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REGULATION OF T LYMPHOCYTE FUNCTION IN TUMORS

The identification of tumor-specific antigens recognized by T lymphocytes on human cancer cells has elicited numerous vaccination trials of cancer patients with defined tumor antigens. These treatments have induced T cell responses but have shown a low clinical efficacy in tumor-bearing melanoma patients. We believe that progress depends on unraveling the different blockages for efficient tumor destruction. The analysis of the T cell responses of melanoma patients vaccinated against tumor antigens has led us to consider the possibility that the limiting factor for therapeutic success is not the intensity of the anti-vaccine response but the degree of anergy presented by intratumoral lymphocytes. We aim at a better understanding of dysfunctions of the immune system in tumors and more precisely T lymphocyte dysfunctions.

PREVIOUS WORK IN OUR GROUP: IDENTIFICATION OF TUMOR ANTIGENS RECOGNIZED BY T CELLS

In the 1970s it became clear that T lymphocytes, a subset of the white blood cells, were the major effectors of tumor rejection in mice. In the 1980s, human anti-tumor cytolytic T lymphocytes (CTL) were isolated in vitro from the blood lymphocytes of cancer patients, mainly those who had melanoma. Most of these CTL were specific, i.e. they did not kill non-tumor cells. This suggested that they target a marker, or antigen, which is expressed exclusively on tumor cells. We started to study the anti-tumor

CTL response of a metastatic melanoma patient and contributed to the definition of several distinct tumor antigens recognized by autologous CTL. In the early 1990s, we identified the gene coding for one of these antigens, and defined the antigenic peptide (1). This was the first description of a gene, MAGE-A1, coding for a human tumor antigen recognized by T lymphocytes.

Genes such as those of the MAGE family are expressed in many tumors and in male germline cells, but are silent in normal tissues. They are therefore referred to as “cancer-germline genes”. They encode tumor specific antigens, which have been used in therapeutic vaccination trials of cancer patients (2). A large set

of additional cancer-germline genes have now been identified by different approaches, including purely genetic approaches. As a result, a vast number of sequences are known that can code for tumor-specific shared antigens. The identification of a larger set of antigenic peptides, which are presented by HLA class I and class II molecules and recognized on tumors by T lymphocytes, could be important for therapeutic vaccination trials of cancer patients and serve as tools for a reliable monitoring of the immune response of vaccinated patients (3-4). To that purpose, we have used various approaches that we have loosely named “reverse immunology”, because they use gene sequences as starting point (5).

Human tumor antigens recognized by CD4⁺ or CD8⁺ T cells are being defined at a regular pace worldwide. Together with colleagues at the de Duve Institute, we read the new publications and incorporate the newly defined antigens in a database accessible at <<http://www.cancerimmunity.org/peptidedatabase/Tcellepitopes.htm>>.

A MECHANISM CAUSING ANERGY OF CD8 AND CD4 T LYMPHOCYTES

The identification of specific tumor antigens recognized by T lymphocytes on human cancer cells has elicited numerous clinical trials involving vaccination of tumor-bearing cancer patients with defined tumor antigens. These treatments have shown a low clinical efficacy. Among metastatic melanoma patients, about 5% show a complete or partial clinical response following vaccination, whereas an additional 10% show some evidence of tumor regression without clear clinical benefit. We believe that progress depends on unraveling the different blockages for efficient tumor destruction.

The tumors of the patients about to receive the vaccine, already contain T cells directed against tumor antigens. Presumably these T

cells are exhausted and this impaired function is maintained by immunosuppressive factors present in the tumor. The T cell response observed in some vaccinated patients reinforce an hypothesis proposed by Thierry Boon and Pierre Coulie: anti-vaccine CTL are not the effectors that kill the tumor cells but their arrival at the tumor site containing exhausted anti-tumor CTL, generates conditions allowing the reawakening of the exhausted CTL and/or activation of new anti-tumor CTL clones, some of them contributing directly to tumor destruction (2, 6). Accordingly, the difference between the responding and the non-responding vaccinated patients is not the intensity of their direct T cell response to the vaccine but the intensity of the immunosuppression inside the tumor. It is therefore important to know which immunosuppressive mechanisms operate in human tumors.

