Immune Theranostics

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As the understanding of how the immune system functions steadily improves, engineers can begin employing principles of rational design to controllably modulate immune responses for therapeutic applications. Key tools in this new frontier of immunoengineering have emerged from biomaterials and nanoscale science, such as theranostics: the combined delivery of therapeutic and diagnostic agents. By providing a means of tracking and quantifying cells that are targeted and modulated during vaccination and immunotherapy, theranostics allows the immune system to be addressed less as a mysterious 'black box' and more as an interlinked network of cells and signaling molecules that can be mapped for improved reproducibility and understanding. Immune theranostics holds promise for realizing technologies that harness the full potential of immunotherapy in the treatment of a wide range of inflammatory disorders.

A NEW FRONTIER FOR ENGINEERING: RATIONAL IMMUNOMODULATION

The immune system is a dynamic and highly responsive network of bioactive molecules, cells, and tissues. This complex system must continuously maintain the homeostasis of its host body within a strict set of physicochemical boundaries while being ever ready to address an equally complex and evolving repertoire of invading pathogens and heterogenous cancers. Adding to this complexity is the uniqueness of each patient's immune system, wherein women, men, children, neonates, the elderly, and the diabetic can each have distinct immune responses to the same stimuli.

Furthermore, prior exposure to particular inflammatory molecules and conditions, such as certain foods or regional infections, can have significant impacts, going so far as to prevent allergic reactions or make some vaccines ineffective in specific parts of the world. While the protective abilities of the immune system have long been tapped for the generation of vaccines, its potential to be directed towards the treatment of cancer and inflammatory disorders has only been explored relatively recently in the form of immunotherapy. But how can we attempt to controllably and reproducibly modulate this system, which not only varies on a person-to-person basis, but also in terms of sex, age and disease state? To address this need, immunoengineers apply principles of rational design, biomaterials science, nanoscale science, systems analysis, and numerous other engineering disciplines to better assess, control and customize immune responses for safe and reproducible therapeutic applications.

Immunoengineering is a relatively new field, but its concepts have always been a core component of biomaterials science. Materials development for biological implants and *in vivo* controlled delivery have historically focused on minimizing inflammation. Biomaterials are therefore usually optimized to inhibit the activation of inflammatory immune cell populations that are found in tissues and biological fluids, with the objective to decrease toxicity, increase therapeutic efficacy of delivered agents, and extend the lifetime of implanted devices. Instead of focusing on preventing inflammation, recent advances in the development of nanoscale biomaterials, i.e. nanobiomaterials (NBMs), now permit the design of materials to directly elicit therapeutically beneficial responses from the immune system (1, 2). The immune system has evolved to interact with NM due to a never-ending battle with viruses, and moreover nanoscale lipid vesicles released by immune cells have been shown to be essential components of cell-cell communication and signaling. As a result, biomimicry of these nanostructures presents a pathway

for probing, modulating, and monitoring immune responses. Theranostics (i.e. combined delivery of therapeutic and diagnostic agents) has thus emerged as a vital tool, presenting a new frontier for identifying and tracking immune cells that are modulated by delivered drugs and immunostimulants (3, 4). Such 'immunotheranostic' strategies are significantly enhancing the ability of engineers to reproducibly generate immune responses by monitoring which components are modulated at the organ and cellular level during immunotherapy and vaccination (5, 6).

PREVIOUS METHODS OF VACCINE DEVELOPMENT: TREATING THE IMMUNE SYSTEM AS A BLACK BOX

Vaccination is fundamentally the process of training the immune system to recognize and eliminate pathogens either prophylactically or therapeutically and can thus be considered one of the first forms of immunotherapy. Although it may seem obvious that immunology should be a key component of vaccine design, this has historically not always been the case. Essentially, rational vaccine design requires an understanding of the immune system that we have not yet achieved, but the urgency to aid the sick and prevent the spread of infection has presented no alternative other than the use of trial-and-error methodologies. As a result, the majority of immunization strategies were developed by treating the immune system as a black box. Antigens, i.e. molecular components of pathogens, and adjuvants, i.e. 'danger signals' that stimulate inflammatory cells, are randomly combined into formulations that serve as the input into the system. The output from the black box consists of the resulting, hopefully lasting and protective, immune response. Usually with little understanding of the mechanism by which the antigens and adjuvants achieve this output, formulations are selected that generate the safest and most effective

prevention or removal of infection with lasting immunological memory to respond quickly to future pathogen exposure. Complex cell-cell interactions occur and dozens of signaling molecules known as cytokines are released by inflammatory cells during an immunization, and it is critical to know which cells contribute to these responses and whether the same cells can be reproducibly stimulated across different human populations. Importantly, different immune cells express different combinations of cytokines, often in amounts proportion to the extent of their exposure to adjuvant, and this network of activated inflammatory cells and released cytokines form an emergent system that can be tailored for specific therapeutic applications. By employing targeted NBMs to control and monitor which immune cells are modulated during vaccination, theranostics provides a means of exploring this black box to better correlate the input vaccine or immunomodulatory formulation with the output immune response.

