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Nanotechnological Approaches to Immunosuppression and Tolerance Induction

Kunal Patel, MD^{1,2,~}, Carl Atkinson, PhD^{1,2,3,~}, Danh Tran, BSc^{1,2}, and Satish N. Nadig, MD, DPhil^{1,2,3,*}

¹Department of Surgery, Division of Transplantation, Lee Patterson Allen Transplant Immunobiology Laboratory, Medical University of South Carolina, Charleston, South Carolina, USA

²Department of Surgery, Division of Transplantation, Medical University of South Carolina, Charleston, South Carolina, USA

³South Carolina Investigators in Transplantation, Medical University of South Carolina, Charleston, South Carolina, USA

Abstract

Purpose of review—Several preclinical studies have engineered nanoparticles for immune regulation, and have shown promising results in the fields of autoimmunity and cancer. In solid organ transplantation, the use of nanoparticle-based immune regulation has only just begun to emerge but holds significant promise for the improvement of our current standard of care immunosuppressive regimens. In this review, we will shed light on the current status of nanoparticle-engineered immunotherapeutics, and the potential application of these technologies to the field of organ transplantation. Further we discuss different strategies for delivery and potential cellular targeting moieties that could be utilized to obviate the need for high dose systemic immunosuppressive regimens.

Recent Findings—Recent studies have shown the potential of immunosuppressive laden nanoparticles to increase bioavailability, drug release, and specifically target immune cell compartments as methods to provide recipient immunosuppressive sparing strategies.

Summary—Nanoparticle centered immunosuppressive strategies hold the potential to usher in a new era in transplant recipient management and could hold the key to minimizing off-target effects of immunosuppressants, along with prolonging transplant survival.

*Corresponding Author: Dr Satish N. Nadig, Department of Surgery, Division of Transplantation, Lee Patterson Allen Transplant Immunobiology Laboratory, 173 Ashley Avenue, Medical University of South Carolina, Charleston, South Carolina, USA, 29425. Tel: 843-792-1716. Fax: 843-792-2464. nadigsn@musc.edu.

~Authors contributed equally to this manuscript

Compliance with Ethical Guidelines

Conflict of Interest

Danh Tran and Kunal Patel declare no conflict of interest.
Satish Nadig reports patents pending (P1446; 10075-034PV; P1577).
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Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Keywords

Nanoparticle; Immunosuppression; Transplant Rejection; Immunity; Targeting

Introduction

Transplantation represents a curative treatment for end stage organ failure, and accordingly, thousands of patients undergo transplant procedures every year. Since the first successful kidney transplant by Joseph Murray in 1954 between identical twins, the past 60 years have witnessed considerable advances in the field, including a greater understanding of the cellular changes that occur during organ storage, ischemia, ischemic reperfusion injury, immune rejection, and the development of immunosuppressive pharmacologic agents to combat rejection.¹ Despite these advances, current clinical modulation of these injurious events is focused only on graft rejection. Solid organ transplants are highly immunogenic, and recipients require aggressive immunosuppression and lifelong treatment to prevent allograft rejection. This places the recipient at substantial risk and shortened life expectancy, not as a consequence of graft failure, but rather due to the high toxicity of immunosuppressive drugs required to stave off rejection. Therefore, in a sense, organ transplant recipients trade off one chronic condition, namely end stage organ failure, for another chronic condition, subsequent lifelong dependence on immunosuppressive medications. Due to the unwanted toxicity profiles and the impact immunosuppression has on the quality of life of transplant recipients, the transplant field has focused heavily on the goal of developing strategies to minimize immunosuppression and, ultimately, to induce transplantation tolerance. Wide spectrums of immunosuppressive therapeutics exist; consisting of broad-spectrum anti-inflammatories, such as steroids, immune cell specific pharmacological agents (Cyclosporine, FK506, Mycophenolic Acid), and more recently monoclonal antibody therapies targeted towards lymphocyte co-stimulatory ligands. All these therapeutics have in common the need for systemic administration, potential toxic side effects, and often the need for combination therapy to balance pro-graft survival versus the recipient drug induced side effect profiles. Recent advances in biomaterial nanoengineering, targeting of biomaterial laden drugs, and improved understanding of the importance of the local tissue microenvironment in immune priming and activation, have led to exploration of biomaterial solutions to immunosuppression, as means to obviate systemic immunosuppression and induce tolerance. In this review we explore the current state of nanotherapeutics in transplantation and discuss the potential approaches to obviate the harmful systemic side effects of immunosuppressive agents as a means to improve survival and quality of life of transplant recipients.

