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Active targeted delivery of immune therapeutics to lymph nodes

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Abstract

Purpose of review—Organ transplantation is a life-saving procedure and the only option for patients with end-organ failure. Immune therapeutics have been key to the success of organ transplantation. However, immune therapeutics are still unable to eliminate graft rejection and their toxicity has been implicated in poorer long-term transplant outcomes. Targeted nanodelivery has the potential to enhance not only the therapeutic index but also the bioavailability of the immune therapeutics. One of the key sites of immune therapeutics delivery is lymph node where the priming of immune cells occur. The focus of this review is on nanomedicine research to develop the targeted delivery of immune therapeutics to lymph nodes for controlling immune activation.

Recent findings—As nanomedicine creates its niche in clinical care, it provides novel immunotherapy platforms for transplant recipients. Draining lymph nodes are the primary loci of immune activation and represent a formidable site for delivery of wide variety of immune therapeutics. There have been relentless efforts to improve the properties of nanomedicines, to have in-depth knowledge of antigen and drug loading, and, finally, to explore various routes of passive and active targeted delivery to lymph nodes.

Summary—The application of nanotechnology principles in the delivery of immune therapeutics to the lymph node has created enormous excitement as a paradigm shifting approach that enables targeted delivery of a gamut of molecules to achieve a desired immune response. Therefore, innovative strategies that improve their efficacy while reducing their toxicity are among the highest unmet needs in transplantation.

Keywords

drug delivery; nanomedicine; nanoparticles; transplantation

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Conflicts of interest

The authors have no conflicts of interest.

INTRODUCTION

Application of nanomedicine in transplantation

Organ transplantation is the optimal therapy for patients with irreversible, end-stage organ disease. Although immune therapeutics have improved over time, a large number of transplant patients, however, suffer from the consequences of allograft rejection. Furthermore, immune therapeutics are implicated in the pathogenesis of organ failure (i.e. calcineurin inhibitor toxicity). Contributing to an increased incidence of post-transplant diabetes, hyperlipidemia, and also playing pathogenesis of accelerated cardiovascular disease, which is the leading cause of death in the transplanted population [1-7]. One of the major clinical approaches to improve long-term transplant outcome is to improve the therapeutic index of immune therapeutics (increasing the efficacy but reducing the toxicity) [1, 5, 8-14]. Therefore, there have been many conventional approaches of sparing, minimizing, or withdrawing calcineurin inhibitor. However, these strategies have limited challenges such as unmodified toxicity, development of new sets of toxicity caused by replacing the drug, as well as higher rejection rates when immunosuppression was reduced [3, 15-17].

Nanotechnology is a multidisciplinary field involving design and engineering of submicron structures. Application of nanotechnology in medicine has rapidly evolved over the last two decades. There are numerous reports on introduction of nanotechnology into different aspects of medicine including diagnosis (biological detection, sensors, and imaging), treatment (drug delivery and tissue engineering), monitoring, prediction, and prevention of diseases [18-21].

Nanocarriers have been developed to treat a wide variety of diseases, mainly because of their specific features that distinguish them from other modalities for instance antibody–drug conjugates [22]. Liposomes, which are nanoparticles composed of a lipidic bilayer enclosing an aqueous core (incorporating drugs, nucleic acids, etc.), were the first nanocarriers to be approved, making them the oldest nanotherapeutic platform [23]. Polymer–drug conjugates, colloidal nanocarriers wherein drug molecules are covalently attached to the polymeric backbone, and polymeric micelles composed of diblock polymers encapsulating drugs, are other examples of nanotherapies that followed clinical approval [23]. These nanoparticles represent the majority of clinically validated and approved nanomedicines [24]. Polymeric nanoparticles, protein-based nanoparticles, and dendrimers are also nanoparticles that are undergoing clinical translation. Other types of nanomaterials used for clinical nanodiagnosics and nanotherapeutic applications include viral nanoparticles, inorganic nanoparticles composed of gold, silica, iron oxide, and hafnium oxide, in addition to oncolytic viruses, which are under clinical investigation [25]. The majority of these nanocarriers are used for oncology applications, as they, in particular, become trapped within tumors through the so-called ‘enhanced permeation and retention’ (EPR) effect [26]. The discovery of the EPR effect in the 1980s suggested that colloidal nanoparticles can accumulate in tumors due to fenestrations in tumor vessel endothelium, as a result of excessive branching, and chaotic vasculature that caused the breakdown of tight junctions and disruption of the basement membrane [27, 28]. Hence, oncology applications of

nanomedicines have been widely studied, resulting in major interest in the design of nanoparticles of different sizes, shapes, and surface characteristics aiming to improve tumor or target-site accumulation [29].