ENGINEERING NANOBIOMATERIALS FOR TARGETED IMMUNOMODULATION

NBMs, broadly defined as any biomaterial with at least one external dimension that is less than 1000 nm, are key tools in this new field of immunoengineering and have attracted much attention for their ability to deliver therapeutics and imaging agents to specific cells and tissues (1, 7). This versatility has demonstrated improved efficacy and deployment of vaccine formulations by providing triggered or bioresponsive mechanisms for controlled release, transporting combinations of bioactives with diverse solubility, and allowing control over reproducibility, speed and cost of production (7). Among the wide range of available NBMs, self-assembled NBMs composed of synthetic amphiphilic polymers are especially advantageous for vaccination

and immunotherapy due to their versatility in chemistry and structure (1). This allows better mimicry of viruses, which possess physicochemical and structural characteristics that dictate their interactions and processing by critical immune cells known as professional antigen presenting cells (APCs).

Professional APCs; which include dendritic cells, macrophages and B cells; are the most frequent targets of immunomodulatory NBMs due to their potency for cytokine release and T cell activation. T cells are the effector cells of the immune system that can directly kill virus infected or cancerous cells (cytotoxic T cells) as well as direct or enhance functions of other immune cells (helper T cells). Using a military hierarchy as an analogy, T cells can be considered both soldiers and non-combat support troops while APCs are the generals that direct their action. NBMs thus function as our direct line of communication to these generals by alerting them of imminent danger (adjuvant) and providing targets (antigen) for elimination. Following internalization by APCs, NBMs are degraded within intracellular compartments that contain a variety of enzymes and redox mediators (8), allowing transported payloads to modulate APC function for activation of T cells.

THERANOSTICS AS A TOOL TO IMPROVE VACCINE DESIGN AND REPRODUCIBILITY

The field of theranostics combines ***thera***py and diag***nostics*** into a single NBM-based strategy. Continued progress in this field will allow early detection of disease, prevent unintended side-effects of drugs, decrease the frequency and amount of administered drugs, and allow quantitative assessment of the accuracy of drug delivery within individual patients. Immunotheranostic nanomedicine may therefore revolutionize treatments for a wide range of inflammatory disorders,

including cancer and heart disease, by providing powerful new approaches not only for therapeutic delivery and diagnosis, but also for personalized medicine and clinically relevant assessment of therapeutic efficacy. Using viruses, bacteria and other pathogens as inspiration, biomimetic NBMs can be engineered with physiochemical properties selected to stimulate or suppress specific APC populations while marking them for detection and quantification *via* multiple diagnostic modalities. As an example, theranostic delivery of a drug regimen to reduce vascular inflammation in cardiovascular disease patients could allow a clinician to monitor the patient's progress during treatment. Since not all patients will have the same response to anti-inflammatory drugs, the clinician could adjust the treatment as necessary by monitoring the levels of critical inflammatory cells within the patient's arteries. NBMs targeting dendritic cells may serve such a function, as the levels of these APCs within vascular lesions directly correlates with the risk of rupture and vascular occlusion (9). There is currently no non-invasive method available to detect such unstable lesions in patients, many of which could suffer heart attack or stroke without warning.

NBMs can be engineered to be amenable to a wide range of diagnostic methods depending on the specific need. Commonly employed modalities include single-photon emission computed tomography (SPECT/CT), positron emission tomography (PET), magnetic resonance imaging (MRI) and fluorescence/luminescence spectroscopy. Out of all clinically relevant imaging modalities, MRI stands out for safety during repeated use, which is in contrast to techniques requiring high dosages of radiation like SPECT/CT and PET. PET has superior spatial resolution (4 to 5 mm) than SPECT (10 to 15 mm) and high sensitivity that can detect picomolar tracer concentrations. Although lower resolution than PET, MRI enhanced with contrast agents, such as gadolinium-conjugated NBMs and superparamagnetic iron oxide nanoparticles, can be used to characterize various features of targeted tissues. While fluorescence is impractical for clinical

applications due to poor tissue penetration, it provides unprecedent quantitative analysis of cellular targeting in animal models, where organs and cells can be routinely extracted for analysis by flow cytometry. This immunotheranostic strategy significantly enhances the ability to reproducibly elicit immune responses by monitoring which components are modulated at the cellular level during the development of vaccines and immunotherapies (10).

CONCLUSIONS AND FUTURE DIRECTIONS

Theranostic NBMs hold great promise for diagnostic imaging and controlled delivery of therapeutics during immunotherapy, providing a much-needed method of mapping and understanding the complex network of inflammatory cells contributing to elicited immune responses. The immediate future directions of theranostics will likely focus on two critical issues. First, APCs will non-specifically remove NBMs from circulation regardless of surface-conjugated targeting moieties like antibodies and peptides, making selective APC targeting difficult to achieve. Avoiding uptake by off-target APC populations will require more advanced engineering of the nano/bio-interface, such as precisely controlling the surface density and affinity of multiple targeting moieties (11), incorporating inhibitory signals like the CD47 ‘don’t eat me’ peptide (12), and optimizing NBM structure and size (13). Second, the scalable monodisperse assembly of NBMs that mimic the complex nanoarchitectures of viruses remains a challenge. Current methods usually involve impractically complex polymers, low yield of the desired nanostructure, and difficulty with therapeutic loading, particularly dual loading of hydrophobic imaging agents and structurally sensitive water-soluble biologics. Recent advances in the commercially scalable technique of flash nanoprecipitation have demonstrated the scalable assembly of complex self-

assembled NBMs from poly(ethylene glycol)-*bl*-poly(propylene sulfide) amphiphilic block copolymers (14). This method of impinging organic and aqueous phases within confined impingement jets mixers achieves highly reproducible and customizable nanoprecipitation conditions for the fabrication of polymersomes and bicontinuous nanospheres (4, 15), which are unique NBMs capable of transporting lipophilic and water-soluble payloads simultaneously.

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