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Metal based nanoparticles as cancer antigen delivery vehicles for macrophage based antitumor vaccine

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ABSTRACT

In the present study, we would like to evaluate the efficacy of modified metal oxide nanoparticles (NPs) as cancer antigen delivery vehicles for macrophage (MΦs) based antitumor vaccine. The cobalt oxide nanoparticles (CoO NPs) were promising tools for delivery of antigens to antigen presenting cells and have induced an antitumor immune response. Synthesized CoO NPs were modified by N-phosphonomethyliminodiacetic acid (PMIDA), facilitated the conjugation of lysate antigen, i.e. cancer antigen derived from lysis of cancer cells. The cancer cell lysate antigen conjugated PMIDA-CoO NPs (Ag-PMIDA-CoO NPs) successfully activated macrophage (MΦ) evident by the increasing the serum IFN-γ and TNF-α level. Immunization of mice with the Ag-PMIDA-CoO NPs constructed an efficient immunological adjuvant induced anticancer IgG responses, and increased the antibody dependent cellular cytotoxicity (ADCC) response than only lysate antigen treated group to combat the cancer cell. The nanocomplexes enhanced the anticancer $CD4^+$T cell response in mice. The result showed that Ag-PMIDA-CoO NPs can stimulate the immune responses over only lysate antigens, which are the most important findings in this study. These NP-mediated Ag deliveries may significantly improve the anticancer immune response by activating MΦs and may act as adjuvant and will balance the pro-inflammatory and anti-inflammatory immunoresponse. The crosstalk between the activated MΦ with other immune competent cells will be monitored by measuring the cytokines which illustrate the total immunological network setups.

1. Introduction

Metal oxide nanoparticles (NPs) have attracted considerable interest in biomedical applications [1], including their use as nanovaccine scaffolds. Because of their tendency to internalize into a wide variety of cell types [2,3], they appear to be particularly suited to deliver antigens to APCs. Immunotherapeutic approaches are the alternative strategies to treat cancer because the conventional chemotherapy treatments have several lethal effects on different body parts. For this purpose, antigen encapsulated nanoparticles are used efficiently in cancer immunotherapy. Encapsulation of antigen within NPs has the potential to prevent Ag degradation by proteolytic enzymes [4], increased efficiency of Ag loading [5,6] and prolong release, all of which might lead to enhanced presentation of MHC-peptide complexes [7,8]. Encapsulation is also associated with improved shelf life of antigen (Ag) and reduced need for adjuvants [8–10]. Recent developmental work largely focused on the use of transition metal nanoparticles like Co, Ni. Cobalt NPs has ability to enter into the cell very rapidly [11] which draws the interest of the researchers toward cobalt NPs based biomedical application system [12]. Cobalt has a physiological role as a co-factor of vitamin B_{12}. CoO NPs are currently attracting enormous interest due to their higher magnetic properties and greater effects on proton relaxation [13].

Several studies have demonstrated anti tumor responses after vaccination with NPs encapsulated antigen-pulsed antigen presenting cells (APCs) in a variety of mouse and human model of cancer [5,9,14,15]. Previously, our in vitro study showed that

Abbreviations: ADCC, antibody dependent cellular cytotoxicity; APC, antigen presenting cells; CoO, cobalt oxide; CTLs, cytotoxic T lymphocytes; CV, crystal violate; DL, Dalton's lymphoma; FT-IR spectra, Fourier transforms infra-red spectra; IFN-γ, interferron gamma; IgG, immunoglobulin G; MeOH, methanol; MLR, mixed lymphatic reaction; NPs, nanoparticles; PMIDA, N-phosphonomethyliminodiacetic acid; PMIDA-CoONPs, N-phosphonomethyliminodiacetic acid conjugated cobalt oxide nanoparticles; TNF-α, tumor necrotic factor alpha.

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PMIDA conjugated cobalt oxide nanoparticles were successfully bound with whole cancer lysate antigen and stimulate anticancer immune response by activating TNF-α [16]. We use the whole tumor lysate as antigen in cancer immunotherapy because the identification of the effective antigen(s) is not required and treatment strategies are feasible even for such malignancies in which only few more or less specific tumor-associated antigens have been characterized [9,16] and secondly, the probable presence of multiple antigens reduces the risk of a tumor cell escape. In this study, we would like to evaluate the *in vivo* anticancer immune response by delivering whole lysate antigen of Daltons lymphoma conjugated with PMIDA-CoO NPs. We have seen that the lysate antigen conjugated PMIDA-CoO NPs are successfully activated the MΦ and induce an anticancer immunoresponse.

