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Delivery of host cell-directed therapeutics for intracellular pathogen clearance

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Abstract

Intracellular pathogens present a major health risk because of their innate ability to evade clearance. Their location within host cells and ability to react to the host environment by mutation or transcriptional changes often enables survival mechanisms to resist standard therapies. Host-directed drugs do not target the pathogen, minimizing the potential development of drug resistance; however, they can be difficult to deliver efficiently to intracellular sites. Vehicle delivery of host-mediated response drugs not only improves drug distribution and toxicity profiles, but can reduce the total amount of drug necessary to clear infection. In this article, we will review some host-directed drugs and current drug delivery techniques that can be used to efficiently clear intracellular infections.

Keywords

Ac-DEX; autophagy; dose sparing; drug delivery; druggable target; host-mediated response; inflammatory cytokines; innate immunity; intracellular pathogens; macrophages; multiple-resistant strains; Toll-like receptors

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Background

Intracellular pathogens contribute greatly to infection and death worldwide. For example, nearly nine million people develop tuberculosis per year (13% of which are co-infected with HIV), caused by the facultative intracellular bacterium *Mycobacterium tuberculosis*, resulting in approximately 1.4 million deaths (four deaths per minute) [1–4]. Twelve million people are infected with *Leishmania*, an obligate intra-cellular protozoa, with nearly 50,000 deaths per year [5]. Moreover, *Salmonella enterica* cause 1.3 billion infections annually [6] with about 200,000 deaths originating from sero-type Typhi [7]. These three pathogens alone result in approximately 2.3 million annual deaths, with additional significant morbidity and mortality as a result of infections due to numerous other intracellular pathogens such as *Plasmodium falciparum, Listeria monocytogenes, Neisseria meningitidis, Pseudomonas pseudomallei, Chlamydia trachomatis* and several viruses.

Intracellular pathogens are characterized as obligate when they multiply strictly within a host cell, and facultative when the organism can grow either intracellularly or extracellularly [8]. When pathogens are contained within a host cell, it adds an additional level of complexity to treatment options. A variety of host cells can be infected by microbes; however, a majority of infections exist in cells with high phagocytic activity such as macrophages and dendritic cells.

Current treatments for infection typically focus on drug action that directly affects pathogens (e.g., protein biosynthesis inhibition, membrane disruption or DNA/RNA synthesis inhibition) [9] and therefore have the highest and most direct efficacy when pathogens are growing in the extracellular environment. Once a pathogen resides intracellularly, there are several factors that can reduce drug efficacy. Limitations to drug transport into a host cell or the activity of a therapeutic once it reaches the intracellular space can result in lack of drug efficacy. Additionally, highly acidic and proteolytic environments found in the lysosome and phagolysosome have been shown to induce partial dormancy in these bacteria reducing the effect of antibiotics [10]. To overcome these delivery barriers, infections are usually treated with high doses of systemic (e.g., oral, intravenous) therapeutics. The need for frequent doses often leads to patient non-adherence to a prescribed drug regimen. In a study of Leishmaniasis, approximately 70% of patients failed to take the prescribed treatment appropriately or for the allotted time, which led to recurrent infections [11]. Noncompliance with drug regimens can lead to suboptimal drug concentrations, allowing mutant strains that are/become resistant to the drug to flourish [3,4,12].

Antibiotic resistance began shortly after the first-generation antibiotics were put into practice [13], but perhaps the most well-known resistant bacteria, Methicillin-resistant *Staphylococcus aureus* (MRSA), has become endemic in hospitals worldwide because of its ability to resist multiple families of antibiotics [14]. Intrinsic resistance mechanisms occur when bacterial genes mutate to result in reduced drug efficacy, whereas acquired resistance mechanisms occur when microbes gain DNA, often horizontally from the host or from other bacteria in the form of plasmids, and are integrated into the bacterial genome [15]. Acquired or intrinsic-resistant mechanisms illustrate that the bacterial genome is sensitive and dynamic. A common example of intrinsic resistance is bacterial efflux pumps. These pumps

actively remove antibiotics from intracellular environments and are associated with multi-drug resistance (MDR), which is one reason why MRSA treatment has become so difficult [16,17].

MDR may occur when druggable targets in the pathogen have been repeatedly exposed to attack from multiple families of antibiotic compounds. A druggable target is defined as a unique structure with which drugs are designed to interact with high affinity, such as the lipid membrane of Gram-positive bacteria for antibiotics in the type-A lantibiotic family [18]. The ability of pathogens to mutate druggable targets like cell receptors, enzymes and ion channels has limited the effect of drugs that directly act on microbes [19].

