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Targeting dendritic cells with biomaterials: developing the next generation of vaccines

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Current vaccine and immunotherapy technology faces ongoing challenges in both efficacy and practicality: many chronic diseases cannot yet be addressed by vaccination, and several vaccines that do function well require multiple injections, which is a substantial limitation in various parts of the world. A possible key to developing the next generation of vaccines is the ability to deliver antigen to dendritic cells (DCs) more specifically and induce the subsequent activation of T-cell immunity. However, antigen delivery to, and activation of, DCs is a complex problem, involving antigen transport to DC-rich areas, DC binding and antigen uptake, and antigen processing and presentation. Addressing these challenges requires novel and multidisciplinary approaches, for example, the application of biomaterials to immunotechnology. Here, we review the latest advances in biomaterial drug vehicles, such as polymer microparticles and nanoparticles, and liposomes, that are being used to target DCs in new strategies for vaccination.

Introduction

Two of the key challenges in vaccine and immunotherapy technology are to increase the potential to generate potent defenses against chronic diseases that evade the immune system, and to develop effective immunity after single injections of vaccine. Recent strategies for developing preventative and therapeutic vaccines have focused on the ability to deliver antigen to dendritic cells (DCs) in a targeted and prolonged manner. DCs are the most effective antigen-presenting cells (APCs), and have a crucial role in initiating T-cell mediated immunity. DCs can control a substantial part of the adaptive immune response by internalizing and processing antigen through MHC class I and class II pathways and, finally, presenting antigenic peptides to $CD4^+$ and $CD8^+$ T lymphocytes [1–3]. Therefore, targeting DCs with an antigen-delivery system provides tremendous potential in developing new vaccines [4].

The field of DC targeting has been dominated largely by *ex vivo* strategies. Approaches consist of isolating DCs from the blood of patients, exposing them to antigen and other maturation stimuli, and, finally, re-injecting them into the patient. Although encouraging results have been obtained, the complexities – medically, economically and logistically – of these approaches are substantial [5–7], and will possibly prevent a major impact on diseases of the developing world. This highlights the need to develop technologies that effect the robust and simple targeting of DCs – for example, with biomaterial vectors.

In vivo DC-targeting strategies have used free antigen, protein fusions and viral gene therapy. Using protein antigens conjugated to DC-specific antibodies, heat-shock proteins or viral replicon particles, these approaches have produced effective results *in vivo* [8–11]. However, complete success depends on overcoming biological delivery challenges and the weak immunogenicity of many antigens. To achieve more-efficient antigen delivery to DCs, researchers today are considering immunogenic properties and physiological transport barriers.

Biomaterial drug vehicles offer a potential solution. For drug delivery, the most commonly used biomaterials are liposomes (vesicles formed from phospholipid bilayers) and polymer microparticles, nanoparticles, vesicles and micelles. Degradable polymers, for example polyesters [poly(lactic acid), poly(glycolic acid) and their copolymers], polyorthoesters, polyanhydrides and polycarbonates, are frequently used [12,13]. Several of these systems degrade rather slowly for using in antigen delivery, and methods have been developed to trigger their degradation during particular steps of the cell-internalization pathway (see next section). Self-assembling block copolymers that have hydrophobic domains (blocks) and hydrophilic blocks are also employed to form vesicles and micelles, within and upon which protein and DNA can be incorporated [14,15]. Ironically, biomaterials scientists have traditionally sought to minimize immunological interactions with materials, or even to induce some form of immunosuppression [16–18]. However, the field has seen a recent shift in direction, as it is becoming evident that certain materials themselves can have a bio-functional role and be used to induce specific and desired immune responses. Polymer particles and liposomes are suitable for conjugation or loading with antigens, and can protect the antigen from degradation *in vivo*. Following internalization of the

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biomaterial vehicles by DCs, the antigens are released intracellularly in a manner that can activate MHC class I and II pathways and, therefore, induce both $CD4^+$ and $CD8^+$ T-cell immunity. Moreover, the surface of the biomaterial vehicle can be conjugated with DC-specific antibodies or ligands to increase targeting specificity. Biomaterials themselves can also function as synthetic adjuvants or 'danger signals' that activate DCs and induce subsequent T-cell immunity, and, additionally, specific molecular danger signals can be bioconjugated to the surface of polymer particles to enhance the adjuvant effects on DCs. Finally, by controlling nanoparticle size, the lymphatics can be accessed efficiently as a delivery route for targeting lymph-node-resident DCs instead of peripheral DCs.

