Advanced Drug Delivery Reviews 99 (2016) 85–96

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Designing liposomal adjuvants for the next generation of vaccines☆

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ARTICLE INFO

Article history:
Received 12 May 2015
Received in revised form 9 October 2015
Accepted 5 November 2015
Available online 11 November 2015

Keywords:
Liposomes
Vaccines
Adjuvants
Antigens
Delivery systems
Microfluidics
QbD
MVA

ABSTRACT

Liposomes not only offer the ability to enhance drug delivery, but can effectively act as vaccine delivery systems and adjuvants. Their flexibility in size, charge, bilayer rigidity and composition allow for targeted antigen delivery via a range of administration routes. In the development of liposomal adjuvants, the type of immune response promoted has been linked to their physico-chemical characteristics, with the size and charge of the liposomal particles impacting on liposome biodistribution, exposure in the lymph nodes and recruitment of the innate immune system. The addition of immunostimulatory agents can further potentiate their immunogenic properties. Here, we outline the attributes that should be considered in the design and manufacture of liposomal adjuvants for the delivery of sub-unit and nucleic acid based vaccines.

Contents

1. Developing new vaccines

Vaccines remain the most cost-effective way to prevent infectious diseases. Due to the development of effective vaccines we have seen the global eradication of smallpox (declared in 1980) and more recently Rinderpest (also known as cattle plague, an infectious viral disease of cattle, declared in 2011). Vaccination has also promoted the dramatic reduction in the instances of polio, diphtheria, tetanus, pertussis, measles, mumps and rubella. Despite this success story, infectious diseases cause approximately 25% of world mortality [1]. In the development of new vaccines, we have a range of vector options available. Live attenuated, can offer lifelong immunity, and strong humoral and cell mediated protection. However, these vaccines are not appropriate for immunocompromised people and there is a risk that live attenuated vaccines can revert to their virulent form. In contrast, inactivated vaccines offer improved safety profiles but cannot provide effective long-term protection from pathogens [2] due to the destruction of the

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Non-Antigenic Regulators-Maiseyeu".

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http://dx.doi.org/10.1016/j.addr.2015.11.005
0169-409X/

pathogen replication and transformation mechanisms [3,4], often resulting in the need for high and multiple dose treatments. Similarly, sub-unit vaccines have a good safety profile but lower potency. To address this issue, adjuvants can be employed to enhance and/or prolong immune responses. Whilst their mechanism of action is yet to be fully elucidated, vaccine adjuvants can function through a range of mechanisms including formation of a depot, enhancing antigen delivery, uptake and presentation to appropriate antigen presenting cells, and induction of stimulatory cytokines and chemokines. There are a range of adjuvant systems already in use including aluminium based adjuvants which have been used in vaccines since the 1930s. More recently new adjuvants such Novartis's MF59 (an oil-in-water emulsion consisting of squalene, Tween 80 and Span 85), GSK's ASO3 (a squalene, Tween 90 and α-Tocopherol oil-in-water emulsion) and ASO4 (aluminium hydroxide and monophosphoryl lipid A), and the virosome system of Berna Biotech have been used in licenced vaccines [5]. However despite these advances, to tackle newly emerging diseases and re-emerging diseases, there is a continued need for new adjuvants.

1.1. Liposomes as vaccine adjuvants

Particulate drug delivery systems offer the potential to act as adjuvants. They offer the ability to incorporate sub-unit antigens within pathogen-sized particles that protect antigens from degradation and facilitate delivery to antigen presenting cells. Of the particulate drug delivery systems available, liposomes were the first system described to offer adjuvant action with their immunological role and adjuvant properties being identified by Allison and Gregoriadis [6]. In these studies, it was noted that negatively charged liposomes incorporating dicetyl phosphate were able to potentiate immune responses against diphtheria toxoid. Since this seminal work by Allison and Gregoriadis into the use of liposomes as adjuvants, all manner of vesicle size, charge and bilayer design have been investigated for their efficacy. Yet, liposomes are not the only bilayer vesicles offering adjuvant properties. Whilst phosphatidylcholines are generally the most common lipids employed, a wide range of lipids have been investigated to prepare vesicles such as niosomes (e.g. [7]) , virosomes (e.g. [8]) and bilosomes (e.g. [9]). These variations on a theme can offer different attributes. For example, incorporation of bile salts into the bilayer of vesicles (to form bilosomes) can improve oral delivery of vaccines by preventing natural stomach digestive enzymes from disrupting the vesicles. Alternatively, virosomes incorporating virus derived proteins promote cell fusion and delivery of viral antigens and have been successfully licenced as adjuvants in vaccines against hepatitis A and influenza [10]. However despite this, the development and application of liposomes as adjuvants are currently limited to two vaccine systems based on virosomes — Inflexal (against influenza) and Epaxal (against hepatitis A).