Enhancing host-mediated responses to pathogens as adjuncts to traditional anti-infectives may provide a more effective pathway for elimination of intracellular pathogens. With an indirect interaction on a pathogen, there is a decreased likelihood for the development of resistance. There are pathways present in hosts which, when activated, result in the production of protective inflammatory mediators such as cytokines and chemokines that enhance microbicidal mechanisms and elicit the recruitment of monocytes, neutrophils and natural killer cells to the site of infection to aid in the removal of pathogens [20]. By exploiting such host-mediated responses, inherent microbial drug resistance may become less of an issue when treating patients resulting in increased pathogen clearance.

Pattern recognition receptors

One method of enhancing host-mediated responses to pathogens is by stimulating host pattern recognition receptors (PRRs) thereby enhancing the immune response. There are a number of PRRs present in human cells, which are conserved from early vertebrates to mammals, allowing for advanced recognition of known pathogen patterns [21,22]. Stimulating the immune system through PRR activation allows for a safer and more reproducible way of clearing invading pathogens without the added danger of introducing a compound that can allow resistant strains to form.

Toll-like receptors

A prototypic class of PRRs is Toll-like receptors (TLRs). To date, there have been 12 different members of the TLR family identified in mammals, most of which have a different function in pathogen recognition and clearance [23,24]. TLRs are all type I integral membrane glycoproteins that have an extra-membranous domain containing leucine-rich repeats and an intracellular signaling domain, which is similar to the IL-1 receptor [25]. Antigen-presenting cells such as macrophages and dendritic cells express TLRs abundantly [26]. TLRs 1, 2, 4, 5 and 6 are expressed on the cell surface, whereas TLRs 3, 7, 8 and 9 are primarily located intracellularly on endosome and phagosome membranes. These TLRs each have different modes of recognition, but once activated, they stimulate a signaling cascade frequently involving partners such as myeloid differentiation factor 88 (MyD88) and ultimately activating NF-κB. These cascades trigger the production of chemokines and proinflammatory cytokines, which make them attractive targets to stimulate an enhanced host response [27].

Although molecules that stimulate TLR responses are typically used as vaccine adjuvants, their ability to upregulate immune responses gives them potential to be used in the clearance of intra-cellular pathogens. Table 1 represents some TLR agonists that have shown efficacy in the treatment of infections and as vaccine adjuvants. For example, US FDA-approved TLR stimulating compounds such as monophosphoryl lipid A (MPL), a TLR4 agonist that upregulates natural killer cells, B cells and monocyte cell surface markers, which have been shown to effectively eliminate *Escherichia coli* in infected mice [28]. MPL is currently used as an FDA-approved adjuvant for vaccine applications; however, some patients have observed intense pain, irritation and other side effects at the injection site [29,30]. Pam_3Cys–Ser–Lys-4 trihydrochloride (Pam_3Cys) is a synthetic lipopeptide, which acts as a TLR2 agonist. It has been shown to enhance the clearance of *S. aureus* [31] and *Lactobacillus crispatus* [32] and has efficacy as a malaria vaccine adjuvant [33]. The trademarked drug Ampligen is a mismatched double-stranded RNA that acts as a TLR3 agonist and has been used in many different viral treatments because of its consistent induction of human antiviral activity [34]. Other TLR agonists such as *Salmonella typhi* flagella (STF2) (TLR5) and RC-529 (TLR4) are used as adjuvants in a wide variety of vaccines, but have not been used clinically in patients with infections.

One mechanism that may account for bacterial clearance after TLR activation is autophagy. Autophagy, which can be stimulated by TLRs and other factors, allows cells to recycle cytoplasmic constituents and destroy intracellular pathogens. The autophagy process first forms a double membrane auto-phagosome that surrounds cellular components or pathogens and then fuses with the lysosome or late endosome for destruction [35]. It can be activated during cellular starvation or through the MyD88 pathway, which has been shown to be activated through TLRs and other PRRs [36,37].

Drugs such as curcumin, clofarabine, metadherin and others have been found to induce autophagy. AR-12 (formerly OSU-03012), a highly hydrophobic drug, has been found to upregulate autophagy in macrophages [38]. AR-12 was derived from Celecoxib, a cyclooxygenase-2 (COX-2) inhibitor, but lacks COX-2 inhibition and is reported to function via phosphoinositide-dependent kinase PDK 1inhibition. AR-12 has shown success *in vitro* with enhanced host clearance of *Salmonella enterica* serovar Typhimurium, *Francisella tularensis* (strains: Schu S4, LVS) and *Francisella novicida* (Figure 1) [38–40]. Reduced hepatic and splenic bacterial burdens and significantly prolonged survival were observed *in vivo* with AR-12 treatment of *S.* Typhimurium-infected mice (Figure 1). Additionally, it has been determined that AR-12 can eradicate intracellular *S.* Typhimurium through both autophagy-dependent and independent mechanisms [39]. However, AR-12 was unable to reach high enough concentrations when administered systemically because of its hydrophobicity and thus would need modulation for clinical application. Drug delivery technologies could be utilized to help overcome the *in vivo* limitations of using hydrophobic compounds like AR-12 or more broadly TLR agonists that have detrimental side effects when delivered systemically at high concentrations.

