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Lymph node targeting strategies to improve vaccination efficacy

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ABSTRACT

With the rapid development of nanotechnology as well as growing interest in immunotherapy, a great number of vaccine delivery vehicles have been explored in order to elicit potent adaptive immune responses against various infections or tumors. Recent studies have shown that targeting vaccine to antigen-presenting cells (APCs) within lymph nodes is an effective strategy for improving antigen-specific adaptive immune response. However, the characteristics of vaccine vehicles, such as size, surface charge and the degree of PEGylation, affect lymph node transfer and subsequent APC uptake, leading to different levels of immune responses. Thus, a comprehensive review of barriers, difficulties and corresponding strategies in lymph node targeting is important. In this review, we summarize the critical factors that affect lymph node delivery and survey recently reported successful vaccine carriers, which may be helpful for the rational design of vaccine vehicles.

1. Introduction

Vaccines, the most cost-effective life-saving medications in history, have protected countless people from infectious disease [1]. Strong vaccine-based protection requires induction of adaptive immunity, which involves antigen uptake, antigen processing by antigen-presenting cells (APCs), and APC activation. Activated APCs prime T and B cells [2], which mount downstream immune responses. Traditional vaccines can elicit good protective antibody responses against many infectious diseases. However, vaccines against major global diseases, such as cancer, AIDS, tuberculosis and malaria, require cellular immune responses, especially a strong $CD8^+$ T cell response. The development of vaccines against these diseases remains a great challenge [3].

For vaccines that aim to induce $CD8^+$ T cell response, a major challenge is that $CD8^+$ T cell training requires antigen presentation by major histocompatibility complex (MHC)-I, but exogenous antigens are normally presented on MHC-II. The MHC-I presentation pathway takes place in the cytosol, so studies have examined the possibility of delivering vaccines to the cytosol using nano-vectors. However, nanoparticles are normally phagocytosed into endosomes, and their endosomal escape into the cytosol is not efficient [4]. An alternative that shows much more promise is to deliver antigen to dendritic cells (DCs), primarily $CD8^+$ DCs [5,6], since these cells can process exogenous antigens into the MHC-I pathway [7]. This cross-presentation ability of DCs, together with their critical and versatile role in T cell immunity, has made them one of the most attractive vaccination targets in recent years.

Diverse vehicles have been developed to target DCs, and these have been extensively summarized in several good reviews [8–10]. The site of vaccination is critical: traditional vaccines are usually injected peripherally, where DCs are much less abundant than other phagocytic cells [10]. Thus, peripheral vaccination is relatively inefficient at inducing an adaptive immune response, especially a T cell response. An effective alternative site of DC-targeted vaccination may be lymph nodes, which are organs of the lymphatic system that initiate and regulate the adaptive immune response and that contain a large number of phagocytically active DCs [11] In addition, $CD8^+$ DC and $CD169^+$ macrophages, which can cross-present antigens, are resident only in lymph nodes. A growing number of studies support the benefit of targeting therapeutic or prophylactic vaccines to lymph nodes for enhancing the adaptive immune response. These vaccines can be delivered effectively to lymph nodes via the interstitium into lymphatic capillaries, which carry them to lymph nodes. Effective lymph node targeting depends strongly on the size of the vaccine vehicle. Other vehicle characteristics (e.g. composition and surface charge) and injection pressure are also important factors.

Delivering vaccine efficiently to lymph nodes is insufficient for inducing a potent immune response: the vaccine must also be efficiently taken up by APCs, and it must strongly activate APCs. For example, if a vaccine vehicle accumulates efficiently in the lymph nodes but does not release its vaccine cargo, a weak immune response will be elicited. Therefore, design of vaccine vehicles should pay attention to lymph node delivery, APC uptake and antigen release. In addition, strategies of lymph node delivery may need to be adapted to the microenvironment

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of the interstitium and lymph nodes, which can vary depending on the disease context.

In this review, we discuss the barriers and difficulties in lymph node targeting after interstitial injection and review the viable strategies and lymph node targeting vehicles that have been reported.

2. Peripheral vs. lymph node delivery of vaccine vehicles

Traditional vaccines are usually administered peripherally. After peripheral injection, antigens are captured by DCs or macrophages in peripheral tissues, and these APCs migrate into lymph nodes, where the processed antigen peptides are presented to T or B cells [10,12,13]. Adjuvants such as aluminum hydroxide [AlO(OH)], incomplete Freund's adjuvant (IFA) and phospholipid-based phase separation gel [14] can help the antigen form a 'depot' such that it is eliminated more slowly from the injection site, prolonging contact between the antigen and immune system and eliciting a stronger immune response than antigen alone [15]. Unfortunately, this strategy does not trigger an efficient T cell response [15], since naïve T cells must be activated by immature DCs, which are present in peripheral tissues in much smaller numbers than other phagocytic cells such as macrophages [10]. For example, peripheral vaccination of mice with ovalbumin (OVA), AlO(OH) and the strong Toll-like receptor-9 agonist CpG induced only weak anti-tumor immunity [16]. It may be possible to improve results by optimizing the adjuvant: one study found that adding a high NaCl concentration to AlO(OH) increased the strength of cellular and humoral immune responses against subsequently injected EG.7-OVA tumor cells, yet these responses were still relatively weaker than those obtained with other approaches [17]. In any event, using peripheral depot vaccines has another disadvantage: it may induce tolerance. For example, peripheral vaccination of mice with gp100 melanoma peptide and IFA created a persistent antigen depot that primed antigen-specific $CD8^+$ T cells around the site of vaccination, but not in tumors [18]. As a result, primed T cells were sequestered and deleted, leading to hyporesponsiveness upon subsequent vaccination. This problem appears to arise when antigens persist in the absence of adjuvant signals: it can be avoided by co-administering a depot-forming vaccine with CpG [19]. The available evidence suggests that inducing potent immune responses via peripheral vaccination may be particularly challenging, which has led to extensive efforts to identify alternative sites of vaccination.

