Review

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Harnessing nanoparticles for immune modulation

Daniel R. Getts[1,2,3], Lonnie D. Shea[4,5], Stephen D. Miller[2], and Nicholas J.C. King[1]

[1] The Discipline of Pathology, School of Medical Sciences, Bosch Institute, Sydney Medical School, The University of Sydney, Sydney, NSW 2006, Australia
[2] Department of Microbiology–Immunology and Interdepartmental Immunobiology Center, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA
[3] Cour Pharmaceutical Development Company, Elmhurst, IL, USA
[4] Department of Chemical and Biomedical Engineering, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA
[5] Department of Biomedical Engineering, University of Michigan, 2200 Bonisteel Boulevard, Ann Arbor, MI 48109, USA

Recent approaches using nanoparticles engineered for immune regulation have yielded promising results in preclinical models of disease. The number of nanoparticle therapies is growing, fueled by innovations in nanotechnology and advances in understanding of the underlying pathogenesis of immune-mediated diseases. In particular, recent mechanistic insight into the ways in which nanoparticles interact with the mononuclear phagocyte system and impact its function during homeostasis and inflammation have highlighted the potential of nanoparticle-based therapies for controlling severe inflammation while concurrently restoring peripheral immune tolerance in autoimmune disease. Here we review recent advances in nanoparticle-based approaches aimed at immune-modulation, and discuss these in the context of concepts in polymeric nanoparticle development, including particle modification, delivery and the factors associated with successful clinical deployment.

Introduction

Nanotechnology is revolutionizing many aspects of modern medicine, including diagnostics and therapeutics [1,2]. The first nanoparticle (NP) therapy was approved by the FDA in 1989. Subsequently, numerous NP therapies have been approved, most of which have focused on optimizing the safety and pharmacokinetic properties of small-molecule agents and hormones [2,3]. More recently, our increasing knowledge of the cellular subsets and regulatory roles of various members of immune system, combined with the emergence of safe, biocompatible nanoparticle platforms, is catalyzing the development of complex, highly adaptable, and programmable NP therapies that are predicted to revolutionize the standard of care of numerous disorders. For example, NPs may be engineered to specifically target cells of the mononuclear phagocyte system (MPS) for the purposes of restoring peripheral immune tolerance or to regulate aberrant monocyte activities during severe inflammation [2,4–7]. Five-hundred-nanometer NPs with negative zeta potential can be harnessed to target circulating monocytes, reducing their potential for causing immune pathology in numerous experimental disease models including West Nile virus (WNV) encephalitis, myocardial infarction, and inflammatory bowel disease (IBD) [8]. The combination of such NPs with specific autoantigens can also be used to restore peripheral immune tolerance in autoimmune models including experimental autoimmune encephalitis (EAE) [5–7,9]. In addition, NPs maybe utilized to mop up extraneous circulating inflammatory mediators.

The functional outcome of NP immune modulation depends on numerous factors that are intrinsic to NPs, such as composition, size, and charge, as well as extrinsic factors such as route of administration. These concepts and how they relate to manipulating immune responses are the primary focus of this review.

Immunological considerations in therapeutic particle design and utilization

NP design

NPs are particles sized between 1 and 1500 nm. They can be made from almost any compound, including poly(amino acids), polysaccharides and poly(alpha-hydroxy acids) as well as non-degradable compounds such as gold, silver, carbon, iron, and silica. The ability to synthesize NPs from biocompatible and biodegradable polymers such as poly-lactide-co-glycolide (PLGA) has revolutionized the use of NPs in the field of immune modulatory therapeutics and this will be the focus here. NPs can be engineered to deliver, alone or in any combination, small-molecule drugs (including immune suppressants and chemotherapeutic agents), proteins (hormones and antibodies), peptides (for vaccine or immune tolerance purposes), DNA (as part of gene therapy approaches), miRNAs, and even machinery to target clustered regularly interspaced short palindromic repeat (CRISPR) components for gene-editing purposes. It is now clear that the physiochemical characteristics of unadorned NPs can also alter immune responses independently of any associated active pharmaceutical ingredient [8].

A primary function of NPs involves the delivery of a specific cargo and numerous methods have been developed due to the challenge associated with the efficiency of

Corresponding author: Getts, D.R. (d-getts@northwestern.edu).

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encapsulation and the properties of this cargo. A straightforward approach is to chemically conjugate the desired active molecule to the particle. Peptide antigens have been chemically conjugated to NPs using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC or ECDI) [6,9], which attaches the amine on the target to a carboxylic acid on the particle. Various chemistries, such as Click chemistry or Michael-type addition, are available and their use is based on the chemical groups within the polymer and on the cargo molecule. Alternatively, the active molecule can be incorporated into the particle directly. Using PLGA to exemplify the approach, if the cargo is either soluble in an organic solvent or stable in a crystalline form when dispersed in an organic solvent [10], the encapsulation can be accomplished using a water/oil single-emulsion method. For delivery of water-soluble molecules, these are incorporated into polymeric NPs using a water–oil–water double-emulsion method [11]. For the double-emulsion method, the aqueous drug is initially dispersed within the dissolved polymer solution and then a second emulsion is formed with an aqueous solution containing an emulsifying agent.

