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Immunological principles regulating immunomodulation with biomaterials ☆

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ABSTRACT

The immune system has evolved to recognize and eliminate pathogens; this recognition relies on the identification of structural molecular patterns within unique tissue microenvironments. Therefore, bioengineers can harness these immunological cues to design materials that modulate innate and adaptive immunity in a controlled manner. This review acts as an immunology primer by focusing on the basic molecular and cellular immunology principles governing immunomodulation with biomaterials.

1. Introduction

The most common and effective approach towards modulating immune responses is vaccination, which has been in practice since the late eighteenth century, when Edward Jenner first used blisters from milkmaids containing cowpox viral particles to immunize a young boy against the similar smallpox virus. Today, vaccines are produced commercially through a number of means and integrate synthetic chemistry, genetic engineering and bioprocess manufacturing. Commercial vaccine manufacturing elucidated the role of biomaterials when batch-to-batch variation was correlated with immunological protection. As vaccine purity was more closely monitored and improved, the effectiveness of the vaccines decreased. It was later realized that these impurities served as vaccine adjuvants or "dirty little secrets", as they were called, which promoted a stronger immune response [1]. At the time, these impurities were contaminating bacteria and aluminum salts from manufacturing vessels. Today, portions of these "contaminants" or adjuvants are added to vaccine formulations in a controlled and deliberate fashion. Beyond basic adjuvants, advanced biomaterials have enabled precise targeting of tissues and cells, as well as controlling the temporal and spatial release of immunological agents [2,3]. These advancements have not only increased the effectiveness of immunomodulatory agents, but also led to a better understanding of immune function and new methods for measuring immune responses.

Evolution has enabled the immune system to sample and recognize pathogens in a finely tuned and coordinated manner. Conversely, pathogens (and cancerous cells) are co-evolving, thus enabling them to generate escape variants that evade immune recognition. In addition, therapeutic biologics, transplants and allergens can undesirably undergo immune-based rejection. Therefore, controlled immunomodulation is critical for a variety of medical interventions, including vaccination of infectious diseases, establishing and maintaining therapeutic tolerance and cancer immunotherapy. Biomaterials have played a prominent role in the innovation of these applications and their continued engineering will help further advance the field of immunomodulation.

While biomaterials possess a tremendous capacity to improve clinical immunotherapy, it is essential for material scientists and bioengineers to understand the molecular and cellular aspects controlling the complexities of the innate and adaptive immune system. The immune system is tailored to utilize and respond to specific cues such as dose, molecular recognition, spatial pattern arrangement, physiological location, cellular trafficking and cell phenotype, which all play roles in the activation and kinetics of an immune response. This review will serve as an immunological primer by focusing on the basic molecular and cellular principles guiding immunomodulation with biomaterials. By harnessing these concepts, bioengineers can advance the field of biomaterial-based immunotherapeutics. For further expansion regarding biomaterials-based design principles that control immunomodulation, the reader is referred to this issue of *Acta Biomaterialia*.

2. Overview of the adaptive immune response

2.1. Antigen-presenting cells

The goal of immunomodulation is typically aimed at inducing specific adaptive immune responses against target antigens. This

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induction is realized through activation of antigen-specific T and B cells (discussed later). The first step in this process is antigen processing and presentation by professional antigen-presenting cells (APCs). Professional APCs are designated as such due to two major features: (1) their capacity to take up, process and display linear peptide epitopes from an encountered antigen (e.g. viral surface protein); (2) their ability to signal and direct T cells to respond in an appropriate manner. There are three major cell types considered to be professional APCs: dendritic cells (DCs), macrophages and B cells. DCs are well established to be the most important cell type for guiding immune responses and serve as a bridge between innate and adaptive immunity [4]. This prominent role has focused the majority of materials-based immunomodulation efforts to be directed at professional APCs.

The signaling ability of professional APCs, such as DCs, is largely centered on the recognition of pathogen-associated molecular patterns (PAMPs) [5,6]. Professional APCs possess pattern recognition receptors (PRR) such as Toll-like receptors (TLRs), named after the initial toll receptors discovered in Drosophila [7]. These TLRs recognize PAMPs, inducing a signaling cascade leading to activation of transcription factors such as NF-κB [8]. The activation of these transcription factors in professional APCs, in turn, induce upregulation of surface-displayed co-stimulatory molecules (e.g. CD80, CD86 and CD40), secretion of cytokines (e.g. TNF-α, TGF-β, IL-12 and IL-1) and increase surface expression of major histocompatibility complex-I and II (MHC-I, MHC-II) [3,9–15]. Therefore activation of TLRs plays a critical role in immunomodulation and is often utilized by biomaterials to enhance immune responses. Example adjuvants and their target TLRs are shown in Table 1.