This review discusses DC targeting with biomaterials and their relevant engineering design considerations as a promising strategy for the next generation of vaccines and immunotherapies.

Biomaterials as antigen-delivery vehicles

Biomaterial vehicles can be engineered to fill various roles in antigen delivery. In fact, they have been studied for this purpose in some capacity for >20 years, after the early demonstration that polymeric carriers could be used to release antigens in a controlled manner [19,20]. However, a focus on the specific delivery of antigen to DCs has emerged only recently. These strategies use biomaterial vehicles because they can bestow longevity on intact antigen to increase the opportunity for DC uptake and processing. For example, biomaterial particles can be loaded with or conjugated to antigens and, thereby, protect the antigen from enzymatic degradation in the tissue. Also, these vehicles can be used to deliver antigen intracellularly to APCs, therefore enabling antigen processing and presentation to occur. It is crucial to consider these properties, among others, when designing biomaterials as antigen-delivery vehicles (Figure 1).

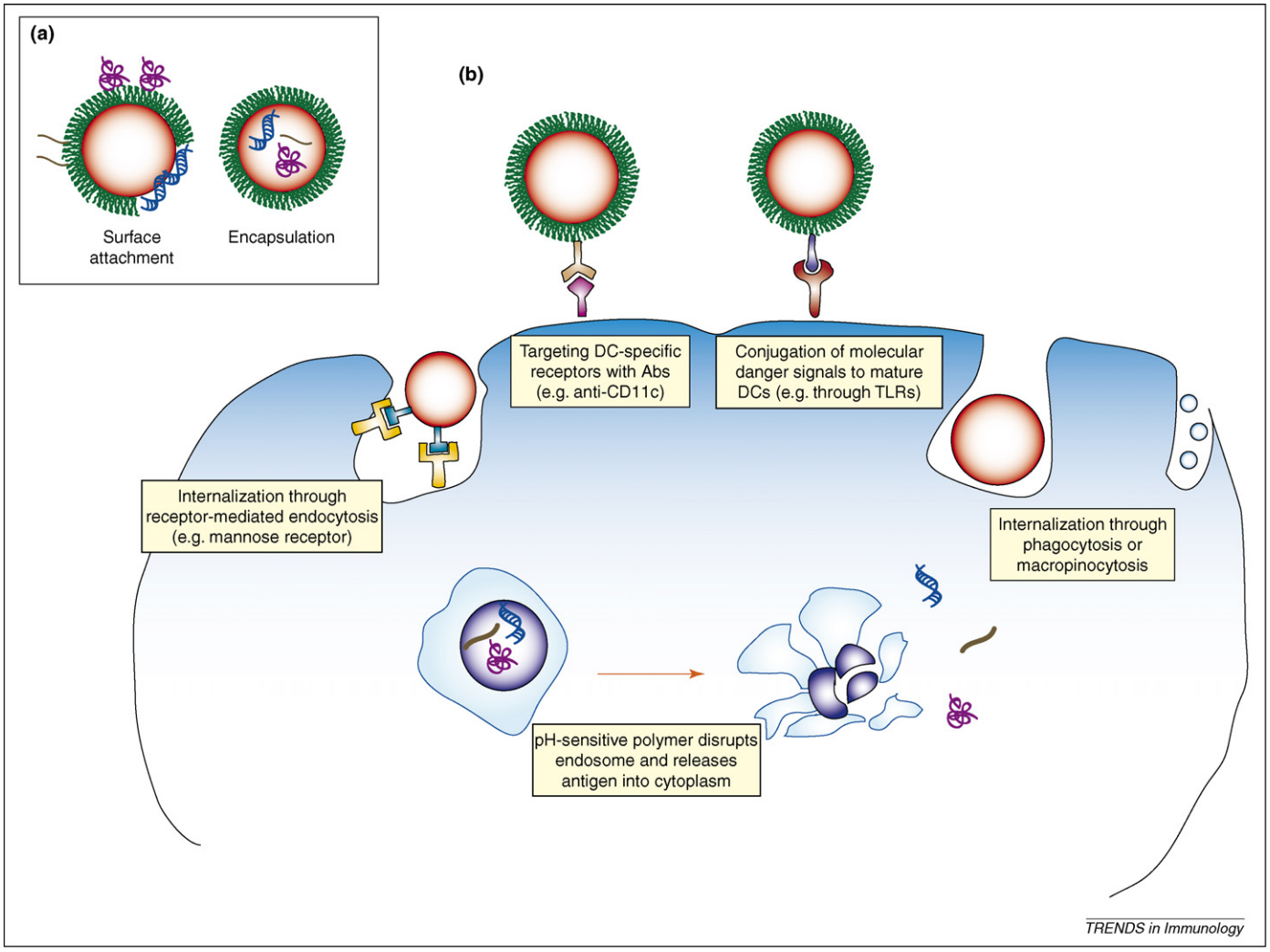

Figure 1. Biomaterial design considerations **(a)** Antigen delivery. Protein (purple) and peptide (brown) antigens can be grafted to the surface of biomaterial vehicles (orange). This binding is often achieved by conjugation to a stabilizing layer of PEG (green). DNA is often bound to the surface of cationic polymers. Antigens and DNA can also be encapsulated within particles and liposomes. **(b)** Biomaterial–DC interactions. Biomaterial vehicles can interact with DCs in several ways. Particles can be functionalized with ligands that bind to receptors that then trigger internalization through endocytosis (e.g. mannose-grafted particles), or, by controlling their size, they can simply be internalized by macropinocytosis (using nanoparticles in the size range of macromolecules) or phagocytosis (using microparticles) without specific recognition. Biomaterial vehicles can be conjugated with DC-specific antibodies (e.g. anti-CD11c and anti-Dec205) to increase targeting. Particles can also be grafted with molecular danger signals that bind to TLRs or cytokine receptors and induce DC maturation. Polymers sensitive to pH can break down intracellularly within endosomes or destabilize the endosomal membrane and release antigen into the cytoplasm. Abbreviation: Abs, antibodies.