Clinical Transplant Immunosuppression

As eluded to earlier, a number of immunosuppressive pharmacotherapeutic strategies are currently employed to deal with acute graft rejection. While these drugs enhance clinical outcomes, particularly in the early post-transplant period, chronic graft dysfunction and complications due to systemic side effects still persist. Here we will provide a brief overview of immunosuppressive compounds, so as to provide background for nanoparticle

discussions. For more detailed information of immunosuppressive drug regimens and mechanism of action, see [reviews²⁻⁹] and Table 1.

Azathioprine, a purine analog, was historically used in conjunction with corticosteroids to block lymphocyte proliferation in kidney transplantation.^{1, 10} It was later replaced by newer generations of immunosuppressive pharmacologic agents, including calcineurin inhibitors, mTOR inhibitors, and mycophenolate. Cyclosporin A and tacrolimus (FK-506), the two most notable drugs in the calcineurin inhibitor group, with tacrolimus being newer and generally considered superior, have enabled success in kidney transplantation, and other organ transplants including liver, pancreas, heart and lung.¹¹⁻¹⁶ This group of immunosuppressives, suppress T cell activity via inhibition of the intracellular enzyme calcineurin, resulting in reduced expression of pro-inflammatory cytokines required for T cell proliferation. While this group of immunosuppressive is the most widely used, its use is associated with severe side effects, which include nephrotoxicity, neurotoxicity, diabetogenesis, and hypertension. Cyclosporin A also causes gingival hypertrophy and hypertrichosis while tacrolimus can also cause alopecia.^{16, 17}

Sirolimus and everolimus, are the two most widely used rapamycin-based mTOR inhibitors, and have been shown to be effective in kidney transplant, and in preventing vasculopathy in cardiac transplantation.^{18, 19} mTOR inhibition leads to regulation of cell proliferation by preventing cell cycle progression from G1 to S1 phase through inhibition of protein synthesis. In addition to its impact on T cell proliferation, rapamycin has been shown to have tolerogenic properties. Rapamycin can influence dendritic and endothelial cell functions, driving them towards a tolerogenic phenotype. Further, in the presence of cytokines, such as TGF- β and IL-2, rapamycin promotes skewing of naïve T cells towards a tolerogenic T regulatory cell phenotype. Building upon these unique mechanisms of rapamycin action, recent studies have shown that utilization of sub-therapeutic doses of rapamycin (insufficient to inhibit graft rejection,) combined with a systemic increase in ex-vivo expanded, adoptively transferred regulatory T cells (Treg) prevents graft rejection. These seminal studies suggest that combined low dose rapamycin with standard immunosuppressive care may prolong graft survival by inducing the recipient's immune system to 'self-immunosuppress'.²⁰ However, rapamycin use is limited to being an alternative to calcineurin inhibitors in patients that fail tacrolimus or cyclosporine immunosuppressive regimes, due mostly to its unfavorable toxicity profile. Rapamycin immunosuppression is associated with life-threatening pneumonitis, malignancy, nephrotoxicity (albeit less severe than calcineurin inhibitors) and diabetogenesis. Further, due to rapamycin's anti-proliferative effects on fibroblasts, early post-transplant administration is not utilized, as therapy severely impairs wound healing.^{19, 21, 22}