2. Experimental

2.1. Chemicals and reagents

Phosphonomethyliminodiacetic acid (PMIDA), MTT, Histopaque 1077, Ethidium bromide, pentoxifylline (POF), N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide (EDC) were procured from Sigma (St. Louis, MO, USA). Anti $CD4^+$ Ab, anti human IgG, IgG1, IgG2a was purchased from eBiosciences. RPMI 1640, fetal bovine serum (FBS), penicillin, streptomycin, sodium chloride (NaCl), sodium carbonate (Na_2CO_3), sucrose, Hanks balanced salt solution, and ethylene di-amine tetra acetate (EDTA), dimethyl sulfoxide (DMSO) were purchased from Himedia, India. Tris–Hcl, Tris buffer, KH_2PO_4, K_2HPO_4, HCl, formaldehyde, alcohol and other chemicals were procured from Merck Ltd., Mumbai, India. All other chemicals of the highest purity grade were purchased from Merck Ltd., SRL Pvt., Ltd., Mumbai.

2.2. Preparation and characterization of the antigen conjugated nanoparticles (NPs)

The synthesized PMIDA coated cobalt oxide nanoparticles (PMIDA-CoO NPs) (1 mg) were dispersed in 1 ml of 0.1 M PBS buffer with antigen and equal volume of EDC and NHS (Supporting document). And then the conjugation was characterized by using SDS-PAGE, DLS, HR-TEM. The protein (antigen) conjugated PMIDA-CoO NPs were isolated by dialysis followed by magnetic separation. The paramagnetic characteristics of CoO NPs were easily isolated by magnetic separation.

2.3. Animal immunization

Six weeks old Swiss mice (6 mice in each group) were immunized (subcutaneous injection) with 100 μl of Ag-PMIDA-CoO NPs dose containing 100, 200, 500, and 1000 μg/Kg body weight (B.W.) in PBS. All the methods were described in supporting doc.

2.4. Immunological changes

The immunological changes such as (i) cytokines, (ii) $CD4^+$ T cell proliferation, (iii) delayed-type hypersensitivity (DTH), (iv) splenocyte proliferation, (v) immunoglobulin (Ig) estimation, (vi) antibody-dependent cellular cytotoxicity (ADCC) assay, (vii) allogeneic T cell proliferation assay, (viii) cytotoxic T lymphocytes (CTL) assay were used to assess the activity of Ag-PMIDA-CoO NPs immunization. All the methods were described in supporting doc.

2.5. Pulsed macrophage based tumor therapy experiment

Mice peritoneal macrophages were isolated and pulsed with 25 μg/ml of Ag-PMIDA-CoO NPs for 24 h. This dose and time period was established in our previous experiment. After incubation, the pulsed macrophages were isolated by three times PBS (1×) washing. Four groups of Swiss mice ($n = 4$ in each group) were immunized with unpulsed macrophage (MΦ), and Ag-PMIDA-CoO pulsed MΦ (2×10^5 cells in each case) weekly for three times in total. Three days following completion of the immunization, mice were inoculated with DLA tumor cells obtained from 90 to 95% confluent cultures (1×10^7) intraperitonially [28]. Tumor size was measured, starting on day 9 and after every 3 days until day 20. The survival time in terms of percentage of increased life span was measured by the following formula

$$\text{Increase in life span} = (T - C) \times 100$$

where the T = number of days the treated animals survived and C = number of days the control animals survived. The data were compared with only cell lysate pulse macrophages.

2.6. In vivo *macrophage based tumor therapy experiment in presences of inhibitors*

Mice peritoneal MΦ stimulation, immunization and pulsed MΦ mediated anticancer immunotherapy in the presences of ROS, p38 MAPK and TNF-α inhibitors ware described in Supporting document in details.

2.7. Protein estimation

Protein content was determined using bovine serum albumin as a standard according to the method of Lowry et al. [17]. Q4

2.8. Statistical analysis

The data were expressed as mean ± SEM, $n = 6$. Comparisons between the means of control and treated group were made by two-way ANOVA test (using a statistical package, Origin 6.1, Northampton, MA 01060 USA) with multiple comparison *t*-tests, $p < 0.05$ as a limit of significance.

3. Results and discussion

The immune system controls the body's homeostatic state against different infections. Cancer is one of the most deadly diseases in the world. The different vaccine provides our body to prevent the infection. The vaccine mediated immune response is enhanced by the application of an adjuvants which may biological or synthetic and may organic or inorganic in nature. The present study was provided novel adjuvants for use in altering, modulating or augmenting immune responses in human or animal subjects against cancer. The inorganic adjuvants most widely used in human vaccines are aluminum containing alum [49], a mineral salt, usually made up as Al (OH) $_3$ or Al (PO) $_4$. But alum has the potential to cause severe local and systemic side-effects, including sterile abscesses, eosinophilia, myofascitis, etc. [50,51]. The present situation hunt for to obviate one or more of the problems associated with the prior art and to provide additional adjuvants suitable for use with vaccines against diseases in which cell mediated immune responses are important for prevention. Antigen presentation by successful vaccination must address some fundamental issues such as efficient delivery of antigen to dendritic cells and subsequent dendritic cell activation to trigger adaptive immunity measured by the number, functionality, and avidity of antigen-specific T cells induced.

Nanoparticles are used as vehicles for the delivery of recombinant proteins, antigens, to generate *in vivo* immune responses. A number of studies have shown that different nanoparticles can be

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