Drug delivery

A major drawback of host-directed drugs compared with pathogen-directed therapies is that they typically have enhanced toxicity toward host cells. To overcome this barrier, as well as many others, a drug delivery vehicle (e.g., micelles, liposomes, polymeric particles) can be employed. Cancer therapy has seen a vast increase in drug delivery research because improved delivery reduces unwanted side effects such as cardiotoxicity and peripheral neurotoxicity [41]. Besides the potential to reduce drug toxicity, drug hydrophobicity is a primary reason to encapsulate in a delivery vehicle. Some efficacious drugs available for topical delivery (e.g., imiquimod) [42] are unable to be delivered systemically at therapeutic concentrations because they precipitate out of an aqueous solution (they are too hydrophobic). Additionally, drug encapsulation into a vehicle has been repeatedly shown to significantly increase the area under the curve (AUC) for a compound [43]. The AUC is a standard method of measurement of the bioavailability of a drug based on a plot of blood concentrations sampled at frequent intervals. Drug encapsulation can lead to extended circulation times and protection from degradation and clearance. Protection from degradation is especially important considering that peptide-based anti-infectives are an emerging area of research [44]. Drug encapsulation can also alleviate the need for a cold chain. Cold chain storage is the requirement for temperature regulation of the drug from the manufacturing plant to the patient's location. This process can increase dosing expenses and minimize drug access in more remote populations. A study by Kanthamneni *et al*. showed that protein encapsulation diminished the need for cold chain storage and enhanced cargo stability over a range of temperatures (−20–45°C), suggesting that storage of encapsulated drugs at constant temperature is not required for protein activity [45]. Thus, delivery of immunomodulatory (e.g., host-directed) compounds using vehicles can improve pharmacological properties such as solubility and efficacy, as well as reduce side effects and enhance storage conditions [46].

Efficient drug delivery can be accomplished through many vehicles including micelles, liposomes and polymeric particles. Micelles (Figure 2) are small, self-assembled, complexes composed of amphiphilic molecules that have a hydrophilic tail and a hydrophobic core, where hydrophobic drugs can be contained. Liposomes (Figure 2) are vesicles that consist of a phospholipid bilayer that can encapsulate both hydrophobic and hydrophilic drugs. Examples of FDA-approved anti-infective therapies for both of these formulations exist for the highly toxic and hydrophobic drug amphotericin B. Amphotec is an example of a FDA-approved colloidal micelle that is used for systemic fungal infections, and Ambiosome is a liposomal formulation of the drug that is used more broadly for parasitic infections. The primary mechanism of action of Amphotericin is to disrupt the membrane of the pathogen and is therefore not a host-directed therapeutic. A newer drug delivery platform is polymeric nano/microparticles (Figure 2). With this platform, a drug is typically encapsulated in a polymer matrix, wherein the drug is fairly uniformly dispersed throughout the polymer and released by diffusion and/or polymer degradation. Nanoparticles are defined by the NIH as being ≤100 nm in size, whereas micro-particles are on the micron scale (>100 nm to >1 μm). An advantage of microparticle systems is that a majority of cells in the body cannot engulf particles on the micron scale, whereas phagocytic cells prefer to phagocytose particles in the micron range (500 nm to 5 μm) [47,48]. Thus, passive targeting of phagocytic cells occurs

rapidly after application of particulate vehicles. Development of polymeric microparticles encapsulating chemotherapeutic agents for cancer therapy, such as BIND-014, has been underway for some time with more than a dozen systems having been approved for clinical trials [49,50]. The application of polymeric particles for anti-infectives is primarily preclinical [51]; however, if the surge in chemotherapeutic polymeric particles is any indication, anti-infective polymeric particles could see clinical trials in the near future.

Although the use of nano/microparticle systems displays great promise, there are limitations ranging from toxicity of the particles to residual byproducts from synthesis. For example, residual dichlorome-thane may cause central nervous damage [52]. With regard to byproducts, liposomal systems are widely regarded as safer; however, the lipid membrane is far more unstable than the structure of polymeric particles [53], lending to the need for tighter storage conditions for liposomes. Since polymeric particles can be produced in batch methods, they have issues associated with polydispersity and variability in drug loading. Scale-up spray drying techniques for polymeric particles [54,55] will help to mitigate some of these limitations with polymeric particles.

