Review

Expert Opinion

Drug delivery technologies for autoimmune disease

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Importance of the field: Targeting autoimmune disease poses two main challenges. The first is to identify unique targets to suppress directly or indirectly autoreactive cells exclusively. The second is to penetrate target tissues to deliver specifically drugs to desired cells that can achieve a therapeutic outcome.

Areas covered in this review: Herein, the range of drug delivery methods available and under development and how they can be useful to treat autoimmune diseases are discussed. Polymer delivery methods, as well as biological methods that include fusion proteins, targeted antibodies, recombinant viruses and cell products are compared.

What the reader will gain: Readers will gain insight into the progression of clinical trials for different technologies and drug delivery methods useful for targeting and modulating the function of autoreactive immune cells.

Take home message: Several tissue-specific polymer-based and biologic drug delivery systems are now in Phase II/III clinical trials. Although these trials are focused mainly on cancer treatment, lessons from these trials can guide the use of the same agents for autoimmunity therapeutics.

Keywords: autoimmune, drug delivery, immunomodulation

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1. Introduction

Autoimmune diseases affect a wide range of tissues and organs and have been classically difficult to manage even when systemic pharmacologic immunosuppression is used. Remission is not common and if achieved it is not stable long term. The treatment of autoimmune disease is complicated by a lack of concrete knowledge about the identity of the initiating trigger and putative autoantigen(s) in many cases. Furthermore, our understanding of immunoregulatory and immunosuppressive cell networks *in vivo* is only now beginning to be unraveled. These are important considerations in the development of drugs tasked with modulating the immune system. Even if the initiating stimulus (i.e., autoantigen for a particular autoimmune disease) is identified, it may be relevant only before clinical onset of the disease. In other words, at the time of clinical diagnosis, the range of autoantigens would be the result of antigen spreading consequent to the progressive destruction of target tissues, and therefore the autoantigen(s) identified and associated with clinical disease may be irrelevant from a therapeutic perspective. Although intervention of the developing autoimmune disease at the time of clinical diagnosis is the highly sought goal of autoimmune therapeutics, intervention before clinical manifestation, by targeting subclinical or preclinical pro-inflammatory processes, would be ideal. This, however, requires the identification of biomarkers that would, without any degree of error, identify everyone who will develop the disease beyond any reasonable doubt. Such markers are unavailable at present, or are predictive in only a minority of individuals. Nonetheless, even at clinical onset, immunotherapy can be useful. For example, targeting cell networks and molecular pathways specific and unique to a given autoimmune disease, or modulating intercellular

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Article highlights.

- A comprehensive overview of current and future drug delivery systems for autoimmune diseases.
- Introduce the concept of targeting common inflammatory pathways to restore systemic immune balance.
- Strategies for site specific drug targeting and their benefits are reviewed.
- A perspective on the timeline of penetrance of drug delivery systems into the clinical setting.

This box summarizes key points contained in the article.

signaling cascades, can provide control and attenuation of the underlying disease mechanisms. In type 1 diabetes mellitus, islet-specific activated T cells could be targeted for elimination by exogenously administered agents, or such agents could in parallel, or independently, activate regulatory suppressive immune cells, which can attenuate or even halt the activity of autoreactive T cells. Drugs to achieve these outcomes need not be unique to a specific disease, and can be combined to target multiple cell networks and pathways concurrently.

A major concern in the development of autoimmune disease therapeutics is the potential for a drug to induce unwanted and unexpected systemic immunosuppression in addition to collateral toxicities. With regard to this concern, drug delivery techniques must carefully determine the proper balance of drug delivery to a site of action versus systemic exposure. One such example is the use of glucocorticoid steroids as anti-inflammatory agents. Despite their benefit in autoimmune diseases, systemic delivery of steroids, even as it can result in the suppression of pathogenic autoimmune inflammation, can also inhibit the body's ability to fend off a bacterial infection in another part of the body. In addition to these effects, glucocorticoids have profound changes on metabolism that may worsen a patient's health if not monitored carefully (i.e., glucocorticoid effects as a metabolic diabetogen). This has led to the field's increased focus on developing drug systems that are targeted to the specific autoimmune-relevant pathogenic site. Direct injection of the drug to a specific site is the simplest solution, but assumes the number of sites is limited, accessible and known. Another approach has been the development of systemic drug delivery systems that can prevent the drug from interacting with nonspecific targets and maintain it in an inert state until the drug reaches its intended site. Concentrating a drug to the target site can facilitate decreasing the amount and frequency of drug administration (which can also improve patient compliance) while maintaining sufficient drug concentration at the site of action. This is particularly important when dealing with systemic autoimmune diseases such as lupus.