Before an antigen can be processed and presented by DCs, the biomaterial vehicle itself must be internalized by DCs. Immature DCs internalize exogenous solutes, particles, and necrotic or apoptotic cells through macropinocytosis, receptor-mediated endocytosis and phagocytosis [21], making DCs excellent targets for biomaterial delivery vehicles that aim to deliver antigen intracellularly. Several studies have verified that DCs can internalize polymer particles and liposomes [4,22–24], and that the internalization mechanism used can be partially controlled by altering properties of the biomaterial vehicle, including size and surface characteristics. This is of particular importance because the mechanism of internalization affects the subsequent processing of antigen (e.g. MHC class I, MHC class II or cross-presentation pathways) [25–27]. For example, protein antigen adsorbed on particles of 1–5 μm is cross-presented at significantly higher levels compared with soluble protein [27,28]. Thus, biomaterials provide a method to mediate antigen processing and presentation. Macropinocytosis is used to internalize extracellular fluid and smaller solutes such as macromolecules [26,29] and particularly small nanoparticles (<50 nm), whereas phagocytosis occurs when larger nanoparticles and microparticles (>500 nm) are taken up [30,31] (Figure 1b). Biodegradable particles made of the polymer poly(D,L-lactic-co-glycolic acid) (PLGA) can bind to or encapsulate proteins and peptides, in addition to DNA [32–34]; these particles are usually large enough to be internalized by DCs through phagocytosis [22,23,30,32–40]. DCs also use lectin-like surface receptors to endocytose ligands with a terminal sugar such as mannose [41]. With this in mind, Copland *et al.* showed that mannosylated liposomes 260 nm in size are internalized through receptor-mediated endocytosis to a higher degree, and present antigen to T cells more efficiently, than neutral liposomes or free antigen [24]. Thus, both the physicochemical and biochemical character of biomaterial vehicles can be adjusted to tailor DC uptake.