Mycophenolic acid (MMF) is an anti-proliferative immunosuppressive, which functions as an inhibitor of the *de novo* purine synthesis pathway by blocking inosine monophosphate dehydrogenase. It has a lower efficacy than calcineurin inhibitors and mTOR inhibitors, and is generally used as an alternative to azathioprine due to its more favorable toxicity profile. Unsurprisingly, MMF also has undesirable complications, which include gastrointestinal tract disturbances, esophagitis, and leukopenia.^{23, 24}

Regardless of the type and class of conventional immunosuppressive pharmacotherapeutic episodes of acute rejection continue to occur, drug associated toxicity are unavoidable, and ultimately chronic rejection will manifest causing graft failure and eventual death.

Traditional immunosuppressive drug delivery requires high systemic drug concentrations, necessitated in part by poor solubility of immunosuppressive drugs in an aqueous environment, non-specific binding to serum proteins, and a drug loss during gastrointestinal passage; to obtain the desired therapeutic response. As such

multiple pre-clinical delivery systems have been recently reported, including, a solid self-nanoemulsifying drug delivery system (SNEDDS) and LCP-Tacro Meltedose, both of which improve bioavailability and which may potentially reduce occurrences of supratherapeutic blood levels.^{25, 26} Additionally, gel formulations for local delivery of tacrolimus to the periphery have demonstrated similar safety, as well as efficacy in preventing rejection in rat limb transplant models.^{27, 28} Advancements to existing drug therapies, such as extended release formulations and enteric coating, which have assisted in developing more predictable pharmacokinetics and a more tolerable side effect profiles, represent the exciting new frontier in transplant medicine, however these are only small forays into the potential of pharmaceutical modifications and administration that could push transplant medicine into the next generation.^{26, 29–31} Recently nanoparticle based drug carriers have been developed, which have been shown to improve drug solubility, cell penetration, improve release kinetics, and drug targeting. All factors that may lead to the generation of therapeutic modalities that obviate harmful systemic toxicity whilst simultaneously promoting graft protection.

Nanotherapy and Transplantation

Recent developments in nanotechnology have increased interest in the delivery of immunosuppressive therapeutic agents to transplant recipients with the goal to ameliorate rejection while simultaneously reducing the adverse side effects associated with therapy. Advantages of nanoparticle (NPs) drug delivery platforms include high encapsulation efficiency despite drug solubility issues, low toxicity due to biomaterial content, drug protection against degradation factors like pH and light, and the reduction of tissue irritation. In order to design an efficient and effective drug carrier, these issues need to be addressed: (1) a tailored surface on the carrier to attach biomolecules for targeted drug delivery; (2) a biocompatible coating which can efficiently encapsulate the hydrophobic drug thereby reducing cytotoxicity; (3) stimuli-induced (i.e., pH) disruption of the carrier agent for slow and controlled drug release to the desired environment. Composition of nanoparticles varies greatly ranging from biodegradable polymers such as polylactide-co-glycolide (PLGA) to heavy metals, such as gold nanoparticles (see Reviews^{32–35}). In addition, lipids have been utilized to encapsulate hydrophobic drugs in liposomes and micelle constructs. NPs can be engineered to deliver drugs, proteins, RNA, siRNA, DNA, and miRNA systemically or by use of specific targeting moieties to specific cell types. Chemical composition and surface properties can be altered to improve cell penetration, protect payloads from environmental induced conformational alterations, and control drug release kinetics. Due to these unique and almost infinitely adjustable properties, nanoparticle technologies are being increasingly explored as a way to deliver higher local doses of immunosuppressive drugs to specific cell

types and tissues thereby obviating the need for toxic systemic concentrations and mitigating systemic side effects. To date three main strategies have been described for the utilization of nanoparticles in transplantation: 1. Systemic delivery, 2. Organ targeted delivery, and 3. Immune system/immune cell delivery. These strategies are discussed in the subsequent text and summarized in Figure 1.

