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Modulation of Immune Response using Engineered Nanoparticle Surfaces

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Abstract

Nanoparticles (NPs) coated with a monolayer of ligands can be recognized by different components of the immune system, opening new doors for the modulation of the immunological responses. By the use of different physical or chemical properties at the NP surface (such as charge, functional groups, and ligand density), NPs can be designed to have distinct cellular uptake, cytokine secretion, and immunogenicity, factors that influence the distribution and clearance of these particles. Understanding these immunological responses is critical for the development of new NP-based carriers for the delivery of therapeutic molecules, and as such several studies have been performed to understand the relationships between immune responses and NP surface functionality. In this review, we will discuss recent reports of these structure-activity relationships, and explore how these motifs can be controlled to elicit therapeutically useful immune responses.

Keywords

nanoparticles; immune response; surface functionality; protein corona

1. Introduction

The immune system is the prime defense barrier that living organisms present when foreign entities try to gain access to the body or when cells or molecules become a potential threat to the body such as in the case of cancer or many types of autoimmune diseases. The ability to modulate the immune response is important in a wide array of contexts, including the prevention of bacterial infection, the treatment of cancer and the suppression of the autoimmune response. [1] Antibodies, cytokines, oligonucleotides, and small molecules have been used as immunotherapy agents. [2] For example, hydrogen sulfide (H_2S) can induce an upregulation of anti-inflammatory and cytoprotective genes, resulting in pronounced anti-inflammatory activity. [3] While “small” molecules are important tools for

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immune modulation, macromolecular systems offer size and structure features ideal for interaction with the immune system. Among these materials, nanoparticles (NPs) hold the promise as therapeutics for modulating the response of the immune system. Important implications of the control of immunological responses range from the prevention of diseases (by the enhancement of vaccines and immunotherapies), to the development of new stealth drug delivery vehicles. [4]

By the precise chemical design, a range of NP platforms have been developed and studied for therapeutic applications. Engineered NPs can protect the payload (drug or antigen) from the biological surroundings, increase circulatory lifetime, reducing cytotoxicity, and targeting cells and tissues. [5] Through choice of particle size, shape, core materials NPs can be engineered to be recognized by the immune system, causing stimulation or suppression of immune reactions. For example, ZnO NPs induce the production of pro-inflammatory cytokines, while CeO_2 and TiO_2 NPs did not cause any effects. [6] Mechanism of uptake can be regulated as well: polypyrrole NPs were internalized into IMR 90 cells via endocytosis, but internalized via both phagocytosis and endocytosis into J774A.1 cells.[7]

Immune activation occurs through multiple mechanisms, including receptors at the surface of cells that recognize specific molecular patterns, and system of proteins that recognize chemical signals, such as the complement system. Different types of immunological responses are triggered depending on how the immune system recognizes foreign entities. [8] Recognition by the immune system can cause elimination of nanomaterials (and a decrease in therapeutic efficiency if the NP is a carrier); however, the same phenomenon can be employed as a tool to generate immunotherapies. As such, exploring the interaction between NPs and the immune system is of critical importance for the development of fundamentally new NP-based therapies.

Modifying the physical and chemical properties of nanomaterials concomitantly alters their immunological response. Size affects cellular uptake pathway, cell penetration, cytotoxicity, and bio-distribution of NPs in the immune system.[9] This review focuses recent efforts to understand structure-activity relationships of immune responses caused by NP surfaces, and how therapeutic benefits can be achieved through appropriate NP engineering. We focus here on the use of intrinsic NP properties as opposed the use of specific antigens of known activity (e.g. oligonucleotides, proteins, long peptides), exploring how these synthetic motifs can be used to elicit therapeutically useful immune responses.

2. Immune recognition

The immune system can be categorized into two distinct processes: the innate and the adaptive arms. The innate immune response constitutes the first, primal, host defense, and is induced by both selective cellular processes (performed primarily by phagocytic cells),[10] and constitutive and non-specific events such as in the case of complement system (a complex multi-component system of proteins) that must be activated to function.[11, 12] Once that these systems are triggered, the second part of the immune system, adaptive immunity, is then able to respond in a highly specific manner against molecular determinants on pathogens in a longer-term process that can proceed over weeks.[13]

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When NPs enter the mammalian body, stimulation of the immune system is normally initiated by the interaction of these materials with cells of the innate immune arm such as monocytes, macrophages and dendritic cells, in a similar manner to a pathogen infection. This interaction leads to signal cascades upon activation of pattern recognition receptors (PRRs).[14] PRRs are proteins expressed by cells of the innate immune arm to identify pathogen-associated molecular patterns (PAMPs) that are associated with microbial pathogens or cellular stress. Other types of receptors are the damage-associated molecular patterns (DAMPs) that are associated with cell components released during cell damage.