Size and shape

The downstream immunological outcome of NP therapy is strongly influenced by the mechanism of cellular uptake. NPs entering cells via pathways that allow access to the cytosol have different immune-modulating capabilities from those taken up via phagocytosis [12–14]. Size and shape influence biodistribution and the mechanism of particle uptake [15,16], but studies using a broad range of standard cell lines (HeLa, CHO, Caco-2, and MCF-7) and NPs (derived from gold, polystyrene, polymer, silicon, titanium, and iron oxide) show that the ideal size and shape for particle uptake depends on the cell type [16,17]. In non-phagocytic cell types, NP <100 nm diameter are most efficiently taken up via caveola- or clathrin-mediated processes [15,16]. For professional antigen-presenting cells (APCs) such as macrophages and dendritic cells (DCs), uptake is impacted more by shape than size, with spherical NPs having more favorable uptake kinetics than rod-shaped NPs, irrespective of NP size [18]. Targeting NPs to phagocytes is a critical aspect of any therapy attempting to manipulate the immune response and is discussed further below.

Size also affects NP toxicity [19]. Nanoparticles <100 nm in diameter tend to interact with cellular organelles, including the mitochondria and nucleus, and these interactions can trigger cellular respiratory and gene toxicity in cells [20]. This risk is reduced with increasing NP size, presumably because larger NPs tend to initiate phagocytosis, which effectively isolates particles from the more sensitive cytoplasmic environment.

Charge

NP charge is a dynamic physicochemical characteristic, with the particle microenvironment, including the protein corona, all capable of altering the surface charge of NPs. Generally speaking, NP charge can be modified by increasing the number of carboxyl (negative charge) or amine (positive charge) groups on the surface of the NP. Studies using different NP charges have clearly shown that this factor influences both uptake mechanisms and downstream immune outcomes. For instance, relative to anionic particles, cationic NPs appear to be taken up more readily via clathrin-mediated processes [21]. Furthermore, positively charged antigen-loaded NPs are significantly more effective at stimulating Th1 responses after either intradermal or mucosal (pulmonary) inoculation, whereas anionic particles stimulate T and B cell responses poorly under similar conditions [22,23]. The ability of cationic particles to stimulate Th1 responses has been associated with preferential DC uptake of such particles and the propensity of cationic particles to regulate positive costimulatory molecules [24]. However, cationic NPs may also alter mitochondrial and endoplasmic reticulum function, triggering the production of reactive oxygen species and proinflammatory cytokines, as well as cell death [25–27]. These events may underlie the adjuvant effects of cationic NPs, but attempts to harness this phenomenon clinically will need to address the consequences of any off-target toxicities.

Anionic NPs, by contrast, have been associated with little to no toxicity [20]. Furthermore, NPs with a charge below −30 mV have been found to have anti-inflammatory properties and when combined with antigen can induce antigen-specific immune tolerance [6,8,9]. This phenomenon is associated with the ability specifically to target scavenger receptors such as MARCO on monocytes and macrophages [6,8,9].

Stiffness and fluidity

Stiffness also affects the biological impact of NPs. NPs made of rigid materials may be associated with increased potential for embolism, while flexible polymer-based NPs that can more easily deform may gain better access to tissues during the complex vascular changes associated with inflammation. The fluidity of NPs, too, affects the ability of antigen-loaded NP to stimulate immune responses. Thus, intramuscular, solid-phase, antigen-containing liposome immunization elicits a more robust Th1/Th17 response than similarly administered fluid-phase liposomes [28]. The stimulatory ability of solid-phase particles is proposed to result from the formation of an immobilized antigen particle depot, similar to that observed for traditional oil-in-water emulsions and aluminum adjuvants [29,30]. This results in a prolonged supply of antigen for APCs and is also associated with upregulation of positive costimulatory molecules such as CD80, which support efficient T cell priming [28]. By contrast, intramuscularly injected fluid-state liposomes are rapidly removed, do not appear to stimulate positive costimulation, and are much less capable of stimulating a T cell response [28]. Whether the intramuscular fluid liposome–antigen combination induced peripheral immune tolerance was not tested, but intraperitoneal (IP) administration of fluid OVA-decorated liposomes can induce antigen-specific IgE non-responsiveness [31,32]. While this was argued to occur in a T cell-independent fashion, the increased levels of IgG after IP administration suggest immune deviation [33]. Notwithstanding this, these findings highlight the importance of understanding the contribution of fluidity and stiffness in NP-mediated manipulation of immune outcomes.