Herein, what the authors consider to be the most promising drug delivery methods potentially useful to direct bioactive molecules or genetic material to tissues and cells involved in autoimmune disease are discussed. Also, the authors list what in their opinion are the approaches that can be readily tested in Phase I/II trials in a period of the next 3 – 5 years.

2. Synthetic polymer-based drug delivery

Polymer-based drug delivery systems use a non-biological method of delivery and are already in clinical use for delivering many small molecules and bioactive peptides. The advantage of the technology is the ability to manipulate the chemical properties of a polymer to accommodate and release a drug under defined conditions. Although many different techniques are used to design polymer-based therapeutics, they fall into two main categories. First are polymers that, alone, are able to elicit a biological response, making them drugs in their own right. These compounds may be naturally occurring extracts from plants or other living organisms or they can be synthetically generated from chemical precursors. Glatiramer acetate, or COPAXONE® (Teva Neuroscience, Missouri, USA), is an example of a successful polymer drug used to treat relapsing multiple sclerosis, an autoimmune disease in which the body attacks and destroys the myelin sheath surrounding axons of the brain, leading to progressive neurodegeneration [1]. Glatiramer acetate has been tested in multiple clinical trials both before and after its FDA approval in 1996, showing reduced multiple sclerosis progression and relapse (**Table 1**) [2-7]. Glatiramer acetate is comprised of four amino acids that are found in myelin basic protein, and it is believed that it may be recognized and targeted by autoreactive immune cells which, when bound by it, enter a state of immune quiescence. Such an approach could be expanded to synthesize polymers of repeating amino acid sequences derived from autoantigens relevant to other autoimmune disease. The limitation is in identifying all of the disease-specific autoantigens and how their expression profiles change (as a result of antigen spreading and other factors) during the course of the autoimmune disease.

Polymers that fall into the second group are immunologically inert and act as drug carriers. These polymers may form very simple or complex multi-chain structures that incorporate other biologically active compounds into them or on their surface. Yet other polymers are attached covalently or non-covalently by means of linkers to bioactive agents, including DNA oligonucleotides, proteins and antibodies [8-10]. The polymer conjugates are typically comprised of three portions: a hydrophilic polymer, a linker molecule and the biologically active compound. The properties of the polymer can be modified to affect the rate of drug clearance, solubility, immune responsiveness and *in vivo* stability [11,12], facilitating decreased drug dosage and frequency of administration. Polyethylene glycol (PEG) is a non-toxic polymer that is a constituent of many clinical and food products [13] to the extent that the process of conjugation linking PEG to its target has been named PEGylation [14]. One of the early PEG conjugations to a biomolecule was to the enzyme adenosine deaminase. The resulting formulation,

Table 1. A list of autoimmune drugs in clinical trials or FDA-approved for use in the clinic*.

Drug	Market name	Type	Phase	Date	Autoimmune disease
Abatacept	Orencia®	Fusion protein	Approved	2005	Rheumatoid arthritis
APL GAD65	Diamyd®‡	Peptide	Phase II	2005	Type 1 diabetes
APL HSP60	DiaPep277®§	Peptide	Phase II	2007	Type 1 diabetes
CD20 Antibody	Rituximab®	Antibody	Approved	2006	Rheumatoid arthritis
			Phase II	2009	Systemic lupus erythematosus
Etanercept	Enbrel®	Fusion protein	Approved	1998	Rheumatoid arthritis
Glatiramer acetate	COPAXONE®	Polymer	Approved	1996	Multiple sclerosis
PEG IFN-β_{1a}	BIIB017®	Polymer	Phase III	2009	Multiple sclerosis
PEG TNF-α	Cimzia®¶	Polymer	Approved	2008	Crohn's disease
			Approved	2009	Multiple sclerosis

*The drug type is listed with reference to the different drug delivery systems discussed in this article.
‡Diamyd Medical, Stockholm, Sweden.
§Andromeda Biotech Ltd., Yavne, Israel.
¶UCB, Brussels, Belgium.