Signaling PRRs include the large families of membrane-bound Toll-like receptors (TLRs) [15] that can recognize such divers molecular structures such as nucleic acids (single and double stranded), bacterial fragments such as Lipopolysaccharides (LPS), and charged phospholipids.[16] In addition, cytoplasmic nucleotide oligomerization domain (NOD)-like receptors (NLRs) recognize cytoplasmic proteins, and have a variety of functions in the regulation of inflammation and apoptotic responses.[17] On the other hand, endocytic PRRs promote the attachment, engulfment and destruction of microorganisms/foreign entities by phagocytes, without relaying an intracellular signal. Endocytic PRRs recognize carbohydrates, and include mannose receptors on macrophages, glycan receptors present on all phagocytes and scavenger receptors that recognize charged ligands and are found on all phagocytes and mediate removal of apoptotic cells.[18]

3. Immunogenicity and nanoparticle surface

3.1 General considerations

The use of NPs as carriers for the delivery of therapeutic molecules is one of the major applications of nanomaterials in biological systems. Substantial research has been directed towards the development of carriers that have minimal interaction with the immune system, with the objective of increasing delivery efficiency. One of the properties that most delivery vehicles share is the presence of a net positive charge at the surface, often use for complementarity with the therapeutic molecule (i. e. nucleic acids and/or anionic proteins), or generated as a result of the surfactant that is used to create the NPs. [19] However, cationic systems induce the activation of inflammatory responses, even when used in concentrations below the cytotoxic threshold. For example, Peer et al. performed a systematic study of the effect of the NP charge in the expression of cytokines, markers of immune activation.[20] They observed that positively-charged lipid NPs produced a strong immunological effect, 10-20 fold higher than that for neutral and anionic NPs. This study and others [21] also suggest that a family of receptors may be involved in the specific recognition of cationic systems. However, this is not the only way by which the immune system can identify foreign charged structures.

Another effect that needs to be taken into consideration is the non-specific adsorption of proteins over the NP surface, namely the formation of a protein corona. When NPs are injected in the mammalian body, this corona tend to include series of proteins called opsonins (such as C1q and C3b) whose sole function is to tag foreign bodies for their rapid elimination.[22,23] The binding of these proteins to charged NP surfaces induces activation of the complement system and macrophage recruitment, initiating the cascade of immune

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responses.[12] As a result of this clearance processes, NPs that form a protein corona (positively or negatively charged) are eliminated faster from bloodstream comparative to particles with stealth capabilities (Figure 1).[24]

3.2 Poly(ethylene glycol) (PEG): The “Stealth” Coating

PEGylation is one of the most popular approaches to control the stability of NPs in biological fluids that not only reduces non-specific protein adsorption, but also improves the circulation time of NPs. After PEG modification, nanoparticles can be decorated with different molecular structures such as antibodies, oligonucleotides, and peptides, allowing their use in active targeting and immunotherapy. However, despite the perception of PEG as the ideal non-fouling coating, PEG derivatives are recognized by the immune system, triggering different immune responses. For example, Jiang et. al. demonstrated that PEGylated NPs could induce the secretion of anti-PEG antibodies, accelerating the blood clearance of these systems (Figure 2a). [25] They also observed that other chemical functionalities that also confer non-fouling characteristics (i. e. zwitterions) did not trigger the formation of antibodies, and hence do not cause accelerated blood clearance. Different reports on the recognition of PEG structures by proteins from the complement system (similar to cationic NPs) have been also presented.[26] These studies suggest that the density and chain length of PEG are crucial determinants for the immunogenicity of these systems, as these parameters influence cellular binding, uptake, and degradation.[27] Likewise, this activation of the complement system (and the adsorption of those proteins in the corona) by PEG has been shown to affect the biodistribution, pharmacokinetics and the overall behavior of nanomaterials in vivo.[28] It is important to note that the use of PEG might also compromise the efficacy and safety of nanomaterials in biomedical applications. As an example, *in vivo* studies showed that PEG (5000)-coated AuNPs induced acute inflammation and apoptosis in the liver of mice. [29]

The immunogenic properties of PEG, however, can also be tailored for use in therapy. As an example, Hubbell et al. used PEG modified NPs as a platform to both deliver and boost the recognition of a specific antigen. [30] Using inverse emulsion polymerization, (PEG) and poly(propylene glycol) (PPG) were copolymerized to obtain NPs that were efficiently and quickly taken into lymphatic vessels and transported to the lymph node, achieving the intended delivery and increasing immunological outcome. The study suggested that the presence of PEG is crucial for this process, as the binding of hydroxyl terminal groups with the exposed thioester of the complement protein C3b initiates complement activation and induced dendritic cell maturation (Figure 2b). As a result, the PEG-NPs induced a strong adjuvant activity when conjugated with ovalbumin (a commonly used model antigen), up-regulating both humoral and cellular immunity, and producing strong levels of anti-ovalbumin IgG.