Liposomes are prone to aggregation, bilayer fusion and drug leakage when stored in an aqueous phase, significantly affecting their shelf life and pharmaceutical outlook [56]. They also have much shorter circulation times than polymeric particles because they bind to opsonins and are phagocytosed more readily [57]. To alleviate some of the concerns for liposomes, surface modifications to the liposome can be performed by adding a hydrophilic polymer such as glycol which increases the ‘stealth’ of the liposome; therefore, decreasing the ability to be opsonized and thereby allowing for longer circulation times [58,59]. Liposomes can also be freeze dried with a cryoprotectant, such as sucrose, and stored in a lyophilized state. This method can increase the shelf life and prevent potential aggregation, loss of stability and maintain the polydispersity [56]. Additional advantages and drawbacks for drug delivery systems can be seen in Table 2.

The most widely used biodegradable polymer for drug delivery is the FDA-approved poly lactic-*co*-glycolic acid (PLGA) [60] and its derivatives polylactic acid (PLA) and poly glycolic acid (PGA). BIND-014 is a PLA particle with the hydrophilic polymer, polyethylene glycol, which is a nondegrading, highly biocompatible, ubiquitously used polymer conjugated to the particle surface of the PLA matrix. Polyesters (e.g., PLGA, PLA, PGA, polycaprolactone), although commonly used generally have degradation times on the order of months and acidic byproducts that can severely lower the pH of the surrounding microenvironment [60]. In this review, we will highlight a wide range of uses for a newly developed polymer known as acetalated dextran (Ac-DEX) that is formed from dextran, an FDA-approved polysaccharide of glucose. Ac-DEX is formed when parent hydroxyl groups on the dextran react to create acetal groups [61]. Extending the polymerization reaction time results in more sustained polymer degradation because of the formation of cyclic acetals rather than acyclic acetals on the parent hydroxyl groups of dextran [62]. We will discuss below some host-mediated response drugs that have been successfully delivered with drug delivery therapies to modulate the immune system.

Expert commentary – delivery of host-mediated response drugs

Imidazoquinolines

Imiquimod and resiquimod are imidazoquinolines of similar structure, which activate TLRs 7/8. This leads to the production of inflammatory cytokines that aid in pathogen elimination [63]. Both compounds are currently used for the treatment of viral skin lesions [64], asthma [65], fungal infections [66], leishmaniasis [67] and many other infections. Resiquimod is a more toxic and potent activator, and thus is used in a lower dose [68]. Although topical efficacy has been shown, both are poorly water-soluble and previously have induced side effects after systemic treatment [69–71]. Using encapsulation methods for targeted and efficient delivery of imidazoquinolines would eliminate solubility issues and further reduce negative side effects of systemic administration.

Bachelder *et al*. showed that encapsulation of imiquimod in Ac-DEX particles significantly increased cytokine and chemokine production when cultured with RAW 264.7 and MH-S alveolar macrophages [72]. Levels of IL-1β, IL-12p70, MIP-1α and IL-6 were significantly increased in bone marrow-derived dendritic cells when cultured with Ac-DEX-encapsulated imiquimod compared with free drug and blank Ac-DEX particles. A dose sparing effect was observed with encapsulated imiquimod, such that a lower concentration of encapsulated drug resulted in increased cellular activation compared with soluble drug. This was the first time imiquimod was used experimentally after encapsulating in a polymeric material.

Using liposomes, Homhuan showed that dendritic cell maturation markers are upregulated on cells treated with encapsulated imiquimod, with respect to control groups [73]. An activated dendritic cell morphology and elevated levels of maturation molecules CD80, CD86 and MHC class II were increased significantly when imiquimod-encapsulated liposomes were introduced (with respect to blank liposomes).

Duong *et al*. created resiquimod-loaded electrosprayed Ac-DEX microparticles and applied them to RAW 264.7 macrophages *in vitro* and to BALB/c mice infected with *Leishmania donovani* [74]. Resiquimod encapsulated in Ac-DEX resulted in significantly higher amounts of nitric oxide than free drug and comparable levels of cell viability *in vitro*. TNF-α levels in cells treated with encapsulated particles were nearly double that of free resiquimod, and IL-6 was significantly elevated in cells treated with encapsulated particles compared with free resiquimod. BALB/c mice administered microparticles containing resiquimod showed a statistically significant decrease of *L. donovani* in the bone marrow compared with controls, which was clinically important because bone marrow is the site of persistent infection.