PEG-ADA (Adagen®, Sigma-tau Pharmaceuticals, Inc., Maryland, USA), was approved by the FDA to treat adenosine deaminase deficiency (ADA) severe combined immune disease (SCID) in 1990 [8,15]. For autoimmune disease treatment, there are at present two PEG conjugates that have undergone clinical trials for autoimmune diseases. The IFN-β_{1a} drug Avonex®(Biogen Idec, Massachusetts, USA) was approved by the FDA for the treatment of multiple sclerosis in 1996 [16]. Phase III clinical trials with a PEGylated form of the drug designated BIIB017 (Biogen Idec, Massachusetts, USA) are now underway [17]. Another bioconjugate of PEG is an anti-tumor necrosis factor-α (TNF-α) antibody, initially tested for the treatment of rheumatioid arthritis and later extended to the treatment of Crohn's disease [18,19]. The conjugate, termed Certolizumab pegol, was FDA-approved for the treatment of Crohn's disease in 2008 and rheumatioid arthritis in 2009 [20]. Despite the widespread effectiveness of PEG drug compounds, recent studies have found their use can lead to the development of anti-PEG antibodies, which may inhibit drug effectiveness [21,22]. Efforts need to be made to understand whether this is a common problem not yet realized or whether it is specific only to certain PEG-compound formulations.

Instead of bioconjuagates, polymers can be used to formulate drugs into polymeric micelles (**Figure 1**). These structures are self-assembling polymers that are usually comprised of a hydrophilic head and hydrophobic tail. They form a spherical structure where the branched hydrophobic tails comprise the core and the hydrophilic heads remain on the outside to interact with the surrounding fluid. A drug can be trapped inside the hydrophobic core of the micelle during assembly or it can be covalently bound to the hydrophobic tail. Unbound drug may be released into the target tissues from the polymeric micelle as the polymer breaks down. Alternatively, covalently bound drugs can have their release rate modified by pH or the amount of energy necessary to break the bond between the drug and the polymer tail. These drugs may or may not be functional in the bound state based on the polymer design. More complex strategies of polymer formulation can be used to regulate the drug release rate from the polymeric micelle core, such as manufacturing drug(s) in multiple layers, multiple polymers and/or different three-dimensional shapes and volumes. Similarly, PEG can be used to coat drug-filled liposomes to increase their stabililty [23]. Polymeric micelle size can affect drug release and also restrict movement of the micelle in different tissues. For example, the ability of macrophages to phagocytose polymeric micelles has been shown to be size-dependent [24]. PEG-poly-DL-lactic/glycolic acid-poly-DL-lactic acid (PEG-PLGA-PLA) micelles have been formulated with synthetic glucocorticoid steroids for the targeted treatment of arthritis in rodent models [25]. The addition of PEG to these polymer micelles reduces their uptake by mononuclear phagocytes while allowing synovial cell uptake to occur [25]. In this fashion, steroids can be administered systemically, yet act in a site-restricted region of joint inflammation. Also, numerous drug delivery systems utilizing polymeric micelles are now in clinical trials for a range of malignancies [8,10]; however, this technique has not reached clinical trials for the treatment of autoimmune diseases.

Biopolymers can also be formulated with nucleic acids, ranging in size from short DNA sequences to entire DNA plasmid vectors [26,27]. One form of biopolymers termed microspheres offers a method to deliver bioactive drugs inside or on the surface of very small, well-defined spherical structures. The authors have shown that a microsphere formulation comprised of PEG, polyvinyl pyrrolidone (PVP) and poly-L-lysine-hydrobromide and short antisense oligonucleotides targeting the primary transcripts of the CD40, CD80 and CD86 costimulatory genes effectively prevents and reverses type 1 diabetes in the NOD mouse model [26]. Although the mechanism is not yet fully clear, the authors propose that the microspheres are taken up following