I. Systemic Nanoparticle Delivery—The mainstay of current immunosuppressive therapy is orally administered drugs which require high serum trough levels to exert their local therapeutic efficacy. Cyclosporine has a strong affinity with serum proteins, particularly albumin, which necessitates its administration at high dose to facilitate function. In the vast majority of cases, nanoparticles can be modified more easily than native drugs to produce the sustained release of medication.³⁶ To this end, cyclosporine and tacrolimus have been encapsulated in poly(ethylene glycol)-poly(D,L-lactide) (MPEG-PLA) co-polymer as a strategy to provide a sustained drug release^{37–42}. The incorporation of poly(ethylene glycol) (PEG) increases surface hydrophobicity, resulting in improved circulating half-life due to reduced serum protein binding³⁹. When characterized in an in-vivo model of liver transplantation, systemically administered tacrolimus MPEG-PLA NPs therapy was associated with longer retention time in the plasma and prolonged graft survival times, as compared to standard tacrolimus drug formulations³⁹. Similar findings were also noted for cyclosporine, using PLGA-based NPs in liver and islet allograft rodent rejection models^{43–46}. In addition to calcineurin inhibition, Shirali et al., 2011⁴⁷ described the construction and characterization of MMF PLGA NPs that the authors demonstrated to prolong survival of full thickness murine skin transplants in fully mismatched allografts. NPs were administered systemically by i.v. tail vein injection and were remarkably effective at a concentration 1000 fold reduced as compared to conventional drug⁴⁷. Importantly, unlike the conventional MMF drug formulation, which induced iatrogenic cytopenia, MMF PLGA NPs had no detectable toxicity. Taken together these studies demonstrate the potential of systemically administered immunosuppressive NPs to modulate rejection and improve drug release kinetics while obviating systemic drug induced toxicity. These bioengineered therapeutics were administered systemically and intended to increase serum half-life concentrations. However, data from Shirali et al., 2011⁴⁷, highlighted the importance of dendritic cell endocytoses of systemically administered MMF PLGA NPs for efficacy. Endocytoses of MMF PLGA NPs by dendritic cells was shown to upregulate PD-L1 leading to a decreased ability to prime alloreactive T cells and protection from rejection⁴⁷. These novel observations demonstrate the potential to modulate alloimmune responses by focusing immune inhibition to specific cell populations or the transplant organ itself, as opposed to total systemic immune suppression.

II. Organ Targeted Immunosuppression—Systemic strategies for delivering therapeutics to affect local responses have led to concerns associated with side effects, however, recent studies have pointed to the importance of local graft factors shaping immune responses. Delivery of therapeutics directly into the local microenvironment, specifically the transplanted organ, may modulate recruitment of immune cells and their phenotype. Upon reperfusion, and during rejection, a wave of inflammatory cells traffic to the graft, infiltrate and differentiate into multiple effector cell types. Local delivery of immunosuppressives

could provide direct access to the tissue/allograft-specific inflammatory cells that infiltrate. This may be of great significance in transplantation. The grafted organ has a number of resident antigen presenting cells, such as dendritic and endothelial cells, which have been shown to be critical in the induction of alloimmune responses. Modulation of these cells at the level of the graft with NP delivered immunosuppressive is an attractive approach to reduce systemic immunosuppressive burden and drug associated side effects. The main challenge to a graft targeted delivery of immunosuppressive drug laden NPs is determining an effective targeting moiety which focuses the NP to the graft. NPs can be constructed to possess surface associated antibody targeting moieties, candidates such as Platelet-Endothelial Cell Adhesion Molecule 1 (PECAM-1) or InterCellular Adhesion Molecule 1 (ICAM-1), which are upregulated on pathologically activated endothelial cells and during graft rejection are attractive options.⁴⁸ However, they are by no means specific for the grafted organ. The production of antibodies directed against donor epitopes, such as HLA antigens would distinguish between recipient and donor graft and could be easily conjugated to drug laden NPs. Though feasible, screening of specific HLA targeting antibodies, generation and personalized production for each transplant recipient is at present technically prohibitive. Therefore, there is a need to identify common targeting moieties specific for the allograft.