Advantages of nanomedicines include, first, ability to deliver hydrophobic drugs; second, improved pharmacokinetics and biodistribution of therapeutics [30]; third, enhanced bioavailability of small therapeutics and biological drugs [22, 31, 32]; fourth, improved tolerability and therapeutic indices of drugs by reducing marginal side-effect and systemic toxicity of the therapeutics by selective delivery to the target site; fifth, delivering therapeutics across biological barriers, for instance, epithelial and endothelial barriers [33-36]; sixth, conjugation of multiple targeting moieties to design nanocarriers with considerably enhanced selectivity; seventh, loading multiple therapeutics into the nanocarriers, another unique advantage of these delivery platforms, without affecting pharmacokinetics of the individual drugs; and eighth, sustained and controlled drug release, which is another well desired feature of nanocarriers [37]. For many applications, notably immune therapeutics drug delivery, slow extended release of the therapeutics is extremely essential to improve therapeutic index.

Nano-sized drug carriers

As mentioned earlier, polymeric nanoparticles and liposomes are two of the most well studied drug nanocarriers. Liposomes are one of the first types of nanocarriers mainly investigated for delivery of hydrophobic drugs. Loading of the drug into a single or double lipid layer increases the solubility and alters the biodistribution pattern of the drug. The size and surface chemistry of the liposomes dictates the biodistribution and not the drug molecules. Yet, liposomes suffer from burst release of the drug, ineffectiveness in intracellular delivery of drug molecules and evading biobarriers [38-41].

Among the polymers developed to formulate polymeric nanoparticles, poly(lactic-co-glycolic acid) (PLGA) has gained considerable research interests because of its favorable properties including biodegradability, biocompatibility, and approval of the Food and Drug Administration as well as European Medicine Agency[42]. PLGA nanoparticles reduce the chance of therapeutic degradation and improve bioavailability of poorly water-soluble therapeutic agents, and facilitate controlled release [32, 43-45].

Biodistribution of nanoparticles, and effect of size, shape, and surface charge

A better understanding of obstacles and barriers nanoparticles face upon administration into the bloodstream has led to the design of nanoparticles with optimized physiochemical properties, prolonged circulation time, organ-specific accumulation, reduced toxicity, and enhanced therapeutic index [46]. Very small nanoparticles, smaller than 5–6 nm in diameter, are exclusively cleared from the body by renal filtration and urine excretion [47]. Slightly larger particles, 10–100 nm in diameter, that escape the renal filtration face the hepatobiliary system and tend to accumulate in the liver by passing through the vascular fenestration measuring 50–100 nm [48]. The splenic macrophages have a significant role in phagocytosis of nanoparticles larger than 200 nm and, lastly, particles in micrometer range become trapped in the capillaries of the lung and mainly accumulate in this organ [49-52].

Various geometries of nanoparticles affect pharmacokinetics and biodistribution of nanoparticles. For instance, cylindrical and discoidal nanoparticles have unique flow characteristics, the former prone to tumbling and margination dynamics favoring nanoparticles binding and adhesion to endothelium [53, 54] and the latter prone to long circulation time owing to the alignment of cylinders in the vessels with blood flow [55, 56]. Another important feature in designing nanoparticles with prolonged circulation time and selective organ accumulation is surface charge [57, 58]. Nanoparticles with neutral or negative surface charge have longer blood circulation time because of less nonspecific protein adsorption and opsonization [59, 60]. On one hand, positively charged nanoparticles accumulate non-specifically in most of the organs, whereas, on the other hand, negatively charged nanoparticles accumulate less in the liver and spleen [61]. Positively charged nanoparticles have been shown to preferentially be uptaken by tumor-associated angiogenic endothelial cells and facilitate tumor accumulation [45, 62, 63].

