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Approaches to enhancing immune responses stimulated by CpG oligodeoxynucleotides ☆

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ABSTRACT

CpG oligodeoxynucleotides (ODN) activate the immune system and are promising immunotherapeutic agents against infectious diseases, allergy/asthma and cancer. It has become apparent that while CpG ODN are potent immune activators in mice, their immune stimulatory effects are often less dramatic in humans and large animals. This disparity between rodents and mammals has been attributed to the differences in TLR9 expression in different species. This along with the sometimes transient activity of ODN may limit its potential immunotherapeutic applications. Several approaches to enhance the activity of CpG ODN have been explored including formulation of ODN in depot-forming adjuvants, and more recently, coadministration with polyphosphazenes, inhibitors of cytokines that downregulate TLR9 activation, and simultaneous activation with multiple TLR agonists. We will discuss these approaches and the mechanisms involved, with emphasis on what we have learned from large animal models.

Contents

1. Introduction

CpG ODN have been shown to be a very strong adjuvant in mice, and promote Th1-type immune responses, often performing better than complete Freund's adjuvant (CFA) which is the gold-standard for inducing cell-mediated immune responses in rodents. CpG ODN have the advantage in that they do not appear to cause severe local inflammatory reactions associated with CFA. CpG can have even greater

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adjuvant activity in mice if formulated or coadministered with other adjuvants [1,2]. This is consistent with an emerging paradigm in vaccinology that multiple adjuvants in combination may be more potent in enhancing immune responses to vaccines than individual adjuvants. This is a deviation from the past where investigators have conventionally tested vaccine preparations containing a single adjuvant. Indeed, evidence is accumulating that multiple adjuvants used in combination have tremendous potential in enhancing the efficacy of experimental vaccines that far surpasses what can be achieved with individual adjuvants [3–5].

Many conventional adjuvants induce good Th2-type immune responses but are not effective at promoting Th1 type immune responses. This is a major limitation in vaccines against pathogens for which Th1 responses are required for protection. Since CpG is predominantly a Th1 adjuvant, it has been of interest to determine whether CpG can modulate immune responses induced by such vaccines. In this regard, addition of CpG ODN to vaccine preparations containing some conventional adjuvants did not compromise the efficacy of these vaccines. Rather, CpG ODN complimented these conventional adjuvants resulting in not only improved magnitude, but also the quality of immune responses [1,3,5]. These include a wide range of substances with known adjuvant activity such as particulates, mineral salts, saponins, liposomes, cationic peptides, polysaccharides and bacterial toxins [2]. The mechanisms which mediate these synergistic responses between CpG and other adjuvants are not fully understood, but several factors may contribute to this synergy including; protection of CpG from enzymatic degradation, a depot effect whereby the CpG is slowly released, and possibly by increasing uptake of ODN by antigen presenting cells (APC) such as dendritic cells (DC).

Enhancing the activity of CpG ODN is even more important in humans and large animals where CpG by itself is not as potent as in rodents. This has created a need to explore ways to potentiate the activity of CpG ODN. The search for ways to enhance the activity of CpG ODN has extended beyond substances conventionally known for their adjuvant activity and now includes novel substances such as synthetic biodegradable polymers, inhibitors of immunoregulatory cytokines and stimulation with multiple TLR agonists.

2. Approaches to enhance immune activity of CpG ODN

2.1. Co-formulation of CpG ODN in depot-forming substances

2.1.1. Adaptive immunity

Early studies by Davis and colleagues [1] revealed that addition of CpG to alum containing Hepatitis B vaccine resulted in synergistic antibody response in mice. We compared a number of depot-forming adjuvants, including non-oil, metabolizable oil and mineral oil based ones, for their ability to enhance adjuvant activity of CpG ODN when formulated with a truncated secreted form of glycoprotein D (tgD) of bovine herpesvirus-1 (BHV-1). The antibody responses induced in mice by tgD and the adjuvant combinations with CpG ODN were significantly higher than those elicited by tgD formulated with CpG ODN alone. An important observation was that while CpG ODN elicited a Th1-type response and all other tested adjuvants induced a Th2-biased immune response, the combinations of CpG ODN and several of the co-adjuvants, including Quil A, Vemulsigen and Emulsigen (EMULSIGEN™, MVP Laboratories, Inc., Ralston, NE, USA) resulted in a relatively balanced Th1/Th2 immune responses [3]. A similar enhancement of immune responses by combining CpG ODN with Emulsigen was observed in rabbits [6] and sheep [3], confirming that the enhancement was not limited to mice.