3.3 Hydrophobic Surfaces for Immune Activation

As described above, the hydrophobic moieties such as aliphatic and aromatic groups found in DAMPs and PAMPs are hypothesized to be involved in the activation of the immune system.[31] As such, it is considered that hydrophobic portions that are normally hidden inside the cellular membrane may serve as one of such danger signals. Following this idea,

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Rotello and Peer functionalized NPs with different degrees of hydrophobicity, and measured cytokine expression after exposing splenocytes to the NPs.[32] Their findings showed a direct correlation between the hydrophobicity of NPs and cytokine expression, while other functionalities such as h-bond donors/acceptors, did not affect the response (Figure 3a). Interestingly, this effect was observed for pro- and anti-inflammatory cytokines both *in vitro* and *in vivo*, indicating a significant control of these immune responses. Similar results were observed more recently by the Santos group by the use of thermally hydrocarbonized porous?silicon NPs, reporting not only an increase in the cytokine expression, but also the increase in the maturation of dendritic cells (DCs).[33]

Likewise, other studies using poly(D,L-lactic acid) (PLA), poly(D,L-lactic-co-glycolic acid) (PLGA), and poly(monomethoxypolyethylene glycol-co-D,L-lactide), polymers that offer different degrees of hydrophobicity while maintaining constant particle size, demonstrated an increase in antigen internalization by dendritic cells for particles of larger hydrophobicity, along with an increase in CD86 and MCH II expression (Figure 3b). [34] Finally, hydrophobic moieties have also been correlated to an increase in adjuvant capabilities by the use of poly (g-glutamic acid) (PGA) with different grafting degrees with to L-phenylalanine ethyl ester.[35] This system showed an increase in antibody generation (along side an increase in inflammatory cytokines) when the grafting degree of the particles was larger (more hydrophobic). Taking together these results suggest the generality of the phenomenon, and evidence the significant potential that hydrophobic portions posses for their use in modulation of the immune responses towards NPs.

4. Perspectives

We have discussed how different chemical functionalities at the NP surface are recognized by the immune system, and how this recognition can be exploited to for therapeutic purposes. However, two important considerations need to be addressed to gain more detailed information on how we can use these interactions during the design of nanomaterials. The first one is how nanomaterials can interact with the complement system. Most of the studies found in literature are limited to *in vitro* analysis of the interactions of NPs with different cells of the immune system. However, it is important to note that when nanomaterials are injected in the body, they not only interact directly with cells from the immune system, but also with the "tagging" system of the complement system. This second process may be the reason of why many *in vivo* studies do not correlate to *in vitro* tests, limiting the scope of the findings. In addition, opsonization of nanoparticles with complement proteins is one of the initial steps for the elimination of these materials from the bloodstream (decreasing therapeutic efficiency), evidencing its central role in the immunological environment. Systematic studies of the activation of the complement by the surface functionality (such as the one depicted for PEG) and by other NP properties are scarce, despite their significance for a better prediction of the *in vivo* behavior of nanomaterials. As such, a better understanding of the rules that govern complement recognition of nanomaterials is of fundamental importance, and efforts should be directed towards this goal.

Another important factor is the fact that most of the immunotherapeutic applications of nanomaterials are directed towards the generation of better vaccine adjuvants (increase an

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immune response, as discussed before), and very few studies attempt the control or reduction of an immunological effect that is already present. This limited scope reduces the applicability of most of the findings to prophylactic applications; preventive therapy that is given when the body is not currently under a stress or a disease. It is unknown if these NPs will possess the same behavior once the system has been challenged by another stressor, for example in the case of inflammation. Inflammation plays a major role in the development of different diseases, and various immunotherapies have been proposed for its treatment. However, even in the case of such a critical immunological challenge, attempts to use nanoparticle-based therapies to gain control are scarce. Furthermore, as no fundamental studies addressing the interaction of nanomaterials and a 'stressed' immune system have been performed, we possess very few clues clues on the fundamental behavior of these systems. This challenge offers an excellent opportunity to not only undercover fundamentals of the immune recognition, and can also open new doors for the development of nanomaterials for non-prophylactic use, a new generation of remedial immunotherapies.