Poly I:C

Polyinoscinic:polycytidylic acid (poly I:C) is a synthetic immunostimulatory compound that imitates double-stranded RNA found in some viral genomes and binds to TLR3 to produce cytokines that elicit a proinflammatory immune response. It has been effective in numerous anticancer therapy studies and for the treatment against viruses [75], *F. tularensis* [76] and *S. aureus* [77]; however, synthetic nucleic acids are poorly taken up by host cells and are prone to rapid degradation [78]. Systemic delivery of poly I:C can also induce autoreactive

T cells and general symptoms of autoimmunity [79]. Since this drug cannot be applied systemically without severe side effects, vehicle-mediated delivery could be beneficial in the treatment of infections by intracellular pathogens.

In a study to enhance the immune stimulation with poly I:C, Smole *et al*. used a transferrin and poly-L-lysine (TfPLL) conjugated vehicle to deliver poly I:C[80]. Poly I:C encapsulated in TfPLL was taken up more efficiently than free poly I:C or poly I:C conjugated to just PLL due to an increase in targeting via receptor-mediated endocytosis. A more significant immune response occurred at lower concentrations of poly I:C encapsulated in TfPLL compared with free poly I:C or poly I:C complexed with PLL demonstrating a dose sparing effect.

Using Ac-DEX to encapsulate poly I:C, Peine *et al*. showed poly I:C can be a potent adjuvant, acting as a TLR3 agonist [81]. Ac-DEX particles were formed from a polymer with varying polymerization times (e.g., 5 min and 4 h) to determine the optimal encapsulation and release rates with this individual compound [61]. In an *in vitro* experiment, RAW 264.7 macrophages were cultured with poly I:C encapsulated in Ac-DEX (5 min and 4 h), poly I:C encapsulated in PLGA and soluble poly I:C. Inflammatory cytokines such as TNF-α, IFN-γ and IL-2 were observed in supernatants in significantly greater concentrations when the macrophages were cultured with Ac-DEX than with PLGA or soluble drug. For successful encapsulation of poly I:C into PLGA, a polycation was required, which can add unwanted levels of toxicity [82]. Ac-DEX required no such polycation for successful encapsulation of poly I:C, demonstrating that the use of Ac-DEX as a vehicle for poly I:C delivery is advantageous.

CpG

Cytosine bonded to guanine on a phosphodiester back bone (CpG) stimulates immune responses by mimicking unmethylated CpG motifs, which are very rare in mammals [83]. This unmethylated oligodeoxynucleotide activates TLR9 found in the endolysosome and activates innate immunity. CpG has been used as an immune response agonist for a number of conditions including cancer [84], allergies/asthma [85], leishmaniasis [86] and Herpes simplex virus-2 infection [87]. Like poly I:C, imiquimod and resiquimod, CpG has significant negative side effects when administered systemically [88,89]. It is also prone to degradation from nucleases and has poor cellular uptake; therefore, delivery with a vehicle would be advantageous [90].

Manoharan *et al*. used silicon oxide nanoparticles coated in polyethyleneimine (PEI) and showed a significant increase in IFN-α cytokine levels with the addition of CpG [91]. Fabrication of these nanoparticles with the addition of PEI increased the surface area up to 83-fold, drastically increasing CpG loading compared to nanoparticles without PEI. Free CpG-induced elevated production of IFN-α at high concentrations and smooth nanoparticles produced moderate amounts; however, flake shelled nanoparticles produced similar levels of IFN-α as free CpG at a reduced concentration.

Using Ac-DEX to encapsulate CpG, Peine *et al*. showed that it can be a successful adjuvant as a TLR9 agonist [81]. RAW 264.7 macrophages were cultured with CpG-encapsulated

Ac-DEX, blank Ac-DEX and free CpG. There was a significant increase in the ability of the macrophages to produce nitric oxide with CpG encapsulated by Ac-DEX compared with free CpG. Cytokine production was enhanced significantly in this study with CpG encapsulated by Ac-DEX compared with free CpG.

Overall, encapsulation of imiquimod, resiquimod, poly I:C and CpG in delivery vehicles shows an advantage over free drug for stimulating an immune response. Furthermore, with microparticle delivery, TLR agonists significantly increase phagocyte activation, cytokine production and immune cell recruitment compared with free drug. Encapsulation of these drugs overcomes poor solubility and allows for the use of lower concentrations (i.e., dose sparing) with higher immunostimulatory effects and fewer side effects. Moreover, toxicity is significantly reduced because of slow extracellular release and passive targeting of phagocytic cells. These studies suggest that the delivery of host-mediated response compounds within a delivery vehicle provides potent agonists that have the potential to work more effectively and safely than unencapsuated drug.

Expert commentary & five-year view

Current treatments for pathogenic infections commonly consist of high doses of antibiotics (often in combination) that induce pathogen clearance. The use and misuse of anti-infectives have resulted in a rise in drug-resistant and multidrug-resistant pathogens, several of which are intracellular pathogens [92]. These multiple-resistant strains pose an enormous health risk because of their ability to survive and proliferate in the presence of strong anti-infective compounds. Ant-infective ‘cocktails’ are administered to combat multiple-resistant strains, but they can result in high medical bills, extreme patient discomfort and damage to vital organs [93]. Use of drugs that interact with the host rather than the pathogen decreases the likelihood of antibiotic resistance and holds promise for future therapeutic approaches.