Following internalization, the biomaterial vehicle must then release the antigen intracellularly in a manner that will enable processing by MHC class I, class II, or both (cross-presentation) pathways. To deliver exogenous antigen for inducing cellular immunity through the MHC class I pathway can be a challenging problem, as internalized particles are initially within endosomes but are trafficked rapidly to lysosomes, where they are then degraded enzymatically, preventing the antigen from being processed and presented intracellularly. To avoid lysosomal trafficking, Murthy *et al.* have designed smart polymers that use acid-degradable acetal bonds to disrupt endosomes in a pH-dependent fashion [42]. These polymers release oligonucleotides and peptides into the cytoplasm as the endosome is acidified before lysosomal fusion; releasing antigen into the cytoplasmic compartment enables processing by the MHC class I pathway instead of the MHC class II pathway (which processes antigens in endosomes) [42]. Poly(ortho ester) microspheres and acid-degradable microparticles have also been engineered to hydrolyze at endosomal pH levels and release DNA inside APCs [43,44]. These strategies demonstrate how biomaterials can be designed to respond specifically to the intracellular environment for efficient antigen release and specific processing.

These recent studies have established the potential for biomaterials to function as antigen-delivery vehicles. Furthermore, they have demonstrated that the different properties of biomaterials can be designed for DC internalization, and antigen processing and presentation. Future work is needed to optimize these biomaterial design properties for more-efficient antigen delivery.

Biomaterials as adjuvants

Protein, peptide and DNA antigens alone are typically insufficiently immunogenic to initiate DC-mediated adaptive immune responses; therefore, it is necessary that they are co-delivered with an adjuvant [45]. Adjuvants function by stimulating the innate immune system, more specifically by activating APCs, which thereby results in the development of a long-lasting, antigen-specific immune response. Therefore, the long-term efficacy of antigen vaccines depends on developing effective adjuvants.

Aluminum-based adjuvants including aluminum phosphate and aluminum hydroxide have been used for >60 years and are the only ones the Food and Drug Administration approved for use in humans in the U.S. [46]. These adjuvants enhance vaccine efficacy by indirectly activating APCs in different ways, including the formation of an antigen depot, antigen aggregation to enhance uptake by APCs, and activating the complement system [47]. These adjuvants have been proven safe in humans but are weak adjuvants for producing antibodies and initiating cell-mediated immunity. There are several other experimental adjuvants that have been shown to activate APCs more directly, including lipopolysaccharide (LPS, a cell-wall component of Gram-negative bacteria) and unmethylated bacterial CpG DNA. These adjuvants are commonly referred to as 'danger signals' because they act directly on DCs, often through binding to Toll-like receptors (TLRs). Danger signals induce DC maturation and, in turn, result in upregulation of MHC class I and class II, the co-stimulatory molecules CD80, CD86 and CD40, and secretion of the inflammatory cytokines interleukin (IL)-6 and tumor necrosis factor (TNF)-α. However, although these adjuvants are effective in animal models, they can demonstrate toxic side effects, for example, systemic inflammation and shock, and are, therefore, not ideal for clinical use in humans. Derivatives of natural molecules such as LPS are being explored to reduce toxicity (e.g. monophosphoryl lipid A) [48]. Other types of adjuvants, including microemulsions (e.g. MF59) and saponins (e.g. QS-21), have shown promise in clinical trials and, in some cases, are approved for use in Europe [4]. However, many hurdles, including adjuvant safety and efficacy on a wide scale, have yet to be overcome. Thus, improved adjuvants that are both non-toxic and strongly immunogenic are required for developing new vaccine technology.

Some biomaterials, particularly polymers that contain hydrophobic domains, can exhibit natural adjuvant behavior. Hunter has investigated the adjuvant activity of block copolymers of hydrophilic poly(ethylene glycol) (PEG) and hydrophobic poly(propylene glycol) extensively, and showed that adjuvant activity increased in proportion to

the size of the hydrophobic block in the copolymer [49,50]. The mechanisms involved with copolymer adjuvants have yet to be elucidated fully, but it is possible that several factors, including polymer hydrophobicity, the complement system and TLRs (discussed below in this section), function in synergy to activate an immune response.