One organ, the lung, offers a unique opportunity for targeted drug delivery. The ability to deliver aerosolized medications to the lung has the potential to appropriately immunosuppress the lung allograft while almost entirely avoiding entering the systemic circulation. Inhaled steroids are already a staple for the treatment of numerous pulmonary disorders, and aerosolized calcineurin inhibitors have been developed for tailored pulmonary immunosuppression.³¹ While aerosolized versions of cyclosporine A have been in development for over two decades, applications for lung transplantation have very recently come close to clinical use, with current Phase II trials ongoing for cyclosporine inhalation solution (CIS) use in transplant recipients suffering from bronchiolitis obliterans (a manifestation of chronic rejection in lung transplants). CIS is a propylene glycol suspension that had been demonstrated pre-clinically to be safe, with favorable pharmacokinetics, in dogs and rats.^{49, 50} Building on this, CIS entered Phase III trials, but failed to show efficacy in disease free survival or overall mortality.^{51, 52} However, that trial did produce valuable information, including the finding that this aerosolized mechanism of delivery can produce high percentages of pulmonary delivery, and substantially lower systemic levels of cyclosporine than oral administration. Equally as important, it appeared that the patients tolerated the therapy well.⁵³ Similar trials focused on inhalational formulations of a rapamycin powder, compounded with lactose for stabilization, was demonstrated to successfully absorb and distribute throughout the lung.⁵⁴ The study, performed in a non-transplant rat model, examined both the crystallized compound and a preparation generated by thin film freezing (TFF), a technique for increasing solubility and bioavailability. Ultimately, both formulations targeted the lung for comparable amounts of time, however systemic levels of TFF were higher.⁵⁴ Similarly early in its development, inhaled tacrolimus tested in a rat lung transplant model demonstrated efficacy in attenuating IRI, despite the fact the study was somewhat hampered by poor delivery of the therapeutic. This phenomenon was attributed mostly to upper airway interference in a spontaneously

breathing animal, a situation circumvented in humans by virtue of endotracheal intubation.⁵⁵ Taken together, these studies highlight the potential for localized lung allograft immunosuppression. While delivery of the immunosuppressives was clearly demonstrated this was not always associated with efficacy. Nanomaterial solutions for localized cell specific delivery of immunosuppression have begun to be explored with the goal to target the cell types that promote acute and chronic rejection. At present, to our knowledge there have been no reports utilizing NPs directly administered to the transplanted lung to modulate rejection. In-vitro studies using gold nanoparticles that had been engineered with everolimus packaged in the hydrophobic compartment of the NP were shown to inhibit mesenchymal cell proliferation⁵⁶. Mesenchymal cell proliferation is a key pathologic event associated with chronic lung allograft dysfunction (CLAD) and therefore the authors hypothesize that local delivery of these novel NPs may impair CLAD development. While not a rejection model, Bayer et al., 2013⁵⁵ demonstrated the benefit of recipient inhaled tacrolimus NPs in attenuation of post transplant ischemia reperfusion injury in a rat isogenic orthotopic lung transplant model. The authors demonstrated improvements in oxygenation, and a significant reduction in intra-graft infiltrating immune cells⁵⁵.

