SUMMARY

Anticancer immunity is a complex network in which innate immunity play an critical role. The immune system can control the onset of tumors by recognizing the changed phenotype of the transformed cells as a non-self phenotype. The active control against cells not presenting self characters is defined by the paradigm of immune surveillance. In recent years, various studies have shown that the immune response against transformed cells start as a localized acute inflammatory response. The cooperation of natural-killer cells (NK) and phagocytes attracted by so-called “danger signals” (pro-inflammatory molecules and chemo-attractants delivered by stressed cells) can lead to total ablation of the harmful cell clone. If the intervention is not completely efficient, immunoediting of the tumor can follow with stimulation of chronic inflammatory responses, mainly mediated by macrophages. The systemic immunity attempt to terminate the uncontrolled inflammation stimulates the intervention of regulatory T lymphocytes and the shift from an antitumor cytotoxic Th1 response to a Th2 inhibitory response. This regulatory mechanism paradoxically assists the tumor development. All the described events needs the interplay and cooperation of both tumor cells and host cells and stromal elements, that altogether form the so-called “tumor microenvironment”. NK cells have various roles inside the tumor microenvironment, especially the possibility to act independently from MHC-restricted antigen presentation, necessary for the activation of the adaptive immunity cells (cytotoxic T lymphocytes CD8+ – CTLs, and T helper CD4+ lymphocytes).

The NK cell activation is bases on the missing-self recognition: cells that not present regular MHC molecule expression (recognized by NK inhibitory receptors – Ly49 in rodents, KIR in humans) permit predominance of activation receptor signaling after binding to proper ligands on the target cell. In consequence to this activation, NK cells deliver cytotoxic granules within the cytotoxic synapse (place of direct contact with the target cell) inducing death of the target cell. The receptors involved in cell activation can be included in families of lectin-like receptors (NKR-P1 in rodents, NKG2 and CD69 in rodents and humans) with capability to recognize carbohydrate molecules. This property raises in importance the products of tumor aberrant glycosylation as a target for immune recognition. In our studies we evaluated multivalent glycoconjugates as possible glycomimetics of putative ligands for NKR-P1A receptor expressed by NK and NK-like cells.

Glycodendrimers based on a polyamidoamine (PAMAM) core, tetra- and octa-branched, decorated with GlcNAc molecules were developed and tested as possible immunotherapeutics in experimental cancers in vivo (chemically induced colorectal adenocarcinoma in rats and B16F10 melanoma inoculated in C57BL/6 syngeneic mice). Reduction of tumor onset was observed in the colorectal cancer model. Melanoma-bearing treated mice showed reduction of cancer growth rate, increase in survival, increase of Th1 cytokines, and appearance of activated (CD69+) cells inside the tumors within 24-48 hrs from the treatment. To trace the glycodendrimer diffusion and targeting of immune cells, the tetravalent glycodendrons (GN4) were linked to a molecule of either fluorescein or rhodamine. We found that the labeled GN4 molecule was targeting NKR-P1 positive cells, with following internalization of dendrimers and receptor. In the
...spleen, fluorescence was localized in areas corresponding to CD69+ expressing cells.

The importance of environments on cancer immunity and progression was studied in my original model of colorectal carcinogenesis in the rat, both under conventional (with microbiota present in the bowel) and germ-free (animals completely sterile since the birth) condition. We considered the changes in immune cell subpopulations induced by cancer development, and the influence of the microbiota on the anticancer immune responses at the systemic level. The drop down of both cytotoxic cell number and function resulted as the key event for the cancer progression. The germ-free conditions resulted to permit a more active anticancer response leading to lower incidence of tumors, with increase in NK, NKT, CTL, B cells and cytotoxicity in the peripheral blood of cancer-resistant animals. This data suggest that the “physiological inflammation” sustained by the presence of intestinal microflora may negatively influence the anticancer immune response (increased tolerance?).

The results obtained from the glycodendrimer stimulation experiments, together with the evidence of an environmental impact on anticancer systemic immunity in CV vs. GF conditions, leaded to a new hypothesis of immunotherapy. To rescue the immune response from the inhibition produced by chronic inflammatory stimuli and Treg cell activity, a paradoxical treatment was performed in melanoma bearing mice (initial and developed tumors) either with antiinflammatory (nimesulide, a COX-2 inhibitor) or immune suppressive (azathioprine) drugs. They were used to temporarily block the deregulated immunity, followed by re-stimulation with administration of glycodendrimers. Results showed constant efficacy in reducing cancer growth by the immunosuppressive treatment, especially in association with glycodendrimers. Instead, the anti-inflammatory treatment showed efficacy only on early tumors and was worsened by the association with glycodendrimers. The functional rescue was evident evaluating the IFN-γ production ex-vivo in splenocytes derived from treated animals: after treatment with glycodendrimers alone and, especially, azathioprine + glycodendrimers important increase was seen when cells were stimulated with tumor homogenates obtained from each own animal. Untreated animals showed instead suppression as expected by the tumor microenviromental components.

The complex of all experiments demonstrated the importance of the local tumor environment as a target of anticancer interventions, its ability to condition the systemic immunity, and the possibility of its modulation by immunotherapies targeting lectin-like receptors of NK cells using glycomimetics of putative physiological ligands.