2.2. Antigen presentation via MHC Class I vs. Class II

MHC presentation serves as a means to communicate with T cells, as the function of MHC presentation is either to activate antigen-specific naïve T cells or to alert previously activated T cells to the presence of their target. There are two forms of antigen presentation by MHC molecules (in humans also referred to as human leukocyte antigen (HLA)). MHC-I presentation has evolved to display endogenous antigen; in other words, this is the mechanism by which peptides from proteins produced within a cell are displayed on the cell surface. The second form of antigen presentation occurs via MHC-II and serves to take up and process exogenous antigen. The molecular basis of antigen presentation will be summarized below, but is primarily important for researchers undertaking minimalistic approaches, distilling antigen components to those required to maintain T cell activation [27]. The fundamental mechanism of antigen presentation is manifested by the APC isolating signature peptides from an antigen and associating them with an MHC molecule (peptide–MHC complex), which is then displayed on the APC surface. Naïve T cells then sample the peptide–MHC complexes with their unique T cell receptors (TCRs). When an appropriate match is found (determined by the affinity of the TCR interaction with the peptide–MHC complex), the T cell recognizes other surface molecules from the APC and cytokines in their local environment, which all cooperate to determine the fate and subsequent function of the T cell (Fig. 1).

All nucleated cells are capable of displaying peptides via MHC-I, which is critical for inducing controlled cell death (apoptosis) of cancerous cells or cells infected by pathogens. However, the initial activation of $CD8^+$ T cells is typically reserved for professional APCs due to their ability to activate T cells via co-stimulation. In the classical, or direct, pathway of MHC-I presentation, proteins synthesized by a cell are subjected to proteosomal degradation in the nucleus, cytosol and finally in the endoplasmic reticulum, where they are assembled with MHC-I molecules and then displayed on the cell surface [28]. The structure of MHC-I molecules presentation is more restrictive than class II presentation due to the closed nature of the MHC class I molecule [29]. This closed structure only allows peptides of specific lengths (typically octamers and nonamers, but up to 13mers have been observed in some alleles) to associate with the MHC-I molecule [28,30,31]. The nature of the peptide–MHC complex allows amino acids in certain positions to associate with pockets in the MHC molecule, thus displaying the remaining amino acids of the peptide to the TCR. The amino acids associating with the MHC molecule are termed anchor residues. Peptides having the correct length and the required anchor residues outcompete other peptides and are preferentially displayed to TCRs. Since MHC-I molecules are closed in structure and the positions of the pockets are well defined, in silico prediction methods are generally effective at determining whether or not a given peptide will be properly displayed, thus enabling T cell activation [32,33]. Researchers solely trying to induce $CD8^+$ T cell responses can take the protein sequence of a targeted antigen (e.g. viral protein) and develop vaccines or cellular therapies focused on predicted MHC-I antigenic peptide epitopes. However, it must also be appreciated that individuals do not carry the same set of MHC class I alleles and the mechanisms controlling peptide processing are not fully understood [34].

Conversely, MHC-II presentation is specific to professional APCs and has evolved to enable T and B cell activation against circulating pathogens. This mechanism of antigen presentation has enabled the success of most clinically approved vaccines to date, allowing for traditional vaccines to be administered and recognized by the immune system. Proteins and particulate matter that undergo phagocytosis are degraded in lysosomes. After digestion of antigens, the resulting peptides replace the invariant class II-associated Ii peptide (CLIP) on MHC-II molecules. The newly assembled peptide–MHC complex is then transported to the surface of the cell for recognition by TCRs from $CD4^+$ T cells [34]. MHC-II molecules are considered to have an "open" structure, which means that there are not strict length requirements for peptide binding. Typically, peptides ranging between 9 and 25 amino acids are found to associate with MHC-II molecules [29]. Similar requirements for anchor residues in MHC-I binding also apply to the MHC-II molecules and thus also serve to enable in silico prediction of T cell epitopes [32].

Table 1
Primary TLR targets, their ligands, and cellular location. Commonly used adjuvants in biomaterials are in bold.

TLR	Adjuvant(s)	Cellular location	References
TLR1/2, 2/6	Lipoproteins	Cell surface	[16–18]
TLR3	dsRNA, **Poly (I:C)**	Endosome	[19,20]
TLR4	LPS, **MPLA**	Cell surface	[21,22]
TLR5	Flagellin	Cell surface	[23]
TLR7	ssRNA, Imidazoquinolines, R848	Endosome	[24]
TLR8	ssRNA, Imidazoquinolines, R848	Endosome	[24]
TLR9	**Unmethylated CpG DNA** (free), ssDNA (encapsulated)	Endosome	[25,26]