The ability to specifically target selected organs and receptors imparts a unique attribute to nanoparticles that makes these therapies particularly valuable. Organ transplantation engenders the truly special circumstance of isolating the organ *ex vivo*, and the opportunity to focus treatment pre-transplant is unparalleled. The *ex vivo* storage and perfusion of kidneys, livers, lungs, hearts and even pancreases has continued to revolutionize transplantation. Hypothermic machine perfusion has been utilized for many years and increasingly, normothermic perfusion has been demonstrated to benefit organs⁵⁷. These modalities have been able to decrease the rates of delayed graft function, expand the donor pools, and even help repair previously damaged kidneys.^{58–61} The relevance of machine perfusion to our discussion largely lies in their ability to deliver novel drug therapies in the unique *ex vivo* setting available in transplantation.^{58, 62, 63} The potential of immunosuppressive therapies applied to the donor organ are made even more impactful in light of findings that donor rapamycin therapy has recently been demonstrated to promote a tolerogenic phenotype to donor endothelial cells (EC) in-vitro and in-vivo. Elegant work has shown that treatment of EC with rapamycin resulted in reduced EC activation, proliferation of allo-reactive memory T cells, cytokine production, and further skewed the T cell response to a graft protective T regulatory cell phenotype in an DC/T cell co-culture system^{64–67}. Furthermore, in *in vivo* studies, human aortic grafts were transplanted into SCID/beige mice and grafts healed-in for 30 days, after which, mice were treated with rapamycin at 3 mg kg⁻¹ day⁻¹ for 3 days⁶⁶. Grafts were then harvested and re-transplanted into humanized SCID/beige mice. Grafts from rapamycin pre-treated donors were significantly protected from graft injury and chronic rejection, as compared to untreated controls, with the protective effect attributed to the induction of donor tolerogenic ECs. Although, these studies set the stage for donor organ pre-treatment, the methods employed are not clinically translatable and therefore strategies to target rapamycin to donor ECs pre-transplantation offer promise for improving post-transplant outcomes. To this end, we have recently designed and constructed rapamycin micelles decorated with cyclic arginine-glycine-aspartate moieties (cRGD) specific for alpha V beta 3 ($\alpha V\beta 3$) integrins located on the surface of endothelial

cells⁶⁸. We demonstrated that membrane targeting facilitated receptor mediated micelle endocytosis, and significantly enhanced micellar specific uptake, as compared to untargeted constructs in *in-vitro* models. Further, we showed that rapamycin micelles inhibited EC cytokine production, and alloreactive T cell proliferation, as compared to free rapamycin drug or untargeted rapamycin micelles⁶⁸. Finally, we were able to demonstrate that incorporation of cRGD targeting moiety to the micelle construct improved aortic graft uptake as compared to untargeted constructs when micelles were added to University of Wisconsin solution ex-vivo. Along a similar theme anti-PECAM/catalase NPs targeted to pig lung endothelial cells were shown to protect grafts donated after cardiac death⁶⁹. Augmentation of the antioxidant capacity of graft ECs with NPs enabled safe transplantation and improved graft outcomes 6 hrs post transplantation⁶⁹. While very much in its infancy, the application of drug laden NPs to specifically target donor cells prior to transplantation as part of the organ preservation process, to modify graft immunogenicity and protect against the development of rejection, has the potential to revolutionize transplantation.

III. Immune Cell Targeting—While intragraft immune cell interactions are important for modulation of the direct pathway of allorecognition, events that occur in the regional lymph nodes promote indirect allorecognition. This interaction initiated by recipient antigen presenting cells, presenting alloantigen to naïve T cells, leads to the generation of graft reactive and damaging alloreactive T cells that play a central role in the induction of acute and chronic rejection. With this interplay in mind, generation of NPs that specifically target to immune compartments, or target antigen presenting cells, has potential to induce focused immunosuppression without the need to systemically inhibit immune responses⁴². In a novel study, Dane et al., 2011⁷⁰ demonstrated that the direct delivery of tacrolimus and rapamycin dual laden micelles to lymph node immune cells prolonged C57Bl/6 recipients of Balb/c donor skin graft survival by more than two fold as compared to free drug controls. Efficacy of this procedure was associated with alterations in lymph node antigen presenting cells and supported the notion that tolerance can be induced when appropriate immune cell populations are targeted. The induction of immune tolerance using NPs is dependent of a number of factors. Firstly, NPs have to be targeted to the correct APCs capable of regulating T cell functions, and optimally promote the induction and/or expansion of Tregs. The appropriate targeting and subsequent tolerogenic stimulation of APCs is crucial for immune tolerance induction, and has been demonstrated in models of skin transplantation⁴⁷. A number of candidate targeting moieties have been investigated to target therapeutics and/or antigen directly to liver, splenic and lymphoid tissue dendritic cells and macrophages. Obvious candidates that have attracted interest thus far are cell type specific markers, such as CD11c, or surface receptor targets, such as C-type lectin and scavenger receptors. C-type lectin and scavenger receptors are ideal moieties, as under normal physiological conditions, these receptors function to internalize antigen and promote re-presentation through recognition of specific structures⁷¹. To date a number of lectin and scavenger receptors have been utilized, which include the mannose receptor (CD206), DEC-205, Dectin-1, C-type lectin domain family 9A (CLEC9A), scavenger receptor class A (SRA) and macrophage receptor with collagenous structure (MARCO)^{71–74}. While a number of targeting moieties exist, incorrect targeting can result in immune stimulation. Previous studies with antigen-loaded NPs, that were designed to target CD40, DEC205, or CD11c present on the surface

