A new Tn-antigen mimetic conjugated to SPIONs is immunoactive

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Tn-antigen, is a tumour associated carbohydrate antigen (TACA) overexpressed in various carcinomas of epithelial origin (including breast, colon, lung, bladder, cervical, ovarian, stomach, and prostate cancer), whose level of expression often correlates with carcinoma differentiation and aggressiveness. Because of its relatively tumour-selective expression, cell surface localization, and role in tumour progression the Tn-antigen has been considered an attractive target for specific active immunotherapy in cancer.
However, the Tn-antigen, having a carbohydrate structure, is a T cell-independent antigen, able to elicit a rapid and long-lasting IgM production, but failing to induce IgM to IgG switch, affinity maturation of IgG, T cell activation, and memory generation. In addition, children under 2 years old and the elderly (above 65 years old) have weak immune responses to this antigen.
Recent evidences showed that carbohydrate antigens may acquire the ability to induce macrophage activation, IgG-class switch, and immune cell activation when presented as clusters, leading to examine the multivalent display of carbohydrates as a new strategy for improving the immunogenicity of T cell-independent antigens.
In this context, we have prepared through a new and highly stereoselective synthetic pathway a chemically stable mimetic of the Tn-antigen, which was then conjugated to superparamagnetic iron oxide nanoparticles (SPIONs) to confer multivalent properties. These compounds were biologically tested on RAW 264.7 macrophage cell lines, under strict endotoxin-free conditions (Limulus amoebocyte lysate assay), for their: 1) biocompatibility (MTT, Calcein-AM, and Trypan blue tests); 2) ability of induce macrophage uptake (Perl's iron stain and FACS); 3) ability to induce macrophage activation (RT-PCR and ELISA assay). No compounds resulted cytotoxic at all concentrations tested (10^{-2}-10^{2} mg/ml). The newly synthesized Tn-antigen mimetic was not endocyted by RAW 264.7 cells, while the SPIONs (3 mg/ml), either functionalized or un-functionalized with this compound, were equally endocyted in a concentration (10^{-2}-10^{2} mg/ml)- and time (1-72 h)-dependent manner. The un-conjugated Tn-antigen mimetic and the un-functionalized SPIONs did not induce TNF-α gene expression/release, while the Tn-antigen mimetic conjugated to SPIONs induced a significant (p£0.01) increase in TNF-α gene expression/release (+2,75-fold increase, and +18-fold increase at 30 mg/ml vs. negative controls (compound-untreated cells), respectively), when compared with the positive control LPS (+2,9-fold increase, and +20-fold increase at 0.1 mg/ml vs. negative controls, respectively).
In conclusion, our results show that: 1) all synthesized compounds are biocompatible; 2) the SPIONs are recognized and endocyted by macrophages; 3) the newly synthesized Tn-antigen mimetic is not recognized by macrophages; 4) the multivalent display of the Tn-antigen mimetic on SPIONs confers properties of immunoactivity.