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Synthetic nanovaccines for immunotherapy

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ABSTRACT

Although vaccination is historically one of the most successful strategies for the prevention of infectious diseases, development of vaccines for cancer and many chronic infections, such as HIV, malaria, and tuberculosis, has remained a challenge. Strong and long-lasting antigen-specific T cell responses are critical for therapy of these diseases. A major challenge in achieving a robust CD8 + T cell response is the requirement of spatio-temporal orchestration of antigen cross-presentation in antigen-presenting cells with innate stimulation. Here, we discuss the development of nanoparticle vaccine (nanovaccine) that modulates the innate immune system and enhances adaptive immunity with reduced toxicity. We address how nanovaccines can integrate multiple functions, such as lymph node targeting, antigen presentation, and stimulation of innate immunity, to achieve a robust T cell response for immunotherapy.

1. Introduction

Vaccines represent one of the greatest medical achievements of modern civilization, and have had a major impact on public health. The first generation of vaccines contains inactivated or attenuated microbes, such as viruses or bacteria. These prophylactic vaccines can induce life-long antibody responses to prevent disease from future exposure. Although these prophylactic vaccines have successfully eliminated or greatly reduced the burden of former epidemics, such as smallpox, poliomyelitis, tetanus, diphtheria and rubella, they do not work well in some patients and have the risk of reversion to virulence [1]. Furthermore, the future impact of vaccination should not only defend against infectious diseases, but also induce immune responses to treat ongoing diseases, such as cancer or chronic infections like HIV, malaria, and tuberculosis. Therapeutic vaccines must overcome pathogen-mediated evasion of the immune response and are likely to require induction of specific cytotoxic T-lymphocyte (CTL; activated CD8 + T cell) responses against pathogens that have already established [2,3]. In the case of cancer, chimeric antigen receptor (CAR) T cell therapy has shown the effectiveness of T cells in killing tumor cells [4], and recently several checkpoint inhibitors (anti-CTLA-4, anti-PD-1 and anti-PD-L1) have been approved, which target the suppression of T cells and enhance anti-tumor T cell response in cancer patients [5]. However, most tumors exhibit low immunogenicity, and a majority of patients fail to generate adequate cancer-specific CTLs and therefore cannot benefit from immune checkpoint therapies, which only remove the inhibition of T cell functions. Therefore, there is an unmet need to develop safe strategies that boost anti-tumor immunity to synergize with immune checkpoint therapy.

Spatio-temporal orchestration (STO) is essential to produce an antigen-specific CTL response (Fig. 1) [6]. (1) Efficient antigen (Ag) delivery to lymphoid organs (e.g., peripheral lymph nodes), cytosolic delivery and cross presentation by the major histocompatibility complex (MHC) molecule in the dendritic cells (DCs) are important. (2) Induction of co-stimulatory molecules (e.g., CD80/CD86) on DCs is critical for T cell activation. Lack of co-stimulation can lead to immune resistance or T cell apoptosis. (3) Cytokine release also plays a critical role in the differentiation of T cells. For example, type-I interferons stimulate the differentiation of naïve CD4 + T cells into Th1 subtype, whereas IL-4 leads to Th2 subtype. For cancer immunotherapy, Th1 and CD8 + CTL responses are desirable [6,7].

Nanoparticle vaccines (nanovaccines) are miniscule particulates (20–100 nm) that target the body's immune system to activate the host's immune response against diseases. Nanovaccines have unique characteristics that can improve vaccine efficiency and modulate the immune response in vivo [8,9]. Using different materials and manufacturing conditions, researchers can precisely control the size, shape, surface charge, hydrophobicity and loading density of antigens and adjuvants. The incorporation of antigens or adjuvants can be achieved by conjugation of these components to the surface or core of nanoparticles, or by encapsulation within vesicles or micelles. In this review, we will discuss how the unique features of nanoparticles affect their

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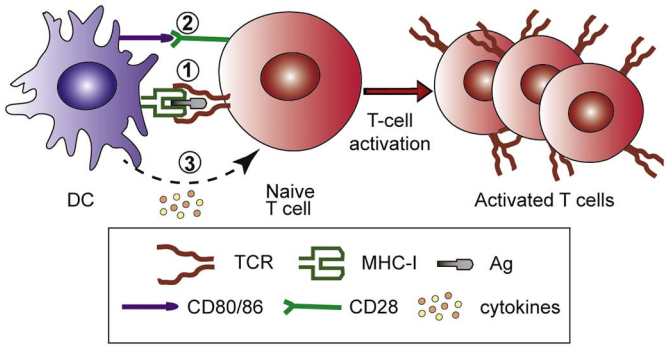

Fig. 1. T cell activation by antigen presenting cells (e.g., dendritic cells). Orchestration of (1) antigen presentation by MHC molecule to the T-cell receptor, (2) CD80/86 co-stimulation, and (3) cytokine signals is necessary to achieve antigen-specific T cell activation.

antigen-presenting cell (APC) targeting, antigen presentation, and how the nanoparticle incorporates different vaccine components to achieve enhanced T cell response. We acknowledge that more parameters, such as shape, rigidity, biodegradability and so on, are all important characteristics that affect the efficacy of nanovaccines. The effect of these parameters has been extensively described elsewhere, and will not be the focus of this review [10–14].