of DCs, was shown to induce robust production of IL-12 and type 2 interferon, as well as CD8⁺ T cell proliferation, features that would promote rejection in the setting of transplantation⁷⁵. However, particles targeted towards MARCO⁺ macrophages in the liver and spleen induced tolerance in a model of autoimmune encephalomyelitis⁷¹.

Immature DCs have been well recognized to induce tolerant states by skewing effector T cell populations to Treg subsets by antigen presentation in the absence of costimulation. Utilization of these tolerogenic properties makes DCs an attractive cellular compartment to target with nanoparticle based immunosuppression. In fact, studies by Haddadi et al., 2008⁷⁶ suggest that delivery of rapamycin encapsulated by PLGA based nanoparticles can downregulate MHCII, CD86 and CD40 on DCs from Balb/C mice when co-cultured with allogeneic C57BL/6 T cells in contrast to DCs treated with soluble rapamycin⁷⁶. Various immunosuppressive payloads have been utilized to target DC aside from rapamycin. Studies have shown that DC-directed therapy with PLGA nanoparticles carrying mycophenolic acid (a standard in immunosuppressive regimens) allows for prolonged allogeneic skin graft survival with the use of 1000 fold less drug³². These studies highlight important differences in different antigen presenting cell populations, and therefore appropriate targeting will be essential for translational success. As previously suggested, rapamycin has emerged as a potent inducer of tolerance in-vitro with several studies demonstrating that delivery of rapamycin to dendritic cells in the form of a NP improves the tolerogenic capacity of the treated DC, and further, prolongs the efficacy as compared to free drug treatment. The mechanisms associated with this phenomenon are incompletely elucidated but it is hypothesized that the slow release of PLGA delivered rapamycin prolongs the downregulation of key T cell co-stimulation molecules expressed on the surface of the treated DC. Horibe *et al.*, 2008⁷⁷ further exploited the DCs ability to promote tolerance and utilized in-vitro rapamycin pre-conditioned, alloantigen pulsed adoptively transferred DCs to promote long-term survival of skin grafts⁷⁷. While in the reported studies DC therapy occurred ex-vivo, it highlights the potential of modifying alloantigen stimulated DCs with rapamycin NPs to promote in-vivo tolerance⁷⁷. In light of these described studies the effective targeting of antigen presenting cells, such as DC, EC and macrophages in-vivo offers a unique opportunity to modify immunity in the transplant recipient. Finally, donor passenger APCs are central to direct allorecognition and graft rejection. The targeting of donor APCs pre-transplantation may be an effective way to stimulate tolerance induction in the recipient. This therapeutic approach has not been extensively explored within the literature and as such is an area that requires urgent exploration.