Research on biomaterial adjuvant potential has been focused largely on determining the degree of DC maturation induced by exposure to liposomes and polymer microparticles [23,24,38]. Immature DCs are extremely efficient at capturing antigen; however, to present this antigen efficiently and activate T cells for an adaptive immune response, it is often necessary for the DCs to be matured by exposure to danger signals. *In vitro* studies have shown that exposing DCs to polymers such as PLGA and poly(β-amino ester) will result in DC maturation by upregulating CD80, CD86 and CD40 [40,51,52]. The exact mechanism of this maturation is not yet fully elucidated, but it is possible that interactions with TLRs have a significant role. For example, TLR4 binds to a variety of ligands that are structurally dissimilar, although many of them (including LPS and bacterial fimbriae) possess hydrophobic domains. Additionally, hydrophobic portions conserved on nucleic acids might be involved, such as those in single-stranded and double-stranded RNA that bind to TLR7 and TLR3, respectively [53–55], and lung surfactant proteins (e.g. SP-A and SP-D) have large hydrophobic domains [56]. Considering all of this, Seong and Matzinger have hypothesized that when exposed, the hydrophobic portions of these ligands might function as danger signals that trigger TLR activation [57]. To extend this hypothesis, it is feasible that the hydrophobic portions of polymers could interact specifically with TLRs and induce DC maturation and adaptive immunity. As mentioned earlier, it is well-established that biomaterials induce greater inflammatory and antibody responses when they are made more hydrophobic [58]. Therefore, when designing antigen delivery vehicles, it is possible that the hydrophobicity of a biomaterial can be used as an intrinsic danger signal that activates DCs.

In vivo studies have demonstrated that DC maturation induced by current biomaterial delivery vehicles alone is not sufficiently strong to activate sustained immunity, and, therefore, it might be necessary to co-deliver additional adjuvants. Van Broekhoven *et al.* targeted DCs with liposomes encapsulating an antigen, and their strategy was shown to provide sufficient anti-tumor immunity only when liposomes were simultaneously loaded with the danger signals LPS or interferon (IFN)γ [59]. Other studies have encapsulated lipopeptides (derived from *Escherichia coli* lipoproteins that bind to TLRs) within liposomes to induce DC maturation [60,61]. An alternative to encapsulation is to conjugate molecular danger signals to the surface of polymer particles. The surfaces of many polymer particles are stabilized with PEG, which enhances the hydrophilicity, resistance to protein adsorption and circulation time of the particles. The common use of PEG has partly resulted in the development of several chemical techniques that enable the efficient conjugation of biomolecules to PEG [62]. In performing this biofunctionalization, it is important to retain biological recognition of the molecular danger signal. Amine functions on the biomolecule are frequently targeted, and it is possible to discriminate between the N-terminal α-amino group and the ε-amino group of lysine residues by their different pK_a values, enabling site-specific bioconjugation [63,64]. These schemes can be employed to conjugate danger signals, such as TLR ligands or cytokines, to PEGylated biomaterial surfaces and, therefore, enhance DC maturation and subsequent T-cell immunity (Figure 1).

Biomaterial vectors provide the potential to improve upon current adjuvant technology by offering maturation stimuli that act directly on APCs. The potential exists to use biomaterials as adjuvants, whether it is the hydrophobicity of a biomaterial functioning as an intrinsic danger signal, or the ability to load or conjugate molecular danger signals. Adjuvants based on emulsions and liposomes have already seen significant progress, and future work should aim to develop further the adjuvant activity of biomaterials and elucidate the molecular mechanisms responsible.

Targeting peripheral versus lymph-node DCs

While antigen delivery and adjuvant activity are important in designing DC vaccine vehicles, another crucial aspect is DC targeting in specific tissues. The location of DCs will affect targeting strategies significantly, as DC phenotype and concentration varies greatly in different tissues. Biomaterials research has focused largely on targeting peripheral immature DCs (Langerhans cells), and new approaches are now being developed to target also lymph-node DCs (Figure 2).

Most microparticle and liposome delivery forms target peripheral immature DCs in the skin, where the materials are taken up to induce DC maturation and migration to lymph nodes, where the DCs activate T cells [65]. The challenge here is that in the skin, immature peripheral DCs are present in extremely low numbers compared with other phagocytic cells (e.g. macrophages); therefore, the ability to enhance DC-targeting specificity becomes crucial to generating a sufficient immune response. For this purpose, Kwon *et al.* conjugated the DC-specific antibody anti-Dec205 on microparticles and showed that anti-Dec205 particles are taken up by DCs nearly three times more effectively than control particles *in vivo* [44]. A similar study used liposomes grafted with anti-CD11c and anti-Dec205 to enhance DC-targeting specificity *in vivo* and the subsequent immune response [59]. Another important factor to consider when targeting peripheral DCs is premature antigen presentation (i.e. a migrating DC that presents antigen on its surface before reaching a lymph node), which can actually result in antigen tolerance [43,66,67]. With this in mind, Wang *et al.* have designed poly(ortho ester) microspheres that delay degradation intracellularly so that their DNA antigen is not released until 24 h after particle phagocytosis [43]. This delay ensures that peripheral DCs do not present antigen until they reach the lymph node. Thus, biomaterials technology can be used to overcome challenges in targeting peripheral DCs.