Human tumor-infiltrating T lymphocytes show impaired IFN- γ secretion

Both human CD8 and CD4 tumor-infiltrating T lymphocytes (TIL) were isolated from tumor ascites or solid tumors and compared with T lymphocytes from blood donors. TIL secrete low levels of IFN- γ and other cytokines upon non-specific stimulation with anti-CD3 and anti-CD28 antibodies (7-10). TCR were observed to be distant from the co-receptors on the cell surface of TIL, either CD8 or CD4, whereas TCR and the co-receptors colocalized on blood T lymphocytes (Figure 1).

Reversing the anergy of tumor-infiltrating T lymphocytes with galectin ligands

We have attributed the decreased IFN- γ secretion to a reduced mobility of T cell receptors upon trapping in a lattice of glycoproteins clustered by extracellular galectin-3. Indeed, we have shown that treatment of TIL with N-

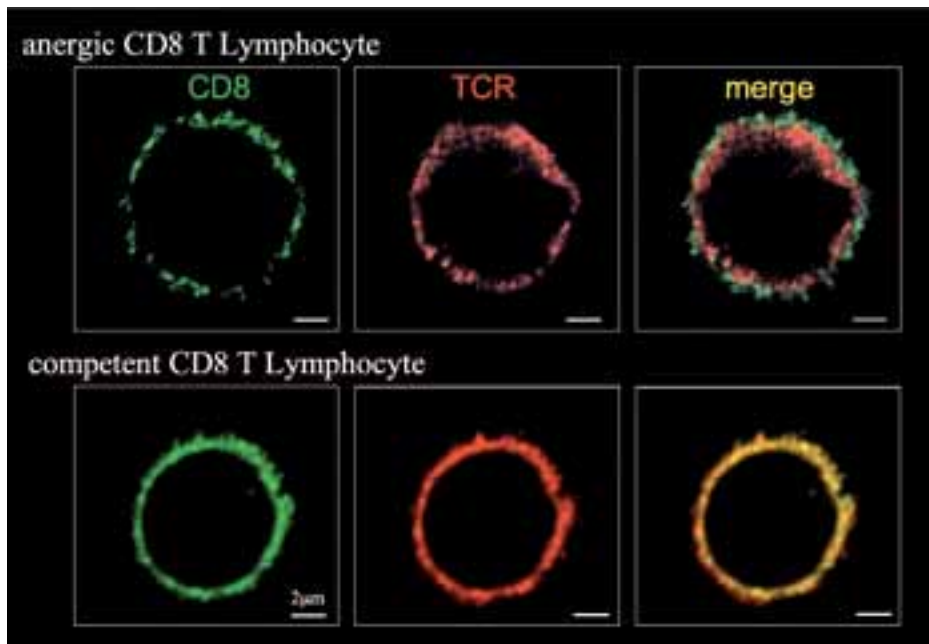


Figure 1. TCR and CD8 do not co-localize on CD8 T cells with impaired functions.

acetyllactosamine (LacNac), a galectin-competitor ligand, restored this secretion (Figure 2). Our working hypothesis is that TIL have been stimulated by antigen chronically, and that the resulting activation of T cells could modify the expression of enzymes of the N-glycosylation pathway, as shown for murine T cells. The chronically activated TIL, compared to resting T cells, could thus express surface

glycoproteins decorated with a set of glycans that are either more numerous or better ligands for galectin-3. Galectin-3 is an abundant lectin in many solid tumors and carcinomatous ascites, and can thus bind to surface glycoproteins of TIL and form lattices that would thereby reduce TCR mobility. This could explain the impaired function of TIL. The release of galectin-3 by soluble competitor ligands would