We have focused on the important role of TLRs in host-mediated anti-infectives, but there are other PRRs present in mammals that can be stimulated to elicit an immune response. Nucleotide-binding oligomerization domain-like receptors (NLRs) are one such receptor family. While TLRs are located in the membrane, NLRs are cytoplasmic proteins that recognize components of pathogens. When NLRs become activated, they oligomerize and activate signaling cascades much like TLRs, producing proinflammatory cytokines and inducing autophagy [94,95]. Muramyl dipeptide, a peptidoglycan fragment of both Gram-negative and Gram-positive bacteria, is an NLR agonist that has been encapsulated as a vaccine adjuvant in ovalbumin microspheres [96], chitosan nanoparticles [97] and PLGA microparticles [98]. NLRs have not been explored as extensively as TLRs in removal of pathogens; however, there is evidence to show that NLRs may contribute significantly to pathogen removal by inflammasome activation [99]. Thus, future studies should look into NLR targeting with regard to pathogen clearance.

In addition to TLRs and NLRs, other PRR agonists could be used in novel therapeutic approaches. Surfactant proteins A and D (SP-A and SP-D) are found in the lungs and act as PRRs that upregulate innate immune responses through enhancing the phagocytic abilities of alveolar macrophages and/or enhancing protective immune responses [100]. These

surfactant proteins are C-type lectins that contain a carbohydrate recognition domain that binds to mannose, fructose and other carbohydrates found only on microbes [101]. The C-type lectin mannose receptor is highly expressed on alveolar macrophages and thus drug delivery vehicles can target recognition by this receptor [100]. Furthermore, PRRs such as RIG-I or RIG-I-like receptors (RLRs), which recognize viral RNA should also be explored as targets for host modulation and removal of pathogens [102]. Combining the delivery of exogenous SP-A and SP-D proteins along with activating PRRs such as RLRs, NLRs or TLRs could prove to be an effective therapeutic approach for infections of the pulmonary tract and elsewhere.

Another potential growth area for host-mediated anti-infectives is combination therapy. Early work with synergistic combination therapies that include low doses of antibiotics with drugs that alter host responses have shown potent clearance of pathogens. Miranda-Verastrequi *et al*. have shown in a clinical trial that topical combination of a lower dose of a current treatment for leishmaniasis (i.e., pentavalent antimony that directly attacks the parasite and has seen immense numbers of resistant strains develop) with imiquimod was successful at fighting off infection [103]. As a follow-up study, Arevalo *et al*. showed that antimony-resistant strains of *Leishmania* could be effectively cleared by co-administration of low-dose imiquimod and antimony more efficiently than either on their own [104]. Using a synergistic combination of vehicle-delivered host response drugs and current treatments will maximize the treatment strength while lessening unwanted side effects and the development of drug resistance.

As previously mentioned, many anti-infective compounds have solubility, toxicity and delivery issues limiting their use. Indeed, many potentially groundbreaking drugs have not passed clinical trials because of toxicity or side effects that occur as a result of the method of application. Drugs unable to be delivered systemically because of side effects or solubility issues are prime candidates for encapsulation. As of 2006, there were over 38 nanotechnology-based products that had been approved for clinical applications with sales of $6.8 billion [105]. As of January 2013, there were 247 nanoproducts (100–400 nm) that are either in clinical trials or already approved [106]. Doxil (PEGylated liposomal doxorubicin), which in 1995 became one of the first nanoparticle systems to be approved by the FDA, has grown to $402 million in US sales in just 2011 alone [107]. These large sales are even more impressive considering the worldwide shortage due to Doxil manufacturing problems. In 2012, BCC Research reported that the global nanomarket was $50 billion in 2011 and is expected to reach $97 billion by 2016 [108]. The anti-infective nanoparticle market was $7.5 billion in 2009 and is expected to reach $15 billion by 2016 [108]. Although cancer drug-particle formulations have been in practice longer than anti-infective particles, we should expect that in the near future drugs that have been FDA approved will begin to reappear in clinical trials in their encapsulated form because of the various advantages of encapsulation.

With the addition of dose sparing, enhanced stability and a wider range of feasible drugs, the uses for encapsulated drugs are growing rapidly. Intracellular pathogens pose a serious threat and with a continual rise in strains resistant to current treatments, there is a need for additional innovation of safer medications/delivery mechanisms. Further research must be

geared toward increasing the efficacy of immune stimulation, enhancing the targeting and types of delivery vehicles and finding better host targets that induce activation of the immune system. With the ability to encapsulate and successfully deliver these drugs, a viable solution may soon become available for eradicating harmful intracellular pathogens.