An alternative to peripheral DC targeting is antigen delivery to DCs that reside within the lymph nodes, and it is possible that this could provide more-potent

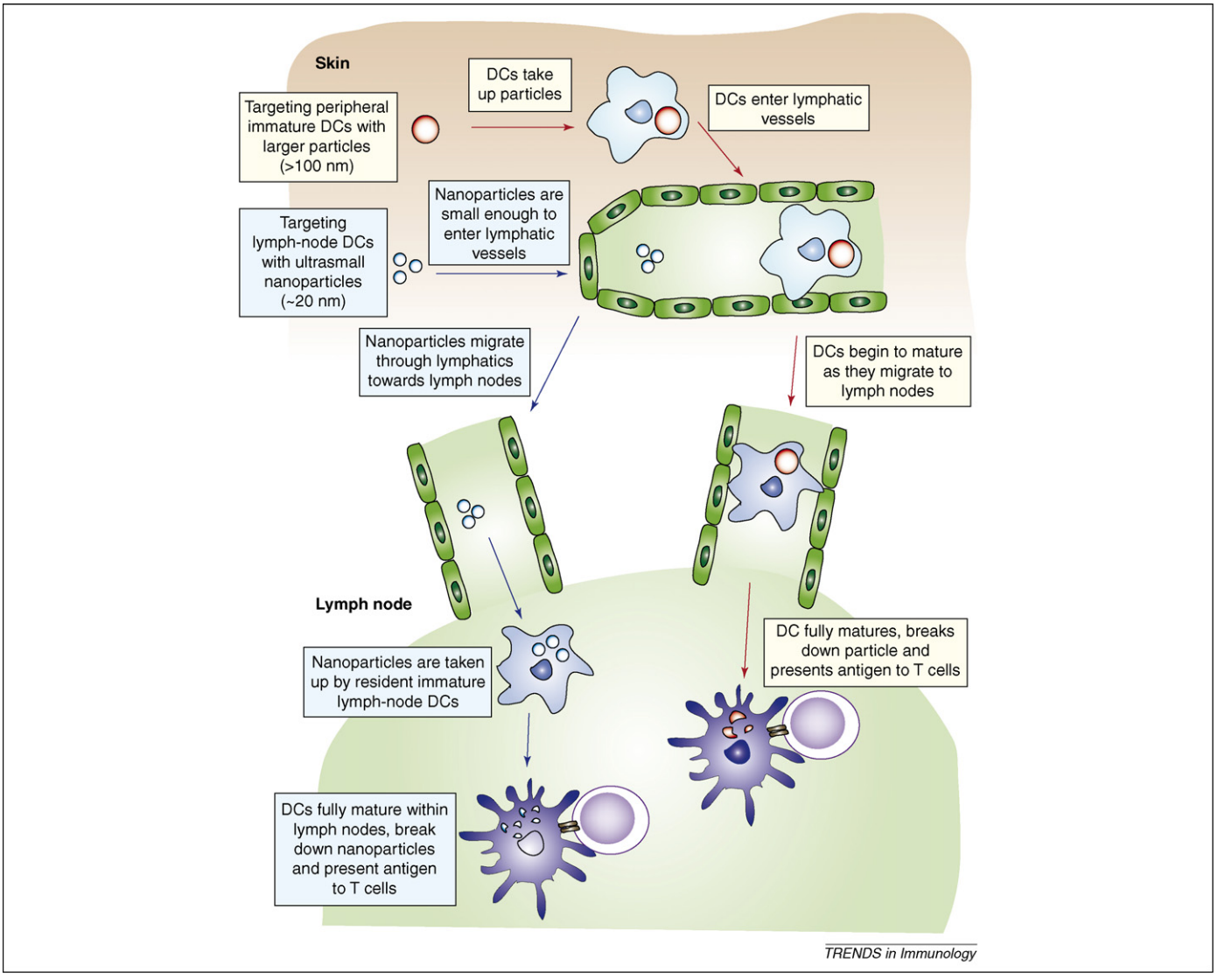

Figure 2. Targeting peripheral versus lymph-node DCs. Immature peripheral DCs take up larger particles and liposomes (>100 nm) and migrate to lymph nodes, where they mature and present antigen to T cells. Ultrasmall nanoparticles can enter lymphatic vessels directly and travel to lymph nodes with the lymphatic flow, where they are taken up by immature DCs resident in the nodes.

immunostimulation. Until fairly recently, it was generally assumed that lymph node DCs were already mature and, therefore, incapable of internalizing or processing new antigen that arrived in lymph nodes. However, recent studies have proven that a substantial fraction of DCs resident in the lymph nodes are immature and still capable of internalizing and processing antigens [68–71]; thus, resident lymph-node DCs (and other APCs) are potential targets for antigen vaccines. There are many theoretical advantages to targeting lymph-node DCs, one potential benefit being avoiding premature antigen presentation because DCs in lymph nodes are already at the site of antigen presentation. Additionally, whereas anti-Dec205 or anti-CD11c targeting might be necessary to compensate for the low DC numbers in skin [11,44,59], no such biomolecular ligand might be necessary with lymph-node-resident DCs because they are present in lymph nodes in large numbers and a substantial fraction are still phagocytically active. Finally, recent work has established that immature DCs within lymph nodes are involved in cross-presenting tissue antigens and inducing self-tolerance [69,72,73]; therefore, lymph-node DCs provide a target for antigen in situations where therapeutic tolerance is useful (i.e. autoimmune diseases and organ transplantations).

For successfully targeting lymph-node-resident DCs, it is crucial to engineer biomaterial vehicles that can be readily taken up into lymphatic vessels after subcutaneous or intradermal injection, and can then be retained in draining lymph nodes [74]. It has been well-established that particle size is among the most crucial factors for lymphatic uptake from the interstitial space [75–77]. Liposomes larger than 170 nm generally remain at the injection site and drain poorly to lymphatic vessels; however, smaller