals were terminated under anesthesia when they displayed significant signs of physiological changes or incorrect behavioral and experimental procedures were realized according to the French and European Regulations and NRC Guide for the Care and Use of Laboratory Animals. Animal facility is authorized by the French authorities (Agreement N° A21231011EA). All procedures using animals were submitted to the Animal Care and Use Committee of Oncodesign (Oncomet) agreed by French authorities (Chreaea agreement N° 811) [1, 2, 3].

Immune cell detection in mice tissues

The tumor and tumor draining lymph nodes were collected for subsequent FACS analysis to study the immune response in mice. Cells suspensions were prepared from tissues either by mechanistic dissociation or by enzymatic digestion. The antigens associated antibodies used for FACS analyses were CD45, CD3 and CD8 for T Cell lymphocytes, were CD45, CD106, Ly6G, Ly6C, F4/80 for tumor associated macrophages and were CD11b, CD4, F4/80 and CD3 for regulatory T Cell lymphocytes. The stained cells were analyzed with a CyFlow® space flow cytometer (LSR II, BD Biosciences) equipped with 3 excitation lasers at wavelengths 405, 488 and 633 nm.

Material and Methods

For mice treated with CTLA-4 mAb 3/10 partial responses and 7/10 complete responses were observed. For mice treated in combination 2/10 partial responses and 8/10 complete responses were observed. For mice treated with PD-1 mAb 1/10 partial responses and 9/10 complete responses were observed. For mice treated with PD-1 or CTLA-4 mAb no change was observed for tumor size.

Conclusions and perspectives

Novel therapeutic strategies are being developed that aim to implicate the immune system in the initiation, development and progression of tumors, by resetting or redirecting the immune effectors against tumors.

Pending new generation of humanized mouse models, the growing interest in immunology as a cancer therapy shows the limitation of xenograft models in immunodeficient animals. A more effective approach is the use of syngenic mouse models that we present here.

We report on a panel of syngenic mouse models our capacity to correlate subpopulation of immune infiltrating cells and the therapeutic effects of new antibodies generation directed against CTLA-4, PD-1 and PDL-1 antigens.

References

5- NRC Guide for the Care and Use of Laboratory Animals.