2. Antigen delivery and presentation

2.1. Major materials and effect

A vaccine that contains only some components of a pathogen is called a subunit vaccine. Subunit vaccines can eliminate the risk of reversion and reduce the possibility of autoimmune and allergic responses compared to inactivated or attenuated pathogen vaccines. However, despite advantages in safety, subunit vaccines have shown weakness in immune stimulation. Nanoparticles are an excellent platform for subunit vaccines, as they can extend the antigen release and circulation time, as well as target antigens to APCs, enhancing the efficacy of these vaccines. Various materials have been used to create synthetic nanoparticles for use in immunotherapy. This section will outline the major classes of materials and the advantages and weaknesses of each.

Many polymer-based nanoparticles have been investigated for their potential efficacy in immunotherapy, and polylactide-*co*-glycolide (PLGA) copolymer has been the most widely studied. PLGA is biodegradable, as its ester linkages are cleaved in vivo to produce two monomers, lactic and glycolic acid, which can be easily metabolized. By adjusting the ratio and positioning of the two monomers or conjugating to other molecules, properties such as size, solubility, and stability can be varied. PLGA is considered safe by the Food and Drug Administration (FDA) for clinical use, indicating its lack of toxicity. PLGA may be coupled to other polymers like polyethylene glycol (PEG) or polyethyleneimine (PEI) to form a block copolymer, which can self-assemble into a polymeric micelle that can encapsulate hydrophobic payloads in aqueous solutions, such as antigens [15] and extend blood circulation time [16]. Antigen-loaded polymer-based nanoparticles of various compositions have shown efficacy in increasing T cell responses, compared to the antigen alone [17].

Liposomes are another common platform for nanoparticulate vaccines. They are comprised of a phospholipid bilayer, which is easily biodegradable. Like PLGA, many liposome-based delivery methods have been approved by the FDA. Liposomes can be easily modified by altering the specific phospholipids used, or by coating the surface with other molecules like PEG [18,19]. While liposomes are able to encapsulate many types of compounds due to their amphiphilic nature, liposomes can suffer from poor loading efficiency and shelf stability [15,20]. Compared to antigens alone, antigens both conjugated to [21] and encapsulated [22] in liposomes have shown increased proliferation of antigen-specific CTLs.

Inorganic materials, such as carbon nanotubes and colloidal gold, have also been investigated for their potential in nanovaccine design. Both carbon nanotubes and gold nanoparticles conjugated to tumor-derived antigens have been shown in murine models to suppress tumor growth in an antigen-specific manner, compared to a vaccine comprised of the free antigen [23,24]. Both materials are easily functionalizable and are readily ingested by immune cells. However, concerns exist, particularly over solubility, long-term toxicity, and nonbiodegradability [24–26]. More work need to be performed to further evaluate inorganic nanoparticles for vaccine use.

2.2. Particle characteristics

Peripheral lymph nodes are a hub for the adaptive immune system as a primary site for antigen-presenting cells, which are key for the generation of antigen-specific T cells [27]. Targeted delivery of antigens to lymph nodes has been shown to increase the adaptive immune response, and nanoparticles provide a novel method of delivering antigens to lymph nodes [28].

In designing nanovaccines to migrate preferentially to lymph nodes, many factors must be considered. One is surface charge of the nanoparticles. It is generally accepted that cationic nanoparticles exhibit more toxicity in phagocytic cells, particularly due to the formation of reactive oxygen species and damage to cellular membranes [27,29,30]. Interstitial fluid contains negatively charged proteins, so charge repulsion causes anionic nanoparticles to drain more quickly to lymph nodes [31]. Phagocytic cells, like antigen-presenting cells, ingest anionic nanoparticles more readily than cationic nanoparticles [32–35]. Therefore, a negative surface charge appears to be preferable.

Hydrophobic nanoparticles have been shown to induce higher levels of antibody titers than hydrophilic nanoparticles [36]. Seong and Matzinger hypothesized that hydrophobic moieties can serve as danger signals to activate the immune system [37]. However, blood and other bodily fluids are hydrophilic, so hydrophobic nanoparticles may not be soluble and can lead to formation of aggregates at the injection site. Indeed, Rao et al. showed a negative correlation between hydrophobicity and nanoparticle uptake and retention by lymph nodes [31]. Thus, amphiphilic nanoparticles, including the use of hydrophilic PEG as a “cloak” for hydrophobic nanoparticles, have become a focus of research.

There is an optimal size range for nanoparticles to migrate to the lymph nodes (Fig. 2) [38]. Nanoparticles smaller than 3–5 nm are cleared by the blood and bypass lymph nodes. Larger nanoparticles are drained by the lymphatic system and traffic to lymph nodes via two distinct, size-dependent mechanisms. The first involves antigen-presenting cells at the nanovaccine injection site, which may take up nanoparticles via phagocytosis and then migrate to the lymph nodes. In the second pathway, nanoparticles transport through lymphatic vessels directly to lymph nodes. Manolova et al. demonstrated the effect of particle size on the delivery method for nanoparticles to lymph nodes. Nanoparticles larger than 200 nm largely followed the first pathway and were delivered to lymph nodes after 18 h. Nanoparticles smaller than this radius drained to lymph nodes in the second, dendritic cell-independent mechanism within 2–3 h [39]. As the second pathway is much faster, nanovaccine development has focused on nanoparticles smaller than 200 nm.

The Hubbell and Swartz groups showed that PEGylated poly(propylene sulfide) nanoparticles smaller than 50 nm had significantly higher uptake and retention by lymph nodes, for up to five days, compared to nanoparticles of 100 nm in diameter. These smaller nanoparticles trafficked to antigen-presenting cells in the lymph nodes with ten times the efficiency of the 100 nm nanoparticles, and were able to induce dendritic cell maturation [40,41].