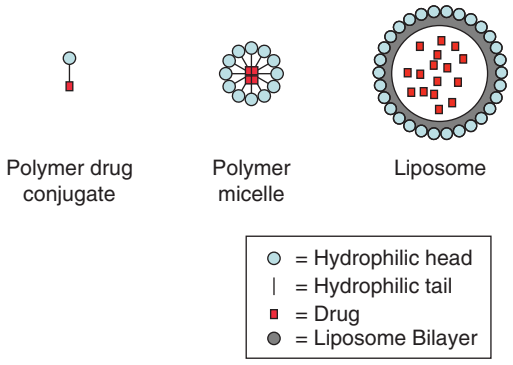

Figure 1. Polymer structures are shown (from simple to complex formations). A drug can be directly conjugated to a polymer tail and used as a therapeutic. Polymer micelles are the formation of multiple polymer units that form a sphere with the hydrophilic heads located on the outside and the hydrophobic tails protected within. Drugs can be trapped in the core during assembly or conjugated to the polymer's tail. Hydrophilic polymers can also be attached to lipid bilayers of liposome drug carriers to stabilize the structure and make it water soluble.

subcutaneous injection (close to an abdominal anatomic site drained in part by the pancreatic lymph nodes) by migratory dendritic cells, which then accumulate inside the pancreatic lymph nodes. These dendritic cells show downregulated CD40, CD86 and CD80 costimulation (as a consequence of the effects of the antisense), effectively being turned into tolerogenic dendritic cells. In the pancreatic lymph nodes, these dendritic cells could promote increased regulatory T-cell prevalence, which, in an antigen-specific manner, could suppress the activation and overall function of autoreactive T cells.

3. Biologicals: proteins and vectors

Methods have been developed that harness and manipulate normal biological and cell functions in order to facilitate effective drug delivery. These methods include specific cell targeting, DNA integration into a cell genome as well as the eradication of specific cell types by apoptotic mechanisms. In this section, how some of these systems have been harnessed for the development of drug delivery and how they have been – and could be –developed further to treat autoimmunity are covered.

3.1 Antibodies

One of the hallmarks of the adaptive immune response is the B-cell production of antibodies. Under normal conditions these antibodies are generated towards specific antigens found on foreign invading bodies entering the host organism. Once the antibody has bound to its target antigen, the antigen itself or the cell that expresses the antigen is marked for destruction. Antibodies are also generated to self-antigens in numerous autoimmune diseases. The ability to home to specific targets and remove them from a disease-relevant site, or systemically, makes antibodies attractive for the production of cell-specific therapeutics. Antibodies are often first validated in animal models and later humanized by modifying the gene sequence to produce a 'humanized' chimeric antibody. Antibody drugs can act in two ways: effective neutralization of an immune-active compound or removal of specific cells. Infliximab is an example of an antibody that is able to do both. Infliximab targets the pro-inflammatory cytokine TNF-α, and by neutralizing and blocking its bioavailability to the type 1 TNF signaling receptor, infliximab is used to treat several autoimmune diseases [28], including Crohn's disease [29], psoriasis [30], rheumatoid arthritis [31] and ulcerative colitis [32]. Infliximab's mechanisms of action differ between rheumatoid arthritis and Crohn's disease. Infliximab clears TNF-α, which is sufficient to treat rheumatoid arthritis, whereas in Crohn's disease it also results in the apoptosis of TNF-α-producing T cells [33]. A fully human antibody targeting TNF-α was later developed, named adalimumab, or Humira®(Abbott Laboratories, Illinois, USA) [34], and has been FDA-approved to treat ankylosing spondylitis, Crohn's disease, psoriasis, psoriatic arthritis and rheumatoid arthritis [35].

Other antibody therapies rely exclusively on removing activated immune cells by binding to specific cell surface molecules. Cytotoxic T cells are responsible for killing virally infected cells and are a good therapeutic target because they are also the cells predominantly responsible for the destruction of cells in autoimmunity. Antibodies against T-cell surface CD3 [36,37] and CD4 [38] have been developed and were able to reverse new onset diabetes in NOD mice. Antibodies targeted to $CD4^+$ T cells produced an immunosuppressive effect without establishing regulatory T-cell populations [38]. Complications may arise from the inability to establish regulatory cell populations, with further danger to existing $CD4^+$ regulatory cells. This may be the reason humanized CD3 antibodies have been extended into clinical trials [39], whereas CD4 antibodies have not yet progressed to any significant degree in human autoimmune disease.

