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Polymeric microparticles for sustained and local delivery of antiCD40 and antiCTLA-4 in immunotherapy of cancer

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ABSTRACT

This study investigated the feasibility of the use of polymeric microparticles for sustained and local delivery of immunomodulatory antibodies in immunotherapy of cancer. Local delivery of potent immunomodulatory antibodies avoids unwanted systemic side effects while retaining their anti-tumor effects. Microparticles based on poly(lactic-co-hydroxymethyl-glycolic acid) (pLHMGA) and loaded with two distinct types of immunomodulatory antibodies (CTLA-4 antibody blocking inhibitory receptors on T cells or CD40 agonistic antibody stimulating dendritic cells) were prepared by double emulsion solvent evaporation technique. The obtained particles had a diameter of 12–15 μm to avoid engulfment by phagocytes and were slightly porous as shown by SEM analysis. The loading efficiency of the antibodies in the microparticles was >85%. The *in vitro* release profile of antiCD40 and antiCTLA-4 from microparticles showed a burst release of about 20% followed by a sustained release of the content up to 80% of the loading in around 30 days. The therapeutic efficacy of the microparticulate formulations was studied in colon carcinoma tumor model (MC-38). Mice bearing subcutaneous MC-38 tumors were treated with the same dose of immunomodulatory antibodies formulated either in incomplete Freund's adjuvant (IFA) or in microparticles. The antibody-loaded microparticles showed comparable therapeutic efficacy to the IFA formulation with no local adverse effects. The biodegradable microparticles were fully resorbed *in vivo* and no remnants of inflammatory depots as observed with IFA were present in the cured mice. Moreover the microparticles exhibited lower antibody serum levels in comparison with IFA formulations which lowers the probability of systemic adverse effects. In conclusion, pLHMGA microparticles are excellent delivery systems in providing long-lasting and non-toxic antibody therapy for immunotherapy of cancer.

1. Introduction

Immunotherapy has been established as a groundbreaking approach to treat cancer [1]. As opposed to conventional cancer treatment strategies which employ methods to eliminate all rapidly proliferating tumor cells, immunotherapy aims to use the immune system to attack the target of interest with high specificity and low toxicity [2–4]. Immunotherapy of cancer embraces several strategies, including application of immunomodulatory antibodies as monotherapy in the treatment of malignancies [5,6]. These antibodies do not directly target cancer cells but instead aim to induce and enhance immune responses against the tumor, particularly by CD8+ T cells which are crucial for tumor eradication [7]. The mode of action of such indirectly acting or immunomodulatory antibodies can be inhibitory or stimulatory, depending on the role of their target in the anti-tumor immune response. Check-point blocking antibodies such as antagonistic antiCTLA-4 have been developed to block inhibitory receptors expressed on T cells [8–10]. Other antibodies such as agonistic antiCD40 function at an earlier phase of the immune response by activating antigen presenting cells (APCs) including dendritic cells (DCs) which are responsible for the activation of tumor-specific CD8+ T cells by cross-presentation of tumor antigens [11].

The cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) is one

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of the key inhibitory receptors expressed by activated CD4+ and CD8+T cells as well as by memory and regulatory T cells [12], and is responsible for "pushing the brake" of the immune system [13,14]. CTLA-4 has a high affinity for CD80 and CD86 on APCs and competes with CD28, a major co-stimulatory signal required for T cell activation, for binding to these ligands. Binding of CTLA-4 to its natural ligands, CD80 and CD86 on APCs, results in decreased cytokine production and T cell proliferation [15]. The inhibitory role of CTLA-4 is crucial to maintain the balance of the immune system and to prevent autoimmunity, whereas cancer immunotherapy aims to reverse the CD8+ T cell inactivation [16]. To overcome the effect of inhibitory immune regulators, CTLA-4 blocking antibodies have been developed as potential anticancer agents [9,17–20].