MECHANISM OF ORGAN DELIVERY

Passive delivery

Developing nanocarriers with prolonged circulation time revolutionized the field of nanomedicine about 30 years ago since nanocarriers would passively accumulate at a particulate site [64]. The passive accumulation solemnly depends on hemodynamics of blood and diffusion, and disrupted endothelia, the need for targeting ligands on the surface of nanoparticles. In the field of cancer nanomedicine, the majority of nanomedicine application relies on the passive targeting of the tumor vasculature [65, 66]. Owing to the leakiness of the tumor vasculature, particles of appropriate size may accumulate passively through EPR effect [26, 67]. Excessive branching of the vasculature in the tumor combined with enlarged gap between the endothelial cells, replacing the tight junctions, has displayed facilitated extravasation of nanocarriers to the tumor site [68, 69].

Several research groups have reported using nanocarriers for delivery of immune therapeutics [70, 71]. Passive delivery of tacrolimus to the lymphatics using PLGA nanoparticles with average size of 200 nm was reported by Shin *et al.* [72]. They showed that intravenously administered tacrolimus-loaded nanoparticles accumulate at the lymphatic sites by passing through the lymphatic capillary fenestrations, although no therapeutic effect was demonstrated [72]. In another study, PEGylated Poly(lactic acid) nanoparticle loaded with tacrolimus (100 nm diameter) were studied in a rat liver transplant model. The tacrolimus-loaded nanoparticles were delivered through gastric perfusion and lead to significant graft survival prolongation [73].

Liposomal cyclosporine nanoparticles were another type of nanoparticles investigated in liver transplantation, showing reduced off-target side-effects in a rat transplant model as nanocarriers trafficked at a higher rate to the liver as the main member of reticuloendothelial system [74].

Look *et al.* [75] have reported targeting dendritic cells *ex vivo* using nanogel-based nanoparticles and solid PLGA nanoparticles. Nanogels loaded with mycophenolic acid showed higher internalization efficacy by dendritic cells and greater immunosuppression.

Immunosuppressive therapy of lupus-prone New Zealand Black/White F1 Hybrid mice with nanogels resulted in extended survival of the animals. Several research groups have investigated encapsulation of rapamycin in polymeric particles *in vitro*. They have reported sustained release of rapamycin over several days [poly(ethylene glycol)-block-poly(caprolactone) micelles] [76], resulting in superior inhibition of dendritic cells maturation [77, 78], and induction of regulatory T cells (Treg) induction by combinational delivery of interleukin-2, transforming growth factor- β , and rapamycin [79].

Targeted delivery

Active targeting is the other method that relies on selective interaction of ligands on the surface of particles with the receptor of interest at the target site [80, 81]. Therefore, the particles are internalized by cells through receptor-mediated endocytosis and deliver their payload at the site of interest [82, 83]. Surface conjugation modality widens the field of application of nanocarriers in medicine. Antibodies, peptides, proteins, small molecules, low molecular weight ligands, and polysaccharides are some examples of targeting moieties to conjugate to the surface of nanoparticles [82-86].

Targeted drug delivery in transplantation

Given the pathobiology of transplant rejection, two sites of lymphoid tissue and organ transplant are of interest for the delivery of immune therapeutics. There have been efforts for the direct delivery of molecules to the organs to suppress early inflammatory responses [87, 88]. However, the focus of this review is on the delivery of immune therapeutics to lymph nodes following systemic administration.

Lymph node delivery of immune therapeutics

The lymph node has been the focus of transplantation research for decades. Lymph nodes are well recognized sites where the alloantigens are introduced to the T cells by dendritic cells. The microarchitecture of lymph nodes is critical to their function [89]. The seminal discovery of Sir James Gowans showing the daily homing of lymphocytes to the lymph nodes has shaped our understanding of immune activation in the lymph node [90-92]. The ability of immune therapeutics to reach the lymph node creates an unprecedented opportunity to augment or diminish the immune activity for the disease of interest. One can envision development of more effective vaccines or enhanced tumor immunity by delivering the antigens or therapeutics of interest to further augment the immune response. The DLN is an ideal target for delivery of immune therapeutics in transplantation as the primary site of alloreactive T-cell activation [93, 94]. Thus far, most of such deliveries solely rely on the subcutaneous injection of materials to reach the DLN of interest.