B cells are another group of immune cells able to be targeted by antibody therapeutics. B cells produce antibodies as part of the adaptive immune response, which can further aggravate and expand autoimmune pathology by means of complement and Fc-mediated mechanisms of target cell impairment and destruction. Rituximab is a chimeric antibody that binds to CD20, a protein found on the surface of B cells. Rituximab was initially designed to clear B cells in non-Hodgkin's lymphoma and was approved by the FDA in 1997 [40,41]. For autoimmune therapies, rituximab has been approved to treat rheumatoid arthritis [42], and Phase II trials are underway and are at various stages of completion for the treatment of systemic lupus erythematosus [43,44] and multiple sclerosis [45]. Rituximab administration into new onset type 1 diabetic patients resulted in some evidence of

functional benefit to residual beta cell mass, after preclinical studies in NOD mice suggested its utility [46,47]. The mechanism of rituximab action in remission of autoimmunity, however, remains unclear.

3.2 Fusion proteins

Fusion proteins are a merger of functional protein domains from two or more different genes to produce a hybrid protein with a bispecific, or multispecific function. The simplest fusion proteins consist of a ligand binding domain of a receptor fused to the Fc fragment of a specific human isotype, often IgG. Also, protein domains that can be actively or passively transported across cell membranes can be linked to biologically active compounds to mediate their entry into the cell.

Individuals with autoimmune disease harbor T cells that recognize a few unique tissue-specific or several generalized self-antigens. T cells recognize such self-antigens presented by HLA class I and class II on antigen presenting cells such as macrophages, dendritic cells or B cells through the T-cell receptor (TCR). If the TCR-specific autoantigen amino acid sequence can be deduced, recombinant altered peptide ligands (APLs) can be generated. APLs are TCR-specific autoantigen-derived peptides that show amino acid sequence variations. T cells, through the TCR, bind APLs without becoming fully activated while preventing access of the endogenous autoantigens to the TCR binding site [48]. This competitive inhibition has been mechanistically linked to the reduction or suppression of autoreactive T-cell function in models of type 1 diabetes using APLs of putative disease-specific autoantigens such as proinsulin [48-51], GAD65 [52] and HSP60 [53,54]. Nevertheless, no APLs have progressed beyond Phase III clinical trials for the treatment of autoimmune disease [55].

Etanercept, or Enbrel®(Amgen, Inc., California, USA), consists of an IgG1 sequence linked to the TNF receptor type 2 and was originally approved by the FDA in 1998 for rheumatoid arthritis [56]. Etanercept functionally binds TNF-α and acts as a soluble ligand 'sink' (or decoy) of the cytokine [57]. Effectively, the rate of contact between endogenous TNF-α and the signaling type 1 receptor is dramatically reduced, if not completely mitigated. In rheumatoid arthritis patients who are unresponsive to etanercept, an alternative fusion protein (Abatacept, Orencia®(Bristol-Myers Squibb, New York, USA)) has been approved [58]. Abatacept is a fusion protein between an Ig and the regulatory cytotoxic T-lymphocyte antigen 4 (CTLA4) protein domain [59]. CTLA4 interferes with the ability of antigen-presenting cells (APCs) to activate T cells through the costimulatory B7 surface protein pathway [59]. Clinical trials have been extended to determine CTLA4-Ig efficacy for active ulcerative colitis [60] and new onset type 1 diabetes mellitus [61].

Ensuring that a fusion protein accumulates specifically on cells expressing a surface protein of interest can also be accomplished without the use of an antibody. Matrix metalloproteinases (MMPs) are produced as pro-enzymes that are not activated until they are cleaved. Linking a MMP cleavage site sequence with that of TGF-β latent-associated peptide (LAP) creates an encasement for a cytokine of interest that is not activated until the MMP cleavage site is acted on at a site of inflammation by proteinases produced as a consequence of the inflammation [62]. This greatly increases the half-life of the cytokine while restricting its activation to sites of inflammation. Although this technique is promising in its ability to achieve local immunmodulation, it has not been adapted to autoimmune diseases. Data collected from continuing clinical trials for cancer treatment will hopefully pave the way for autoimmune disease therapeutics [63].