Even though there is no linear correlation between the animal size and CpG ODN dose, it is obvious that higher doses are needed for CpG ODNs to be effective in larger species. For example, in cattle, the amount of CpG ODN required to induce a similar immune response to one of our established mineral oil-based adjuvants, VSA3, was between 10 and 50 mg [7]. Although the cost of CpG ODN may not be a major factor for human vaccines, such high doses of CpG ODN would be uneconomical in animal vaccine. This makes it even more important to formulate CpG ODN with a co-adjuvant as this may reduce the dose of CpG required to induce immune responses. We observed that formulation of tgD with CpG ODN and an oil-in-water adjuvant, Emulsigen, significantly enhanced serum neutralizing antibody responses and protection from BHV-1 challenge in calves when compared to formulation with CpG ODN only; indeed, even if the CpG ODN were formulated with Emulsigen at a low dose of 250 μg, the immune responses and protection were still stronger than with 25 mg CpG ODN alone [8]. This demonstrates that in addition to improvements in the immune responses and protection induced, formulation of CpG ODN with a co-adjuvant leads to a reduction in the dose and hence the cost of CpG ODN. Furthermore, formulation of tgD with both Emulsigen and CpG ODN leads to "antigen sparing", as a reduced tgD dose elicited equivalent immune responses when compared to the antigen with Emulsigen alone (unpublished observations). Antigen sparing can be an important factor in vaccines such as influenza where large doses may be required within a short period of time. In this regard, Cooper et al. [9] demonstrated in human subjects that addition of CpG 7909 to a flu vaccine may allow the use of reduced doses of the flu vaccine with no detrimental effect on the immunogenicity.

We have also used CpG ODN as an adjuvant to develop vaccination strategies for respiratory syncytial virus (RSV), which causes upper and lower respiratory tract infections in humans of all age groups, but primarily targets infants and young children. In fact, RSV is the most common viral pathogen causing lower respiratory infections in infancy and childhood. Although RSV infection usually leads to T cell responses and viral clearance, under certain conditions, RSV can create a Th2 cytokine microenvironment that supports an immunopathologic IgE response. Consequently, an effective RSV vaccine should induce balanced immune responses, as well as local immunity at the mucosal surfaces of the respiratory tract. This is a challenge in newborns, who have difficulty developing a cell-mediated immune response. Additionally, maternal antibodies may interfere with vaccination in this age group.

Since bovine RSV (BRSV), one of the major respiratory pathogens in calves, causes very similar disease symptoms as human RSV, we chose to use BRSV to test vaccine formulations. This allowed us to first use the mouse model and then transfer promising formulations to calves. Previously, a formalin (FI)-inactivated RSV vaccine was tested in young children and did not induce protection, but instead resulted in enhanced pathology in children subsequently infected with RSV [10–13]. This was attributed to a Th2-biased immune response, and influx of granulocytes into the lung, resulting in bronchiolitis and pneumonia. Thus, it became very clear that a Th1-biased or balanced immune response is critical for induction of protective immunity to RSV. We have demonstrated that, while FI-BRSV with Emulsigen elicited a Th2-biased immune response in mice, FI-BRSV formulated with CpG ODN, or both Emulsigen and CpG ODN, induced a Th1-type or balanced immune response, characterized by enhanced IgG2a and IFN-γ production. Furthermore, mice immunized with FI-BRSV formulated with Emulsigen produced eotaxin and IL-5, as well as eosinophils in the lungs, which is indicative of an immunopathologic response, while the animals immunized with FI-BRSV and CpG ODN or Emulsigen and CpG ODN did not [14]. The amount of virus produced in the lungs upon challenge with BRSV was most dramatically reduced in the mice that received the CpG ODN formulated vaccines. Importantly, the ability of CpG ODN to balance the immune response to FI-BRSV formulated with Emulsigen, and enhance protection from viral challenge, was confirmed in newborn calves [15], strongly suggesting that the beneficial effect of CpG formulation is not restricted to mice.

2.1.2. Innate immunity

We explored whether Emulsigen would have any impact on the innate immune responses. Like in humans and mice, CpG is a potent inducer of 2′5′A synthetase in sheep and this is a reliable biomarker for