Functional nanoparticles for dendritic cell-mediated cancer immunotherapy

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Autologous dendritic cell (DC)-based tumor vaccination still represents matter of study because of negligible clinical benefits, although encouraging in vitro and in vivo experimental results exist. Among unresolved problems there is the achievement of an adequate number of mature DCs able to support an effective immune response against tumor in oncological patients. Nanoparticles (NPs) have been proposed to use them as vectors of self-tumor antigens to reach a full and effective autologous DC stimulation. Given their tolerability, Silicon (SiO_2)- or gold(Au)- NPs represent nowadays a very interesting pharmacological tool. NPs can be absorbed by antigen-presenting cells (APCs) and modulate immune responses, with low immunogenicity in themselves and low acute toxicity. However, the interactions of several types of NPs with antigen presenting cells, such as DCs, are still poorly known. The aim of the present study was to address the effects of NPs of various composition and size on human DCs generated in vitro and to obtain an optimal phenotypical and functional DC activation using SiO_2- or Au-NPs functionalized with human tumor lysates. Au-NPs were synthesized from gold citrate as previously published [1]; SiO_2-NPs were synthesized through Stöber's process following Thomassen et al. method [2], rhodamine was added to the alkaline hydro-alcoholic solution to produce fluorescent NPs. Au- and SiO_2-NPs (average diameter 24.2 nm and 106.8 nm respectively) and rhodamine conjugated SiO_2-NPs (143.1 nm) were administered to DC cultures at concentrations 10 and 100 μg/ml. The same types of NPs, coated with 10 or 100 μg/ml of human ovarian carcinoma cell's line lysate, were used at a concentration of 37 μg/ml to have the same exposed area to lysate binding.
Human immature dendritic cells (iDCs) were isolated as reported [3]. After 5 days of culture, NPs were added for 48h, with or without maturation-inducing cytokines, before harvesting the cells for analysis by phase contrast, fluorescence and electron microscopy. Phenotypical and functional maturation of DCs were respectively evaluated by flow cytometry and CFSE assay evaluation of allogeneic lymphocyte proliferation. The results show that NPs enter DCs by endocytosis, concentrate in the paranuclear area and do not exert harmful effects on cells. Using rhodamine-conjugated silica NPs it was possible to show progressive increase in cell fluorescence between 30 min and 24 h from administration of particles to cultures. After 24 h fluorescence was intense and widespread to the whole cytoplasm, independently of the addition of maturation-inducing cytokines, less intense in cells exposed to protein-coated nanoparticles. Flow cytometry analysis demonstrated that neither type of NPs influenced DC maturation either upon or without stimulation by maturation-inducing cytokines. When NPs were functionalized with 10 μg/ml of tumor lysate there were an increasing expression of DC maturation markers respect DC exposed only to the same concentration of tumor lysate. If preliminary data of phenotypical maturation should be confirmed by CFSE assay we should hypothesized that adsorbed tumor lysate specific antigens could activate an effective DC-based immune specific T-cell response potentiated by NPs as vectors. These data could propose functionalized NPs displaying tumor associated self-antigens as a potential vaccine for cancer immunotherapy.

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