Subcutaneous injection is an extensively studied route of administration of therapeutic carriers, specifically liposomes, for delivery to the lymph node [95]. Size of the liposomes and the injection site are the most important factors influencing lymph node targeting via subcutaneous injection. Phagocytosis by macrophages is the driving force behind the targeted delivery of liposomes to the regional lymph nodes [95-99]. Dane *et al.* [100] have encapsulated tacrolimus inside micelles with average diameter of 50 nm, using amphiphilic poly(ethylene glycol)-b-poly(propylene sulfide) block copolymers. The efficacy of

tacrolimus micelles was investigated in the allogenic C57BL/6 tail skin transplantation model and graft survival prolongation was only achieved when tacrolimus/rapamycin micelles combinational therapy was used.

Azzi *et al.* [101] designed polylactide–cyclosporine A nanocarriers and coupled them *ex vivo* to dendritic cells for targeted drug delivery to the lymph nodes. The dendritic cell–nanocarrier combination injected into the footpads of mice showed enhanced drug delivery and suppression of T-cell proliferation in the lymph nodes.

Active targeted delivery to the lymph node

On the contrary, delivery of molecules to the lymph nodes following systemic administration has been scarce, thus far, as it is a more daunting task compared with passive delivery. There is a need for pragmatic approaches that would allow delivery to the deeper chain of lymph nodes in the body. Visceral lymph nodes are primary sites of interest, for instance, not only for transplantation but also for cancer therapy. Lymphocytes interact with specialized vasculature present in lymph nodes referred to as high endothelial venules (HEVs). This vasculature was long recognized by their bubbly appearance as compared to the conventional venular cells that are flat [102]. L-selectin present on naïve T cells interacts with a series of sugar-coated molecules called peripheral node addressing (PNAd) molecules [103]. PNAd molecules are collectively recognized by a monoclonal antibody called MECA-79.

We have designed microparticles (MPs) which can follow the foot steps of lymphocytes. MPs containing tacrolimus were coated with MECA-79 that specifically homed to the DLN. Our study indicates that such delivery allows to achieve prolongation of heart allograft survival with much lower circulatory levels of tacrolimus [104■■]. There is a need for future studies to improve the trafficking of nanoparticles to lymph nodes. The efficacy of such delivery in various disease models needs to be tested. Furthermore, transplant organs suffering from chronic rejection develop tertiary lymphoid tissue (TLO) that share many features of lymph node including PNAd expressing HEVs. Pathogenic effector memory T cells residing in TLO play a key role in the pathogenesis of chronic rejection [105-112]. Interestingly, blocking TLO reduces chronic rejection [113]. Again, our HEV nanodelivery platform enables the delivery of immune therapeutics to TLO to target pathogenic T cells. Moreover, our delivery of immune therapeutics to transplant organs has the potential to inhibit both inflammatory responses and effector and memory T cells within the organ.

CONCLUSION

The exploitation of innovative strategies for the active targeted delivery of immune therapeutics to the DLN in transplantation is a crucial clinical necessity. Despite rapid expansion of nanomedicine in the field of cancer therapy, less is explored in immunotherapy and transplantation. Therefore, more studies are needed to identify targeting molecules and effective delivery platforms without posing systemic toxicity.

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KEY POINTS

- Targeted delivery of immune therapeutics using nanoparticles has great potential for developing systems to achieve desired immune response.
- Immune therapeutics–nanoparticle drug delivery platforms can enhance the therapeutic index of immune therapeutics along with lowered systemic toxicity.
- Immune therapeutics–nanoparticles platform carries considerable potential to deliver a wide variety of immune therapeutic molecules.
- Targeted delivery of immune therapeutics to draining lymph node (DLN) and the transplanted organ has the potential to suppress immune response as well as inhibit the inflammatory response.