Lymph nodes, in contrast to peripheral tissues, contain a large number of phagocytically active DCs [11], including lymph node-resident $CD8^+$ DCs [5,6], which can cross-present antigens. In contrast, DCs in peripheral tissues (Langerhans or dermal DCs) cannot [20]. Lymph nodes also contain $CD169^+$ macrophages, which can cross-present antigens [21]. These macrophages play a critical role in the adjuvanticity of QS-21, which is a component of the US Food and Drug Administration-approved adjuvant AS01 [22]. Several studies have shown that delivering vaccines directly to the lymph node induces stronger immune responses than delivering them to peripheral tissues. Poly(DL-lactide-*co*-glycolide) (PLGA) microspheres containing OVA and poly(inosinic:cytidylic acid) [poly(I:C)] induced much stronger IgG antibody and $CD8^+$ T cell immune responses when injected directly into lymph nodes than when injected intramuscularly [23]. Intranodal injection of microspheres induced a robust IgG2a response after a single administration, whereas subcutaneous administration failed to elicit a substantial IgG2a response, and intradermal or intramuscular administration generated only intermediate IgG2a titers [24]. The available evidence suggests that delivering vaccine to lymph nodes can induce better immune responses than peripheral vaccination.

3. Methods for delivering vaccines to lymph nodes

While intranodal injection provides the most direct and efficient vaccine delivery to lymph nodes, it usually requires surgery [24] or guidance through the use of ultrasound [25] or tracer dyes [23], which makes the procedure more complex and brings potential risks. In addition, lymph nodes are small, limiting the injection volume. These issues make intranodal injection impractical for widespread prophylactic vaccination.

An alternative is to promote the entry of vaccine vehicles into lymphatic capillaries after interstitial injection; the vehicles then drain from the capillaries into lymph nodes. Interstitial administration can be achieved through subcutaneous, intramuscular and intradermal injection. The size of a vehicle strongly influences how efficiently it can enter the interstitial fluid and subsequently the lymph nodes. In vascular capillaries, a basement membrane underlies endothelial cells connected by tight junctions [26]. As a result, only vehicles smaller than approximately 10 nm can enter vascular capillaries [27]. Larger vehicles, in contrast, can enter lymphatic capillaries because initial lymphatic vessels have a discontinuous basement membrane, lack smooth muscle and exhibit wide, button-like interendothelial junctions [12]. Vehicle size is also limited by the 100 nm diameter of water channels in the interstitium, which carry the vehicles to capillaries [27,28]. Vehicles larger than this do not arrive efficiently at lymphatic capillaries because of reduced diffusion and convection through the interstitium. At the same time, vehicles cannot be too small: vehicles smaller than 10 nm enter vascular and lymphatic capillaries easily, but because flow rate is 100–500 times faster through vascular capillaries than lymph capillaries, these small particles tend to be cleared away from the interstitium via vascular capillaries (Fig. 1A) [26]. One study based on dendrimers suggests that materials with diameters larger than ~ 9 nm tend to enter the lymphatic system, while materials with diameters smaller than ~ 6 nm tend to drain into the blood [29]. Thus, particles with diameters of 10–100 nm may be suitable for allowing efficient transfer through the interstitium and entry into the lymphatic capillaries and ultimately the lymph nodes (Fig. 1B). Vehicles unable to enter lymphatic capillaries can reach lymph nodes only after being phagocytosed by APCs in the extracellular matrix (Fig. 1C). However, this route to lymph nodes is much less efficient.

In addition to size, other characteristics of vehicles affect their ability to target lymph nodes, including composition and surface charge. Interstitium is composed mainly of entangled collagen fibers and glycosaminoglycans, and the major glycosaminoglycan is

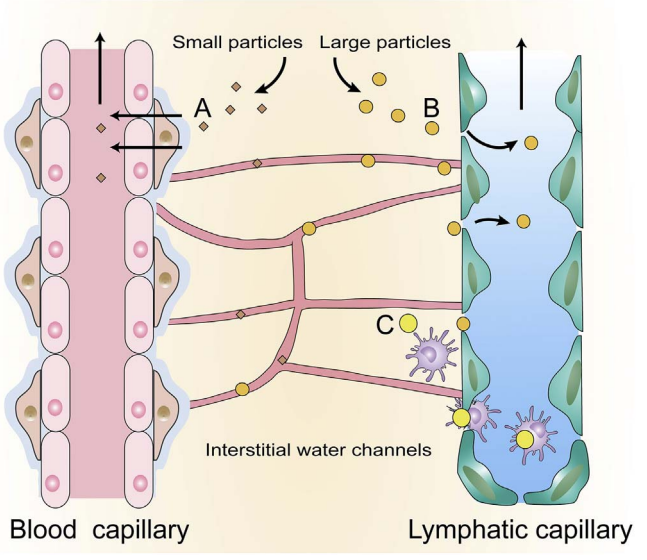

Fig. 1. The fate of vaccine vehicles administered interstitially depends strongly on vehicle size. (A) Only vehicles smaller than 10 nm can cross the tight junctions between vascular endothelial cells and enter the blood capillary. (B) Vehicles with diameters of 10–100 nm can passively diffuse through inter-endothelial junctions and enter the lymphatic capillary. (C) Vehicles larger than 100 nm cannot enter the lymphatic capillary from the interstitium. Instead, these large vehicles can be taken up by dendritic cells, which then migrate to the lymph nodes.