5. Conclusion

Taken together, we have come a long way to understanding the interaction between NPs and the immune system. NP surfaces can now be engineered to achieve desired immunological responses by the use of different chemical functionalities that avoid or trigger a distinct immune activities. Surface coverages such as PEG and zwitterions provide "stealth" functionality, important to the delivery community. Surface engineering to generate immune responses, however, presents a huge area for potential therapeutics. We have already witnessed the development of new nano-adjuvants to boost the responses of antigens. This imunnostimulation is just the tip of the iceberg. Nanomaterials have the potential to provide tailored responses that would enable targeted pro- and anti-inflammatory responses that could revolutionize address health concerns as diverse as autoimmune disease, infections, and cancer. Clearly, however, there is much to be learned fundamentally before we can truly tap the potential of NPs for immunomodulation.

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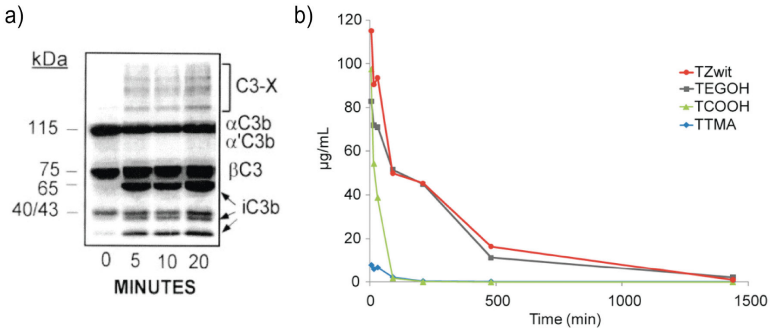

Figure 1.

a) SDS page of Doxil® particles after incubation with human serum evidencing the adsorption of complement proteins. b) Nanoparticles that form a protein corona (TTMA and TCOOH, with positive and negative charged respectively) are eliminated faster from the bloodstream comparative to neutral nanoparticles (TEGOH and TZwit). Reproduced with permission from references 23 and 24 respectively.

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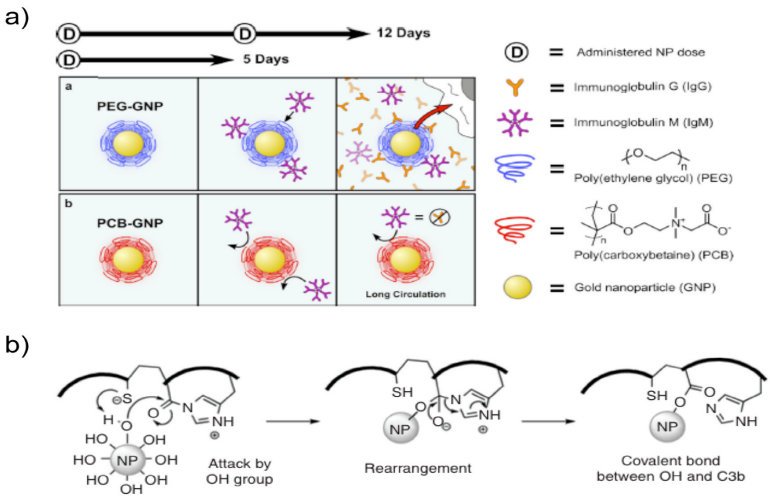

Figure 2.

a) Schematic illustration of the sequence of events after PEG-AuNPs and zwitterionic-AuNPs enter the blood stream. b) Proposed mechanism of the binding of PEG-NPs with proteins of the complement system. Reproduced with permission from references 25 and 30 respectively.

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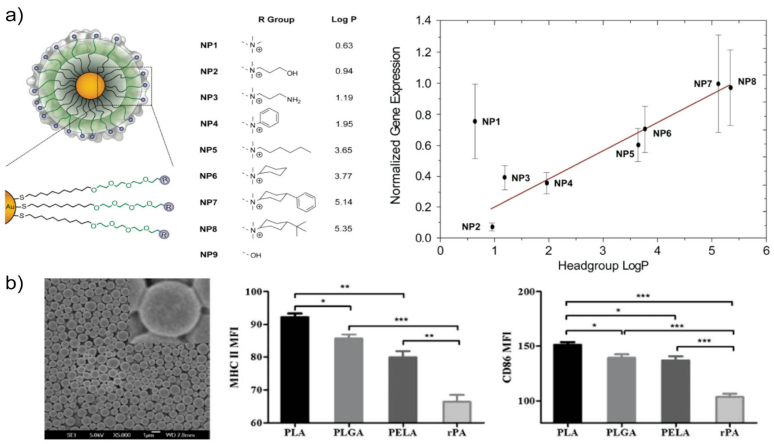

Figure 3.

a) Cytokine expression (Tumor Necrosis Factor, TNFα) of a series of NPs bearing end groups of different hydrophobicity. b) MHC II and CD86 expression (stimulation of dendritic cells) by the use of polymeric NPs of different degrees of hydrophobicity. Reproduced with permission from references 32 and 34 respectively.