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Nanoparticle-based autoimmune disease therapy

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Abstract
The goal of immunotherapy against autoimmunity is to block pathogenic inflammation without impairing immunity against infections and tumours. Regulatory T-cells (Tregs) play a central role in maintaining immune homeostasis, and autoimmune inflammation is frequently associated with decreased numbers and/or function of these T-cells. Therapies harnessing Tregs to treat autoimmune inflammation remain under-developed with caveats ranging from the lack of antigenic and disease specificity to the potential phenotypic and functional instability of *in vitro*-expanded Treg cells *in vivo*. Here, we review nanotechnology-based approaches designed to promote immune tolerance through various mechanisms, ranging from systemic or local suppression of antigen-presenting cells and deletion of antigen-specific T-cells, to the systemic expansion of antigen- and disease-specific Treg cells *in vivo*.

1. Introduction

Autoimmune diseases result from an attack of the immune system against specific tissues, compromising their structural and/or functional integrity. In most, if not all autoimmune diseases, autoimmune inflammation is orchestrated by cognate interactions between antigen presenting cells (APCs) and autoantigen-specific T-cells [1]. Whereas recognition of self peptide-MHC (pMHC) complexes displayed by APCs in steady state (non-inflammatory) conditions tends to promote T-cell tolerance, engagement of these complexes by autoreactive T-cells in the context of inflammation tends to promote T-cell activation [2–5]. Consequently, It is not surprising that many therapeutic approaches against autoimmunity aim to promote tolerance by targeting the APC-T-cell interaction.

In general, therapies designed to induce antigen-specific tolerance include the targeting of relevant autoreactive T- and B-cell specificities via their antigen receptors. In this context, the term 'tolerance' has been used to describe a broad range of outcomes, including clonal deletion, anergy [6,7], changes in cytokine profile [8], and induction of

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regulatory T- and B-cells [9,10]. Altered peptide ligands (APLs, amino acid-substituted variants of autoantigenic peptides) [11] have been used since the early 1990s to induce antigen-specific tolerance in animal models of autoimmune disease. APLs typically function as partial agonists or antagonists of key T-cell specificities and therefore exploit the specificity of the TCR–pMHC interaction to modulate the function of those autoreactive T-cells thought to play a critical role in disease initiation and/or progression. Certain APLs induce T-cell anergy [12] or clonal deletion [13], whereas others trigger immune deviation [14–16]. In some cases, APL immunotherapy inhibited autoimmune inflammation, but in others, it accelerated disease onset [17] or triggered life-threatening anaphylactic responses [18]. The immune response to a specific APL can hardly be predicted [19]. This caveat, coupled with the antigenic complexity of most autoimmune diseases and the need to target multiple autoreactive T-cell specificities simultaneously, represents important limitations of traditional antigen-specific approaches for the treatment of autoimmune disease.

Here, we review nanotechnology-based therapeutic avenues that have sought to improve the outcomes of traditional antigen-specific therapies. Both micro- and nanoparticles have been used as vehicles for drug and/or antigen delivery to phagocytes as well as direct T-cell-targeting compounds. In some designs, the physical and chemical properties of the particles contribute to the pharmacokinetic and toxicological behaviour of the compound but not to its tolerogenic activity; in others, the nanoparticle is an integral part of the active product.

2. Harnessing the physical and chemical properties of nanomaterials for cellular targeting

Biologically relevant nanomaterials, usually nanoparticles, are defined as structures smaller than 100 nm in diameter with physical and/or chemical properties that have the potential to alter biological processes driven by specific cell types [20]. Physical and chemical parameters such as nanoparticle size, shape, and surface chemistry impact on the biodistribution, cellular tropism and toxicity of nanomaterials [21–23]. These parameters must therefore be fine-tuned to maximize its desired biological and therapeutic activities and limit the off-target toxicity of individual designs. For example, particles larger than 500 nm in diameter tend to accumulate in the spleen, and size is inversely proportional to the speed with which nanoparticles reach the lymph nodes upon intravenous delivery [22].

In the context of autoimmunity, cellular toxicity associated with certain nanomaterials can be exploited to kill or inhibit pathogenic cell types (selective block-by-toxicity). For example, inhaled multi-walled carbon nanotubes promote systemic immunosuppression without causing significant lung damage, through a mechanism dependent on the secretion of tumor growth factor-beta (TGF-β), prostaglandins, and IL-10 [24–26]. Likewise, inhaled carbon nanotubes can inhibit lung-resident dendritic cells (DCs) and promote lung immunosuppression [25]. The importance of nanoparticle chemistry on cellular tropism and biological activity is further highlighted by the description of nanotube composites for cancer immunotherapy that promote T-cell activation, rather than immunosuppression [27]. Other nanomaterials, like citrate-coated gold [28,29] and cerium oxide nanoparticles [30–32], have been shown to have anti-oxidative and anti-inflammatory properties with limited organ toxicity.

Nanomaterials with the desired cellular tropism can be further engineered to preferentially accumulate into (or deliver cargos to) specific subcellular compartments of immune cell types, such as by using surface chemistries that promote cell entry but inhibit nuclear translocation (e.g., citrate-stabilization of gold nanomaterials) or that promote accumulation in the nucleus and mitochondria [33].

Shape and size also affect the organ and cellular tropism of nanomaterials [34] (Fig. 1). Some shapes promote and others inhibit phagocytosis by macrophages. For example, whereas sphere-shaped (vs. anisotropic) nanomaterials are easily internalized by macrophages, disk-shaped structures are not, despite readily binding to their surface. This property could be harnessed to promote the transport of the nanomaterial by macrophages to other locations like tumors or lymphoid organs [35]. In addition to shape, the size of the nanomaterial also influences its cellular tropism, such as by facilitating or inhibiting actin polymerization and wrapping of the cell membrane around the nanomaterial. Functionalization with 'don't eat me' ligands [36] (like CD47) is another strategy that could be used, for instance, to inhibit the accumulation (and dumping) of certain nanomaterials into macrophage-rich organs like spleen and liver.

More complex nanoparticle designs involve the functionalization of nanomaterials with specific ligands and/or the loading of their cargo compartments with immunoregulatory molecules. By narrowing the bioavailability of these molecules to cell types capable of recognizing and internalizing these compounds, these structures have the potential to enhance the desired biological activity and limit the off-target toxicity of anti-inflammatory drugs. These nanoparticle designs typically involve the use of chemically inert materials, to reduce unwanted chemical reactivity between the nanomaterial and the anti-inflammatory cargo or components of the target cell. For example, superparamagnetic iron oxide nanoparticles (Fe_3O_4 or Fe_2O_3 SPIONs) [37], which can be rendered soluble in biological fluids by coating their surface with dextran starch or polyethylene glycol, meet these requirements. SPIONs are highly biocompatible and biodegradable; the iron core is recycled into soluble ferritin iron or hemosiderin [38]. In fact, SPIONs have been approved for clinical use in the form of Ferumoxides (dextran SPION), Ferucarbotran (carboxydextran SPION), and Ferumoxtran-10 (dextran SPION) [39,40].

The development of nanoparticles with surfaces having several different physical and chemical properties—also known as Janus nanoparticles [41]—enable the production of multi-functional compounds, hence the simultaneous modulation of various processes in target cells.

3. Nanoparticles as vehicles for delivery of anti-inflammatory compounds to phagocytes

Nanomaterials have been engineered to deliver cargos to APCs to modulate antigen presentation and downregulate innate immune signals, which collectively promote activation of adaptive autoimmune responses. Some compounds