Whereas protein and peptide delivery has been validated, oligonucleotides with therapeutic capacity face extra hurdles to be and to remain active inside a cell. Major impediments to cell entry are not only penetration of the cell membrane, but also accumulation inside the desired location of the cell. The endosomal pathway is particularly tricky because endosomes possess the machinery to destabilize and destroy the oligonucleotides unless a method of endosomal escape out of the late endosome/lysomome compartments is imposed. To address the first hurdle, penetration of the cell membrane, one method relies on protein transduction domains (PTDs), or cell-penetrating peptides (CPPs), short peptides that can cross the plasma membrane apparently by means of passive mechanisms. Animal studies demonstrated the ability of double-stranded NF-κB oligonucleotide decoys linked to CPP domains to treat several murine autoimmune disease models, including type 1 diabetes mellitus, inflammatory bowel disease, collagen-induced arthritis and muscular dystrophy [64]. Clinical trials using a CCP fused with cyclosporine A, called PsorBan®(Progen Pharmaceuticals, Queensland, Australia), for the treatment of psoriasis was an early example of CCP-based fusion proteins focused on autoimmune disease, but failed to make it to market [65,66]. CCP fusion proteins can also be paired with MMP cleavage sites to induce limited specificity, as with LAP [67]. Other methods include the development of cell-specific CCPs linked to pro-apoptosis factors such as for the targeted destruction of synovial fibroblasts in rheumatoid arthritis [68] or HIV-infected cells [69]. It is also theoretically possible to design a cell surface-specific-CCP-therapeutic protein/oligonucleotide hybrid that will bind to a cell-specific protein, cleaved by a proteinase to release a CCP-fusion protein that will then enter the cell through the peptide transduction domain to deliver the therapeutically relevant moiety inside the cell.

3.3 Naked DNA and viruses

Gene therapy offers the possibility of conferring new or augmented capacities to cells either by overexpression of an exogenous gene inside a cell population of interest or by genetic manipulation of the endogenous expression of a therapeutically relevant gene. Alternatively, gene therapy can also attenuate or silence expression of one or more genes in a cell

population. In its simplest form, DNA plasmids are administered *in vivo* to an anatomic site where they are taken up by cells. The expression of the transgene is transient, but short-term gene expression may be all that is required to elicit a biologic response favorable to disease correction. Thus far, there are no confirmed reports of successful DNA plasmid-based modulation of human autoimmunity, although many mouse studies have had successful outcomes for several autoimmune conditions [70]. Viral vectors possess several attributes that make them more useful for gene delivery. They have the potential for stable long-term gene expression and may provide a method of treatment for chronic autoimmune diseases that have been classically difficult to control or to respond to therapeutics designed to boost regulatory immune cells. Unlike plasmid DNA, the key feature of a viral vector is its ability to transport a packet of genomic material into the cell nucleus for expression. Viruses commonly used for gene therapy include adenovirus, adeno-associated virus, herpes simplex virus and retrovirus [70]. Each virus has its own benefits and shortcomings and targets different cell types depending on the particular envelope protein complement. A significant hurdle for viral vectors is the immune response that they can elicit, not only precluding repeated administration, but also promoting the complete eradication of transduced cells.

Adenovirus is considered to be the 'workhorse' of gene therapy in general. It is a robust virus capable of a high rate of infection in both dividing and non-dividing cells and can be generated in high titers. The generation of non-replication competent virus has partially mitigated its immunogenicity [71,72]. Herpes simplex virus and adeno-associated virus (AAV) share some characteristics with adenovirus, but can offer a longer duration of gene expression and a larger genomic insert, even as their mode of infectivity differs. AAV, in some instances, can integrate into the genome [73,74]. Gene therapy utilizing the adenovirus has been used successfully to treat animal models of autoimmune disease such as uveitis [75], type 1 diabetes [76,77] and rheumatoid arthritis [78]. Similarly, AAV has been used successfully to treat type 1 diabetes [79] and rheumatoid arthritis [80]. Interestingly, a recent study has demonstrated the benefit of AAV that delivers short hairpin inhibitory RNAs (shRNA) for the treatment of rheumatoid arthritis [81], showing the proof-of-concept that viral vectors can be used successfully to suppress gene expression in a stable manner without resorting to repeated administration of naked siRNA or antisense DNA. Direct administration of siRNA and antisense oligonucleotides in general is feasible, but the rate of cell uptake and their potential for nonspecific activity (general gene knockdown in cells and activation of Toll-like receptors) require careful consideration [82]. Despite the beneficial use of adenovirus and AAV-encoded transgene delivery to autoimmune animals, these viruses have not been used in clinical trials for the treatment of autoimmune diseases in humans. This may be largely owing to concerns of immunogenicity of these viruses as well as the nonspecific effect on hepatic function, sadly revealed following the death of one of the volunteers of an adenovirus-based clinical trial [83].