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Key issues

- Intracellular pathogens have the ability to rapidly mutate due to exposure to suboptimal concentrations of pathogen-targeting drugs, resulting in antibiotic resistant strains.
- Host-directed drugs allow for a decreased chance of pathogen mutability.
- Encapsulated drugs are shielded, which minimizes side effects in the host.
- Dose sparing allows for a smaller dosage of drug with the same or greater effects.
- Targeted drug delivery allows for enhanced uptake into macrophages and/or dendritic cells.
- Encapsulation of drug increases the stability of the compound and lessens the need for a cold chain.
- Encapsulation allows drugs with solubility problems to be distributed systemically.
- Stimulation of Toll-like receptors allows for an exceptional immune response to combat infections.
- Combination therapies allow for smaller amounts of drug and increased pathogen clearance.

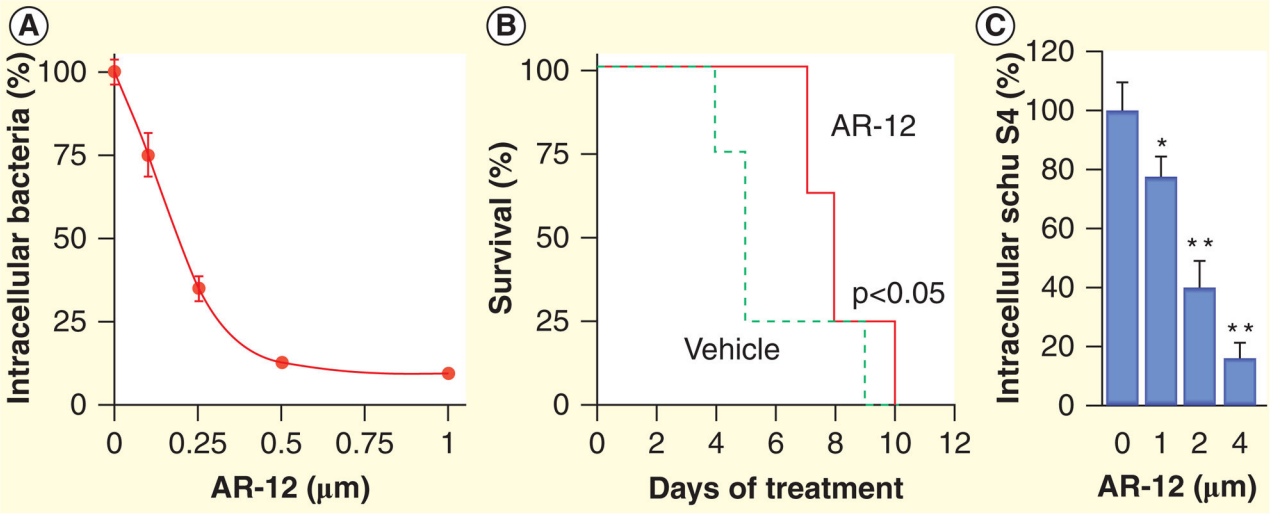

Figure 1. AR-12 effect on bacteria in infected macrophages

(**A**) *Salmonella enterica* serovar Typhimurium survival in RAW264.7 cells after 8 h of treatment with various concentrations of AR-12. (**B**) Survival of mice infected intragastrically with *S.* Typhimurium and treated orally (24 h post-infection) with AR-12 at 2.5 mg/kg or vehicle, once daily for the duration of the study. (**C**) Intracellular survival of *F. tularensis* (a type A strain, *Schu S4*) in THP-1 macrophages with AR-12 treatment 3 h post-infection.

(**A**) Data taken from [39].

(**B**) Data taken from [39].

(**C**) Data taken from [38].