particles (<40 nm) show significant uptake into lymphatic vessels [75,78]. We have designed polymer nanoparticles with a hydrophobic rubbery core of cross-linked poly(propylene sulfide) (PPS) and hydrophilic surface of PEG that can be synthesized to extremely small sizes [79]. Intradermal injection of 20 nm particles resulted in rapid and highly efficient uptake into lymphatic vessels, and retention in lymph nodes for up to 120 h post-injection

[80]. We also found that PPS nanoparticles were internalized within the nodes exclusively by DCs and macrophages. Moreover, we demonstrated that a significant fraction (~50%) of resident lymph-node DCs had taken up nanoparticles without using any targeting ligand. Therefore lymphatic targeting of lymph-node DCs with ultrasmall PPS nanoparticles might offer another promising biomaterial strategy for vaccination.

Concluding remarks and future perspectives

Although using biomaterials in vaccine formulations has been studied for many years, the recent emergence of targeting antigen specifically to DCs and their subsequent activation with biomaterials has demonstrated exciting potential for developing new vaccine technology. Polymer microparticles and nanoparticles, and liposomes, offer the ability to protect antigen from degradation and deliver specifically to DCs, and novel chemical strategies can be employed to release antigen intracellularly so that it can be processed by both MHC class I and class II pathways. Polymers can also be used as synthetic adjuvants that use different mechanisms to induce DC maturation and initiate adaptive immune responses. Moreover, bioconjugation strategies can be used to attach molecular danger signals to particles to amplify the immune response while providing a potential non-toxic alternative adjuvant technology. Finally, using ultrasmall nanoparticles (~20 nm) offers an antigen-delivery system to unlock the potential for targeting lymph-node-resident DCs for inducing adaptive immunity and therapeutic tolerance. The development of biomaterials for immune-cell targeting continues at a rapid pace, and future studies should focus on optimizing antigen delivery and adjuvant activity. The combination of polymer chemistry, molecular immunology and lymphatic physiology will help to design a successful DC-targeting system and might lead to the next generation of vaccines.

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