Retroviruses make up the other group of commonly used gene therapy viruses and are characterized by low immunogenicity, long-term gene expression and genomic integration of their transgene cargo. Retroviruses have been used to modify several cell types *ex vivo* in animal models for the treatment of arthritis [84,85], diabetes [86] and multiple sclerosis [87]. Early arthritis animal models focused on delivery and potentiation of the anti-inflammatory cytokine IL-4 directly [84] or indirectly by blocking IL-12 production [85]. The technology has advanced to the clinic, with a retrovirus targeting synovial fibroblasts to produce the interleukin-1 receptor antagonist protein, which blocks the pro-inflammatory cytokine IL-1 [88,89]. Interestingly, this is the same target that the protein drug Anakinra, Kineret® (Biovitrum, Stockholm, Sweden), targets and has been approved by the FDA for the treatment of arthritis [90]. Most retroviruses are only able to infect dividing cells, but the lentivirus subgroup is more complex in structure and has the ability to infect both dividing and quiescent cells. Lentiviruses unfortunately can have low rates of infection and do pose some safety concerns [70]. They carry a risk for insertional mutagenesis, which in clinical use has been associated with activation of oncogenes leading to leukemia following the reconstitution of lentiviral-transduced hematopoietic cells of X-linked SCID patients [70,91].

3.4 Transplantation of *ex vivo*-engineered autologous cells

Transplantation of cells engineered to show modified gene expression or even normal 'wild-type' cells can be used to treat several human diseases. This technique has two advantages over standard drug delivery technologies. For autoimmune disease, transplantation of cells in a number large enough to overcome the effects of endogenous homonymous cells, either in their natural state or in an engineered state, may help a tissue that is chronically dysfunctional or even destroyed; for example, transplantation of insulin-producing cells in type 1 diabetes to replenish and restore a minimal beta cell mass able to maintain normoglycemia. In type 1 diabetes, as much as 80% of beta cell mass can be lost during the asymptomatic period of the disease [92], which can make a 'reversal' of impaired glycemic control to pre-disease levels difficult. Unless some means of restoring a minimal beta cell mass able to maintain normoglycemis is available, controlling beta cell-directed autoimmunity alone will not be enough to restore the patient's glucose regulation. The second advantage cell transplantation offers is the ability to modify the cells *ex vivo* without complications that may arise from engineering cells and tissue by means of delivery of drugs *in vivo*. Systemic administration of viruses or drugs may result in collateral effects to unintended targets or unexpected drug breakdown products being generated in the liver. From a logistical perspective and a cost–benefit analysis, an 'off-the-shelf'

medicament is easier to manufacture and to establish a standard quality control monitoring program in contrast to harvesting cells from the patient, modifying the cells and reintroducing them into the patient. However, if one envisages cell therapy, a large, universal and self-renewing supply of stem or progenitor cells with a capacity to differentiate in large numbers to a population desired by the therapist could significantly reduce the cost of such therapeutics.

As concerns immunotherapy, dendritic cells make ideal agents for autoimmunity immunomodulation because they are crucial regulators of the immune system in maintaining peripheral tolerance to self-antigens [93]. One proven way to stabilize a state of tolerogenic activity in dendritic cells is to impair their ability to provide costimulation to T cells. When dendritic cells acquire antigens under inflammatory conditions, they undergo a series of maturation steps and migrate to the local draining lymph node where they interact with and activate T cells by presenting the antigens to the TCR and providing activation through costimulation [94,95]. Downregulation of costimulation signals prevents T-cell activation in several models [96-98]. The authors have developed a method to stabilize autologous dendritic cells in a state of low costimulation capacity, and therefore to generate a tolerogenic population by *ex vivo* treatment of autologous dendritic cells with a mixture of antisense oligonucleotides targeting the primary transcripts of CD40, CD80 and CD86. These dendritic cells, administered into NOD mice before and immediately after the onset of clinical type 1 diabetes, prevent the disease and re-establish euglycemia in new onset disease recipients [99,100]. This approach is now in Phase I clinical trials in human diabetics [93].

