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Carbon nanotubes as vaccine scaffolds ☆

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ABSTRACT

Carbon nanotubes display characteristics that are potentially useful in their development as scaffolds for vaccine compositions. These features include stability in vivo, lack of intrinsic immunogenicity, low toxicity, and the ability to be appended with multiple copies of antigens. In addition, the particulate nature of carbon nanotubes and their unusual properties of rapid entry into antigen-presenting cells, such as dendritic cells, make them especially useful as carriers of antigens. Early attempts demonstrating carbon nanotube-based vaccines can be used in both infectious disease settings and cancer are promising.

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1. Introduction

Vaccines are drugs designed to elicit a specific, active immune response in a host that will prevent a disease from starting, or lessen the effects of a disease after it has begun. In the field of infectious diseases, nearly all vaccines are administered prophylactically. Such approaches have provided some of the most important advances in human health over the last century. Many attempts also have been made to generate "therapeutic" cancer vaccines for patients who already have cancer, which could be used to reduce tumor burden, or prevent or slow recurrence of cancers. No such effective specific cancer vaccines are marketed in the USA today.

The immune system is designed to recognize and react with foreign antigens and vaccines are drugs that are engineered to mimic these foreign molecules, so as to direct the specific immune response for therapeutic purposes. Antigens presented by microbes and bacteria are typically more potently immunogenic than self-antigens derived from cancers. In cancer vaccines, the antigens are not foreign, and are usually over-expressed proteins or sugars on the surface or inside the tumor cell. Intracellular proteins from microbes, viruses or cancers can be

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presented on the cell surface in the context of MHC molecules for T cell recognition [1,2]. Presentation by dendritic cells (DCs), which are the most effective antigen presenting cells (APCs) [3,4], drives the initiation of a strong response. Self-antigens are generally weakly immunogenic and the immune system of animals or patients with cancer either does not recognize the antigen, is tolerant to it, or cannot mount an adequate cytocidal response [5,6]. Soluble molecules are usually weak immunogens; therefore, delivery formulations that include carrier molecules and adjuvants can make more effective immunization strategies. Adjuvants promote a more potent immune response, such as cytokines [7], saponins, CpG motifs [8], and heat shock proteins [9]. Synthetic and natural materials, such as dendrimers or keyhole limpet hemocyanin (KLH), have been used as carrier molecules [10,11].

Vaccine carriers that efficiently deliver the antigens into professional antigen presenting cells such as dendritic cells would be most useful, as presentation of peptide antigens by the MHC molecules of dendritic cells (APCs) is essential to mounting a potent immune response. Particulate vaccines are a promising approach to improve the immunogenicity of proteins, as the particles improve immune responses. Interestingly, the response is highest when the particles are on the nano-scale, which may relate to the ability of cells to best interact with the vaccine particle of this size [12–14]. Nanoparticulate formulations also can serve as an extracellular or intracellular depot of antigen, which prolongs immune activation.

Carbon nanotubes (CNTs) offer a number of features that make them interesting candidate materials for vaccine compositions, as scaffolds to carry the specific antigenic target and to facilitate its presentation to the immune system. CNT are relatively inert and non-immunogenic and non-toxic [15–18] by themselves. They are highly stable on the shelf and in vivo. Their unique structures allow highly efficient conjugation of many antigens, simultaneously, and to multiple different antigens at once, to their surfaces. Interestingly, 100% of the structure of single wall CNT is surface atoms. In addition, CNT can be made particulate and insoluble, thereby prolonging effects in vivo as a depot, and promoting engulfment by phagocytic cells involved in the generation of the immune response. Finally, CNTs have the interesting property of rapid entry into cells, including dendritic cells, which are essential to the stimulation of effective immune responses [19–21].

In this review we discuss various approaches to vaccine development, the current methods to isolate and purify CNT for this purpose, methods to covalently functionalize them with biologically active molecules such as protein antigens, and the early attempt to make vaccines with CNT. The biomedical applications of CNT, including their use as carries for antigens, as immune stimulants, or as inflammatory molecules, have been reviewed several times recently or in this issue [15,18,22–24]. We encourage readers to seek these papers for a broader look at the biomedical applications of CNT.