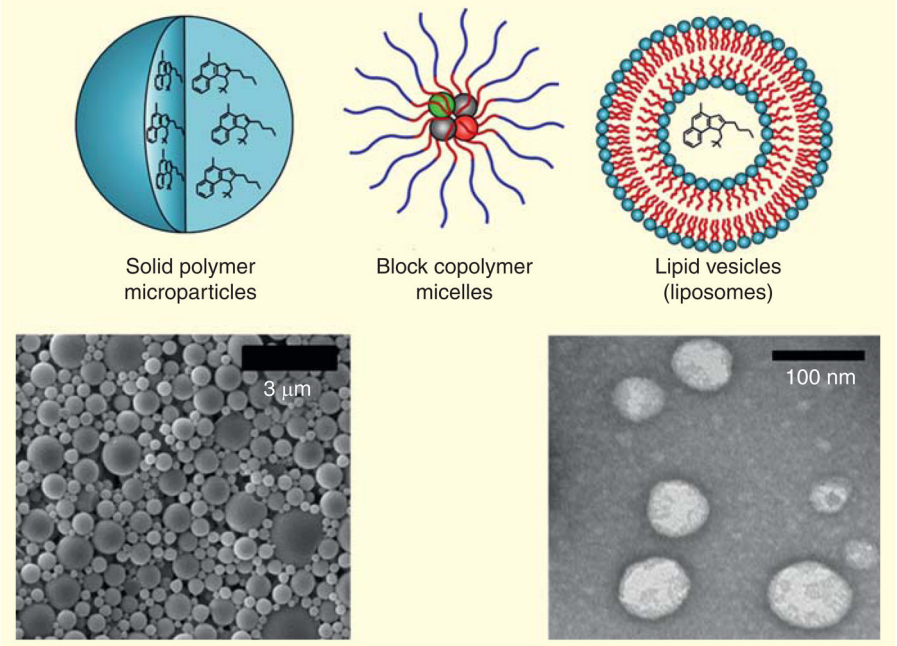

Figure 2. Nano/microparticle vehicles that can be used to deliver toxic drugs to the host cells while minimizing the drugs toxicity by shielding them from the body

Pictured on the left is a solid polymer microparticle and its scanning electron microscopy image with a scale bar of 3 μm. Pictured in the middle is a block copolymer micelle, which can be highly functionalized by numerous polymer groups for different specializations. On the right is a liposome and its transmission electron microscopy image with a scale bar of 100 nm.

Table 1

TLR agonists that have been used to treat different pathogens and encapsulated into various particulate carriers.

Drug name	Target	Clinical uses	Particle encapsulation	Ref.
Imiquimod (Zyclara, Aldara)	TLR7/8	*Leishmania braziliensis*	Acetalated dextran (Ac-DEX)	[67,72]
		Anti-viral	Poly lactic-*co*-glycolic acid (PLGA)	[96,109]
		Anti-fungal		[66]
Resiquimod (R848)	TLR7/8	Anti-viral	Ac-DEX	[64,74]
		Viral-induced asthma		[65]
		Schistosoma mansoni		[110]
Cytosine bonded to guanine (CpG)	TLR9	Anti-viral	PLGA	[111,112]
		Microbe induced asthma	Ac-DEX	[85,81]
		Leishmania donovani	Polyethyleneimine (PEI)	[86,113]
		Herpes simplex virus-2		[87]
Polyinoscinic: polycytidylic acid (Poly I:C)	TLR3	Antiviral	PLGA	[114,115]
		Francisella tularensis	Poly-L-lysine	[76,80]
		Staphylococcus aureus	Ac-DEX	[77,81]
Pam_3Cys–Ser–Lys-4 trihydrochloride (Pam_3Cys)	TLR2	Anti-viral	Trimethylchitosan	[33,116]
		S. aureus	PEI	[31,113]
		Lactobacillus crispatus	PLGA	[32,117]
Monophosphoryl lipid A	TLR4	*Escherichia coli*	PLGA	[28,120]
		Anti-viral		[118]
		Antifungal		[119]
Poly $I:C_{12}U$ (Ampligen)	TLR3	Antiviral		[29]
RC-529	TLR4	*Neisseria meningitidis*	PLGA	[98,121]
Salmonella Typhi flagella (STF2)	TLR5	Antiviral		[122]

Table 2

Advantages and drawbacks to drug delivery systems.

	Advantages	**Drawbacks**
Polymers	Large and tunable range of particle sizes available Long shelf-life Can be stable outside the cold chain Can encapsulate hydrophilic and hydrophobic drugs Can have scalable and continuous fabrication methods available Can be biocompatible	Limitations to lower limit of particle size Some fabrication methods are batch and not scalable Potentially undesired degradation byproducts Pharmacokinetics of multiple compounds (e.g., polymer, degradation products and drug)
Liposomes	Tunable size through manufacturing methods like extrusion and sonication Can have cellular uptake by fusion with cell membrane Can encapsulate hydrophilic well Biocompatible	Typically batch fabrication method Increased instability over time and outside the cold chain Variable hydrophobic drug encapsulation Rapid clearance if not PEGylated Pharmacokinetics of multiple compounds
Micelles	Usually nanometer in size (10–100 nm) Can be biocompatible Structure is more kinetically stable than liposomes Encapsulates hydrophobic drugs	Batch fabrication method Rapid clearance Does not encapsulated hydrophilic compounds Pharmacokinetics of multiple compounds
Free drug	Pharmacokinetics of only single compound and its metabolic byproducts	Fixed retention time Hydrophobic drugs have limited solubility