2. Liposomal adjuvants — how can we use our knowledge of their mechanisms of action to drive their development?

By limiting microbial growth, the innate immune system is a powerful system that is essential in the early stages of defence against immune challenge. However, it also drives the development of adaptive immune responses that are essential to enabling the body to clear any given pathogen. The innate immune system comprises many factors, both cellular (e.g. dendritic cells, macrophages, mast cells and neutrophils) and soluble (i.e. humoral) factors that can coordinate cellular responses. It is the integration with the adaptive immune system that underlies the functional significance of the innate immune system.

When developing novel vaccines, the ability to stimulate the innate immune responses needs to be considered. For live vaccines, this happens naturally with growth of any live attenuated organisms. However where no live, active infection occurs, the immune system requires additional stimulation in the form of adjuvants. Liposomal adjuvants have been known to function by offering both protection and enhanced delivery of the vaccine antigen and depending on their design they can promote antigen presentation and/or facilitate the formation of a depot resulting in attraction of antigen presenting cells that engulf antigen and become activated (Fig. 1).

2.1. Physico-chemical attributes that can impact of liposomal adjuvant action

To improve antigen delivery to antigen presenting cells there are a wide variety of lipids available ranging from natural or synthetic, cationic or anionic, unsaturated or saturated, long or short chain, single or double chain; and these can all be used in a range of combinations. The choice of lipid used in the formulation and the manufacturing method can all influence the physico-chemical attributes of the liposomes formed. This in turn influences their adjuvant action; it is recognised that cellular uptake, antigen processing and the presentation by antigen presenting cells are partially dictated by these particle characteristics [11]. There are a range of physico-chemical factors that should be considered in the design of liposomes as adjuvants. For example, the choice of lipid used can impact on the fluidity of the liposomes bilayers. The location and degree of hydrocarbon chain saturation, in addition to hydrocarbon chain length, all affect the strength of the van de Waals forces that hold adjacent chains together within the bilayer. Hence longer chain length lipids tend to form rigid ordered bilayer structures whilst those with shorter tails will become fluid and disorganised. To consider the impact of bilayer fluidity on liposomal adjuvant activity, Maxumdar and Ali [12], investigated the protective efficacy of liposome encapsulated *Leishmania donovani* antigens. They tested three different liposome formulations prepared from distearyol derivative of L-α-phosphatidyl choline (DSPC) (with a liquid crystalline transition temperature of 54 °C), dipalmitoyl phosphatidyl choline (DPPC) (transition temperature of 41 °C) and dimyristoyl (DMPC) (Tc 23 °C) for their ability to entrap *L. donovani* membrane antigens and to potentiate strong antigen-specific antibody responses [12]. The authors demonstrated improved adjuvant activity with DSPC liposomes (95% protection in mouse challenge studies), with almost no protection in mice immunised with antigen in DPPC or DMPC liposomes. This effect of changing membrane fluidity may affect the adjuvant activity through both cellular interactions and biodistribution. Within our studies we also demonstrated that rigid liposomes prepared using dimethyldioctadecylammonium (DDA) bromide lipid promoted stronger immune responses that more fluid liposomes prepared using the unsaturated analogue dimethyldioleoylammonium bromide (DODA), which contained one unsaturated C = C bound in each of the lipophilic acyl chains [13]. In biodistribution studies, the rigid DDA-based liposomes were shown to promote higher levels of antigen at the injection site, resulting in a continuous attraction of antigen-presenting cells that expressed elevated levels of the co-stimulatory molecules CD40 and CD86 [13]. Indeed the rigid, DDA-liposomes induced 100-fold higher Th1 responses than the fluid DODA liposome counterparts.

Inclusion of cholesterol within liposomes is also known to influence bilayer fluidity and is commonly incorporated within liposome formulations for drug delivery, as it can enhance liposome bilayer stability by inserting in the lipid bilayer and stabilise the system [14]. However, in terms of the impact of liposomal adjuvant action the effect of cholesterol is unclear; whilst some studies have shown improvements in the immune response [15,16], others have noted reduced responses [17,18].

Vesicle size has also been shown to influence liposomal adjuvant efficacy and studies have shown that vesicle size can influence the development of the immune responses towards a Th1 or Th2 cytokine profile via a range of routes [19–23]. For example, studies have described enhanced Th2 responses after administration of smaller particles whilst larger particles promote IFN-γ and typical Th1 responses [19,20]. This may be a result of differences in particle trafficking to local lymph nodes and uptake by antigen presenting cells, with larger vesicles (650 nm) showing improved antigen tracking, processing and antigen