2. Vaccines and adjuvants

Development and application of vaccines against pathogens have achieved a significant success in controlling and preventing life-threatening infectious diseases in the past century [25,26]. Similarly, the development of cancer vaccines has been of intense research interest in the past few decades and has provided approaches to adjuvant therapy in conjunction with other anti-cancer therapies [27]. Vaccines normally consist of three components. The first component is represented by one or more specific antigens that can be encoded by DNAs, or are peptides and proteins, or carbohydrates, derived from immune-dominant epitopes identified in pathogens or cancer cells. Upon vaccination, these specific antigens are able to generate specific and long-lasting immune responses against the host cells, whereby destroying either pathogens or cancer cells. The second component of vaccines, which is not always necessary, is a carrier. This is a scaffold, which may be immunogenic on its own and that is used to deliver the antigen to appropriate cells in vivo or retain it at a site. The third important component of vaccines is the adjuvant, which is required for effective vaccine delivery and for inducing robust inflammatory responses.

Effective adjuvants act through multiple mechanisms, including the generation of antigen depots, activation of antigen-presenting cells (APCs) via pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) and enhancing the presentation of vaccine antigens by APCs [28,29]. In addition to adjuvants with biological functions, the carrier delivery systems included in the vaccine formulation can also help to achieve desired targeting ability, depot and inflammation [30]. Adjuvants can be generally divided into two categories: immune-stimulatory molecules and antigen delivery vehicles. However, they can exhibit both characteristics simultaneously. Vaccines and adjuvants used in earlier studies are either attenuated pathogens (for example: Coley's toxins) or adjuvants that are the mixtures of bacterial walls and mineral oil, such as complete or incomplete Freund's adjuvants. Although it had been a wide-spread practice for immunologists to use adjuvants together with specific vaccines to generate effective immune responses, the mechanisms of their action were poorly understood. The discovery of the TLRs in the 1990s significantly increased the understating of how adjuvants stimulate innate immunity and bridge it to adaptive immunity. Recent advances in nanotechnology have also made it possible for specific, effective and controlled delivery of the vaccines to immune system [30].

2.1. Immuno-stimulatory adjuvants

2.1.1. TLR agonists

The immune system recognizes pathogen-associated molecular patterns (PAMPs) expressed by wide variety of infectious microorganisms, by PRRs, which include TLRs, NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs). The use of TLR agonists as adjuvants is based on the knowledge that several TLR ligands are known as PAMPs, and engagement of receptor-ligands activate DCs, macrophages and other innate immune systems that express TLRs on their cell surface or in their intracellular endosomes. Activation of DCs and macrophages results in enhanced phagocytosis of antigens, cytokine production, and up-regulation of co-stimulatory molecules. Subsequently, the host mounts an adaptive immune response, characterized by the expansion of antigen-specific T and B cells, activation of T helper and cytotoxic T cells and production of antibodies, that insure long-lasting immunological memory to protect against infection and cancers. Twelve TLRs and their ligands have been identified in humans [31]. For example: TLR-3 binds to double strand RNA (dsRNA) in virus, which has been known to induce type I interferons, enhances antigen presentation and the cytotoxicity of NK and T cells. The synthetic dsRNA polyinosinic–polycytidylic acid (Poly I:C) has been tested in pre-clinical studies and in numbers of human clinical trials as an adjuvant to anti-cancer vaccines [32,33]. TLR4 binds to bacteria lipopolysaccharide (LPS), which is a potent non-specific immunostimulator and is presented in various adjuvants. TLR5 binds to flagellin [34] and TLR9 to unmethylated CpG oligodeoxynucleotide bacterial DNA. TLR-9 agonist CpG-OD represents the most studied and advanced adjuvant candidate [35]. When used as an adjuvant, CpG motifs stimulate cells that express TLR-9, primarily plasmacytoid DC and B cells, to produce Th1 cytokines, enhance antigen presentation and induction of long-lasting CD8 T cells and Ab production. CpG-ODN has been evaluated as adjuvant in a wide variety of preclinical models and in human clinical trials in both infectious diseases and cancer vaccine therapies [35–40].

2.1.2. Cytokines

Cytokines produced by immune cells are important immune-modulators in both innate and adaptive immune responses. Therefore, a number of cytokines have been used as adjuvants to enhance vaccine efficacy [41]. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is the most widely used adjuvant in human trials, primarily because it can activate macrophages and induce DC differentiation,