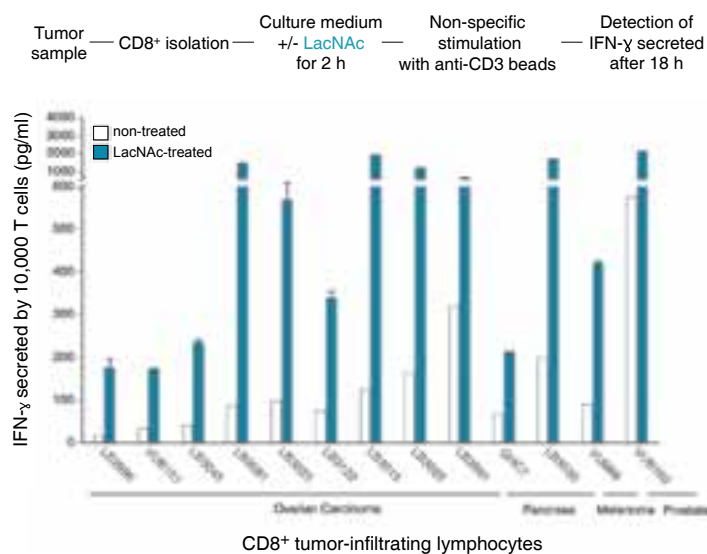


Figure 2. Treatment of tumor-infiltrating lymphocytes with a galectin ligand reverses anergy.

restore TCR mobility and boost IFN- γ secretion by TIL. We recently strengthened this hypothesis by showing that both CD4 and CD8 TIL that were treated with an anti-galectin-3 antibody, which could disorganize lattice formation, had an increased IFN- γ secretion compared to untreated cells.

Towards a clinical trial combining vaccination and galectin-binding polysaccharides

Galectin competitor ligands, *e.g.* disaccharides LacNAc, are rapidly eliminated in urine, preventing their use *in vivo*. We recently found that a plant-derived polysaccharide, currently in clinical development, detached galectin-3 from TIL and boosted their IFN- γ secretion. Importantly, we observed that not only CD8⁺ TIL but also CD4⁺ TIL that were treated with this polysaccharide secreted more IFN- γ upon *ex vivo* re-stimulation. In tumor-bearing mice vaccinated with a tumor antigen, injections of this polysaccharide led to tumor rejection in half of the mice, whereas all control mice died. In non-vaccinated mice, the polysaccharide had no effect by itself. These results suggest that a combination of galectin-3 ligands and therapeutic vaccination may induce more tumor regressions in cancer patients than vaccination alone. Translation of these results to the clinic was unfortunately impossible because the company producing this polysaccharide got bankrupted. We recently identified another plant-derived polysaccharide that binds to galectins and was already used in combination with chemotherapy in phase II clinical trials in colorectal cancer patients. This compound was as effective as LacNAc in boosting the secretion of IFN- γ by treated TIL. A clinical trial with this new compound, in combination with anti-tumoral vaccination, will hopefully be launched in 2011 in clinical centers close to the Brussels Branch. We are currently trying to understand the very early activation events that are defective in TIL.

IS THE SPONTANEOUS ANTI-TUMOR T CELL RESPONSE OF BREAST CARCINOMA PATIENTS A CLINICAL PROGNOSTIC FACTOR ?

Several retrospective studies suggest a correlation between the survival of patients with ovarian or colorectal carcinoma and infiltration of their tumors by immune cells. So far, prospective data validating these observations do not exist. In collaboration with Dr Javier Carrasco (Grand Hôpital de Charleroi) and Dr Jean-Pascal Machiels (Cliniques Universitaires St-Luc), we set out a prospective study aimed at looking for a correlation between the clinical outcome of patients with non-metastatic breast carcinoma and their spontaneous anti-tumor T cell response. Considering our experience in quantitative approaches to detect very weak T cell responses in the blood of melanoma patients, Danièle Godelaine set out the evaluation of the frequencies of anti-tumor CD8 T lymphocytes in the blood of breast cancer patients recruited in the two clinical centers. Frequencies are evaluated by mixed lymphocyte-peptide cultures, carried out with HLA-A2 and A3-restricted peptides derived from HER2/neu and hTERT, two proteins usually overexpressed in breast tumors, followed by detection of specific cells with HLA-peptide tetramers. Blood samples are collected before and after surgery. Tumors removed at surgery are analyzed by immunohistology for infiltration by immune cells, and fragments are frozen for further analysis. This prospective study foresees to include 172 patients whose clinical evolution will be followed up over a 5-year period. So far, 25 patients have been included. Eight of them have a frequency against the targeted antigens ranging from 10^{-5} and 10^{-6} among blood CD8 T cells, whereas the frequency in healthy donors blood was estimated to be lower than 5×10^{-7} . We hope to identify the patients with a better prognosis in order to offer them an adapted care avoiding unnecessary heavy treatments.

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