CD40 is a receptor on APCs as well as on several other cells and binds to its ligand (CD154-also called CD40L) on activated CD4+ Th cells [21]. The CD40–CD40L interaction is essential for maturation of DCs (up-regulation of co-stimulatory molecules, increased secretion of cytokines) and consequently for CD8+ T cell priming and induction of CD8+ T cell response [22]. Earlier studies have shown that the CD40L signal from CD4+ Th cells can also be provided by agonistic antiCD40, encouraging their use for the induction of a robust T cell response [23]. Despite the promising results obtained with clinical trials using immunomodulatory antibodies in advanced stage cancer patients [18,24], after systemic administration, immune related adverse effects such as autoimmune and inflammatory reactions and cytokine release syndrome have been observed [20,25–30]. To minimize these adverse effects, Fransen et al. used Montanide ISA 51 to prepare a sustained-release water-in-oil emulsion for local delivery of an agonistic CD40 antibody in a preclinical mouse model. Unlike systemic antibody administration, this allowed local treatment with a lower dose of antibody, abrogating systemic toxicity while remaining effective in activating T cells [31,32]. In a study the effect of different administration methods on anti-tumor efficacy and toxicity of antiCD40 was evaluated in adenovirus protein E1A-expressing tumor-bearing mice. It was shown that the antitumor efficacy of 30 μg antiCD40 administered locally either in saline or Montanide was comparable to 3 consecutive intravenous injections of 100 μg antiCD40 (survival 70–80%) while single intravenous injection of 30 μg antiCD40 showed minimal tumor growth reduction (survival 30%). In addition, local treatment with low dose of antiCD40 resulted in lower toxicity than high dose intravenous treatment and sustained release formulation of antiCD40 in Montanide caused the lowest adverse effects, which was characterized by organ histology and liver enzymes in the blood [32]. Montanide ISA 51 is a commercially available mixture of light mineral oils (similar to incomplete Freund's adjuvant (IFA)) with mannide monooleate (as surfactant) and has been used extensively in clinical trials [30,33]. Nevertheless, administration of Montanide ISA 51–based emulsion and similar formulations has been associated with several side effects such as inflammation and swelling, painful granulomas at the injection site, fever, cysts and sterile abscesses [34]. In order to provide a safe formulation for local delivery of immunomodulatory antibodies, microparticulate formulations loaded with CTLA-4 blocking antibody and CD40 agonistic antibody were developed in this study using the biodegradable polymer (poly(d,l lactic-co-hydroxymethylglycolic acid) (pLHMGA)). Although similar in backbone to pLGA, pLHMGA possesses pendant hydroxyl groups which increase the hydrophilicity of the polymer. This results in less acidification inside the particles upon degradation and protects the protein/peptide from chemical modification [35,36]. As a result pLHMGA and similar hydrophilic polymers have shown better protein/peptide compatibility and complete release of encapsulated proteins/peptides as compared to pLGA [36–38]. Moreover, these polymers have been successfully used locally as antigen or drug delivery systems *in vivo* without showing toxicity [39,40]. In the present study, first, pLHMGA microparticles were optimized using – for economic reasons – polyclonal human IgG, to obtain a formulation with the desired particle size and antibody release profile. Because these particles were intended for local and sustained release of the antibody and not to be taken up by e.g. macrophages, the desired particle size should be larger than 10 μm [41]. Next, based on experience with the IgG formulations, antiCD40 and antiCTLA-4 loaded microparticles were prepared and characterized. The anti-tumor efficacy of the obtained microparticles was compared with that of IFA formulations in tumor-bearing mice. Antibody serum levels were monitored during treatment for potential systemic toxicity and the site of injection was studied for local reactions.

2. Materials and methods

2.1. Materials

Poly(lactic-co-hydroxymethyl glycolic acid) with a copolymer ratio of 50/50 was synthesized and characterized as described previously [40,42] (Supplementary data, Fig. S1 and Table S1). IRDye680RD N-hydroxysuccinimide ester (NHS ester) was obtained from LI-COR Biosciences, USA. Polyclonal human IgG (50 mg/mL in glucose 5%) was a gift from Sanquin, the Netherlands. Polyvinyl alcohol (PVA; Mw 30,000–70,000; 88% hydrolyzed) and dimethyl sulfoxide (DMSO) were obtained from Sigma–Aldrich, USA. Sodium dihydrogen phosphate (NaH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were obtained from Merck, Germany. Dichloromethane (DCM) was purchased from Biosolve, the Netherlands. Sodium azide (NaN_3, 99%), sodium hydroxide (NaOH), dipotassium hydrogen phosphate (K_2HPO_4) and sodium dodecyl sulfate 20% (SDS) were purchased from Fluka, the Netherlands. Bicinchoninic acid assay (MicroBCA) reagents were obtained from Thermo Fisher Scientific, USA. Phosphate buffered saline (1.8 mM NaH_2PO_4, 8.7 mM Na_2HPO_4, 163.9 mM Na^+, 140.3 mM Cl^-, pH 7.4) (PBS) was obtained from B Braun, Germany. Pyrogen-free water was obtained from Carl Roth, Germany. Polyclonal anti-rat antibody (BD biosciences, USA) was used for analysis of antiCD40 by ELISA and antiCTLA-4 was analyzed by biotin-labeled mouse anti-hamster antibody (clone 192-1) (BD biosciences, USA). Chemicals were used as received without further purification, unless otherwise stated.

2.2. Labeling IgG with NIR fluorescent dyes

Given the limited availability of immunomodulatory antibodies, human IgG was used as a model antibody to optimize the pLHMGA microparticle formulations. In order to accurately characterize the release kinetics of the formulations, IgG was labeled with IRDye680RD (IR680) by coupling the NHS ester of the dye to the protein. In a typical procedure, the medium in which the IgG was provided (50 mg/mL in glucose 5%) was exchanged to PBS (B Braun, Germany, pH 7.4) using a Zeba™ spin desalting column (7 kDa, Thermo Fisher Scientific, USA). Next, the pH of the antibody solution was adjusted to 8.5 by adding 0.1 mL of K_2HPO_4 1 M pH 9.0 to 1 mL of IgG in PBS. The IRDye680RD NHS ester was dissolved in DMSO (4 mg/mL) and 0.67 mL of this solution (2.7 mg of the dye) was added to the IgG solution yielding 2:1 molar ratio of dye/IgG. The reaction was carried out at room temperature for two hours. The unreacted dye was subsequently removed using Zeba™ spin desalting columns (7 kDa) equilibrated with HEPES buffer 50 mM pH 7.0 in two consecutive steps and IR680-IgG was collected in HEPES buffer and kept at 4 °C. IR680-IgG was characterized by gel permeation chromatography (GPC) as described previously [40].