Conclusions

Taken together, these differing delivery systems, targeting, and therapeutic NP approaches represent an exciting and rapidly growing field of drug delivery that may well dictate the future of transplantation medicine. While our discussion has been compartmentalized into specific organ-and immune compartment targeted therapies, it should be noted that many of these developments will invariably cross over into other (or all) disciplines of transplantation. For instance, many of the same principles discussed regarding modulation of rejection can be used to optimize donor organs and apply therapeutics to prevent ischemia reperfusion related injuries. By adjusting the targeting moieties on the nanoparticles, or by

adjusting and combining therapeutics, the delivery vehicle can be targeted to surface molecules specifically expressed on different organ systems and at different time points across the transplantation process to modulate IRI, cellular, humoral and chronic rejection. With these novel strategies to deliver the current FDA-approved drugs, the next few decades may witness a new era of better targeting agents that not only can solve the allograft rejection issues in the field of transplantation but also can improve patient compliance by minimizing drug side effects.

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Figure. 1.

Nanoparticle drug delivery systems for the prevention of allograft rejection. Diagram summarizing the varying nanoparticle types, and systemic versus targeted approaches for immune regulation.

Table 1

Summary of immunosuppressive drug regimens and mechanism of action

Immunosuppressants						
Drug name	Class	Used for	Physiologic effect	Route of admin.	Half-life	Excretion
Cyclosporine A	Calcineurin inhibitors	-Transplantation -Certain autoimmune disorders	Binds cyclophilin	IV, Oral	6hrs Peak level: 1–4 hrs Wide distribution	Bile Met. by CYP 450 (liver)
			Drug/protein complex binds & inhibits activity of calcineurin , a phosphatase that is part of the intracellular signaling cascade that ultimately stimulates T cell proliferation via transcription factors that induce cytokine production... ∴ inhib. T cell function			Renal toxicity -Gingival hyperplasia Clearance... -↓ by drugs that ↓ p450 - ↓ by amphotericin B, erythromycin, ketoconazole
Tacrolimus (FK506)			Binds FKBP		Peak level: 2–4 hrs	Met. in liver
			<i>Tacrolimus is 100× MORE potent than cyclosporine A</i>			↓ side effects than eye. A b/c of ↓ potency
Siroliimus (rapamycin)	Cytotoxic (antiproliferative or antimetabolic) drugs	Transplantation -Cancer -Stents (↓ vascular smooth muscle proliferation) ➡ both investigational		Oral	Peak level: ~1 hr	Feces Met. by CYP 450 (liver)
			Drug/protein complex binds mTOR (which regulates <i>mRNA translation</i>) - SRL-FKBP- mTOR stops entry into G1 or S stage of cell cycle ➡ ↓ T cell activation/proliferation			Renal toxicity - Hyperlipidemia NOT directly nephrotoxic BUT can ↑ <i>cyclosporine, tacrolimus</i> nephrotoxicity
Azathioprine		Transplantation (w/ cyclosporine A & or prednisone)	Prevents B & T cell clonal expansion	Oral, IV	Peak level: 1–2 hrs	Cleaved to 6-mercaptopurine <i>in vivo</i>
						↓ rapidly dividing cells (bone marrow, GI mucosa) ➡ leukopenia, thrombocytopenia, GI problems

Immunosuppressants						
Drug name	Class	Used for	Physiologic effect	Route of admin.	Half-life	Excretion
			mercaptapurine Inhibits: - De novo purine synthesis -Nucleotide salvage pathways Growth of cells			
Mycophenolate Mofetil		Kidney transplant (w/ <i>cyclosporine A</i> & <i>corticosteroids</i>)	Prodrg of mycophenolic acid (MPA) Inhibits de novo purine synthesis via inhibition of IMP deHase	Oral	"rapidly absorbed"	Urine Active metabolite is <i>mycophenolic</i> <i>acid (MPA)</i>
Cyclophosphamide		To suppress humoral immunity (<i>bone marrow</i> <i>patients</i>)		Oral	3–12 hrs	—
Methotrexate		Autoimmune disease (e.g. arthritis)	Inhibits purine , thymidylate & methionine synthesis → inh. <i>dihydrofolate</i> <i>reductase</i>	Oral, IV	8–15 hrs (for↑ dose)	Kidney CI: pregnancy, breast-feeding