4. Conclusion

The traditional oral, systemic and parenteral routes of drug administration have now been supplanted by new delivery routes and vehicles that consist of polymers, biological and non-biological vectors. Polymer-based drugs allow for a greater control of drug delivery and diffusion rates in the body. This greater control may allow for lower therapeutic doses of conventional drugs and greater patient compliance with reduced dosage per day owing to increased drug half-life. Antibody and fusion protein-based drugs offer new ways in which to target selectively biologically active agents to desired locations. The treatment of autoimmune disease at present necessitates a balance between reducing pathogenic inflammation and maintaining the body's ability to respond to foreign antigens. This balance is exceedingly hard to maintain using conventional immunosuppressive drugs administered systemically. New targeting techniques could allow for systemic drug delivery while having the drug localize or become active only at the desired target site of action. Through a combination of these approaches, therapeutic intervention can be more aggressive while reducing side effects on unintended targets. Likewise, the development of immune cell transplantation grants us a new method to restore directly the balance between pro- and anti-inflammatory elements of the immune system. The combination of these technologies provides us with the opportunity to go beyond the symptomatic treatment of autoimmune diseases and seek a true cure.

5. Expert opinion

The drug delivery methods useful for autoimmune disease fall at present into three different categories, with potential for overlap: non-biological synthetic polymers, targeting antibodies/fusion proteins and biologics (DNA, cells and viruses). The current developmental status of each of these different techniques ranges from the stage of basic research to fully approved FDA drugs. At present, synthetic polymers are at the forefront of drug delivery systems for the treatment of autoimmune disease, with FDA-approved PEGylated drugs for the treatment of Crohn's disease, multiple sclerosis and rheumatoid arthritis. There are two likely paths of advancement that this technology may possibly follow in the clinic. First could be the approval of synthetic polymer micelle and microsphere delivery systems. These structures are capable of delivery of larger payloads of drugs and are now in different stages of clinical trials. Second could be the continued refinement of targeting systems allowing for more tissue-restricted drug delivery. Improvements in this area may draw heavily from current technology used in antibody fusions, such as incorporating antibody into the shell of micelles and microspheres for targeted delivery, effectively merging the two technologies. Through these advancements and the continued refinement of polymer chemistry, this area of therapeutics will probably dominate technology development for the treatment of autoimmune disease over the course of the next 5 – 10 years.

Cell therapy implies personalized medicine and, unlike polymers which offer the potential of 'off-the-shelf' products, it faces regulatory as well as expanded biological and physiologic impediments. Nonetheless, hematopoietic stem cells, T cells and dendritic cells are at the forefront of biological therapy in cancer therapeutics, and as a significant number of regulatory issues are already being addressed, it is the authors' opinion that some of these cell populations are ready to be tested in autoimmune conditions. They believe, based on experience and continuing Phase I clinical trial for type 1 diabetes, that dendritic cells will lead in this area because they have the ability to affect multiple aspects of the immune system, including cytotoxic T-cell activation, the balance of pro- and anti-inflammatory T-helper cells, the establishment of anti-inflammatory T regulatory populations, and regulation of cytokine production. At present, a non-regulatory impediment for generalized implementation of personalized cell therapies is cost. The harvesting of progenitor cells from

patients, the engineering *ex vivo* under GMP/GLP conditions and the monitoring could be cost-prohibitive for many centers that do not possess the expertise and facilities for conducting cell therapy. Although universal stem cells that overcome allogeneic rejection could provide an 'off-the-shelf' source of progenitor populations, clinical translation of such an approach is far off at this stage, especially because there are no preclinical studies demonstrating proof-of-concept, even though the technology exists to realize this approach. These two factors could possibly prevent the widespread application of this approach in the near future.

In spite of its feasibility, gene therapy using viral delivery technology still carries with it some legitimate safety concerns, especially for methods that introduce the vector systemically. Early expectations in the field of gene therapy have been largely unfulfilled despite an expansive body of literature on successfully treating autoimmunity in mice and rats. The possibility that an integrating virus will lead to a mutational insertion will need to be compared with the dangers posed by a lifelong autoimmune condition that could be maintained by conventional immunosuppression. Until safety can be demonstrated in a breakthrough clinical study, this technology will largely remain in the basic research setting.

The authors believe that it is now time to move past humanized antibodies and chemical pharmacologics. Phase I safety testing of cell therapeutics, as well as the expansion of polymer-based therapeutics, need to be started for the treatment of autoimmune diseases, especially given the overwhelming data from cancer trials demonstrating safety of the delivery vehicles.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

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