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REVIEW ARTICLE

Biomaterials for Engineering Immune Responses

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Abstract | The last few decades have seen rapid progress in the fields of drug delivery systems, implantable scaffolds, and tissue engineering. Broadly referred to as biomaterials, these inter-related areas of research have many diverse applications. One such application is in the area of immunology, where biomaterials may be used as tools to modify specific immune responses. Here, individual components of the immune system are described, followed by a discussion of the recent advances in biomaterial-based strategies for the modulation of immune responses.

1 Introduction

The immune system has many diverse functions in the human body. While it is well recognized that the immune system protects against invading pathogens, recent literature has highlighted its key role in also maintaining tissue homeostasis[1, 2]. Hence, perturbations in immune cell numbers or function result in a wide variety of disorders that range from increased risk of infections to cancer[3] and even cardiovascular diseases[4, 5]. Treating these pathophysiological conditions involves the modulation of specific immune responses, which may be achieved through a variety of therapeutic strategies. Approaches that are currently used in the clinic generally rely on the systemic administration of drugs (small molecules and biologics) or adoptive transfer of engineered cells, which are associated with drawbacks such as toxicities and lack of adherence, or the need for high-end infrastructure and technical expertise.

Material-based nano-, micro- and macro-engineering tools developed over the last few decades offer solutions to the aforementioned problems, and a number of researchers have utilized these to establish new methods for engineering immune responses (Table 1). Based on the immune component that is being modulated, these biomaterial-based approaches may be broadly classified as: cell-specific (involving alteration of cell function or utilizing cells for delivering therapeutics), protein-specific (modifying amount or function of extracellular proteins of the immune system), or a combination of both. Centered on this classification, the review is divided into three major sections, each discussing current biomaterial technology for engineering the function of specific immune components. A final section provides perspectives on current research in the area of immuno-modulatory biomaterials and suggestions regarding thrust areas for the future.

2 Cells of the Immune System

2.1 Neutrophils

Neutrophils are the most abundant immune cells in human blood, are the first to respond to inflammatory signals in wounded tissues, and participate in a number of ways to clear pathogens[6, 7]. In the context of engineered systems, the action of neutrophils against foreign bodies, such as biomedical implants, is well explored and has been reviewed recently[8]. However, the idea of modulating or utilizing neutrophil activity for engineering immune responses is underexplored. One reason for this could be the relatively short half-life (< 1 day) of circulating mature neutrophils[9], which provides very little time to alter responses of these cells and expect to see an effect on other cells and tissues. Nevertheless, the ubiquity of these cells in circulation and their ability to infiltrate most inflamed tissues makes them a useful target to engineer, especially for applications involving targeted delivery of drugs. Indeed, Chu et al. utilized these characteristics of neutrophils to deliver drugs to inflamed lungs using crosslinked albumin nanoparticles that are phagocytosed by neutrophils and transported

Adherence: Regular intake of 'medicines'.

Materials: In this context, defined as any natural or synthetic substance that has been manipulated or processed in some manner.

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Table 1: Summary of biomaterials developed for modulating immune responses

Biomaterial size	Base material used	Drug or biological substance/s	Immune component targeted	Application	Refs.
Nano (≤ 100 nm in at least 1 dimension) particulates	Denatured BSA	2-[(aminocarbonyl)amino]-5-(4-fluorophenyl)-3-thiophenecarboxamide: TPCA-1	Neutrophils	Neutrophil-mediated drug delivery	10
	Crosslinked BSA	Piceatannol	Neutrophils	Modulating neutrophil function	15
	Self-assembling peptides	Various protein antigenic fragments	B cells	Periodic antigen presentation for activating adaptive immunity	96–99
	Liposome	Paclitaxel	Neutrophils	Neutrophil-mediated drug delivery	11
	Cationic lipids	siRNA against complement regulatory proteins	Complement	Anti-tumor therapeutics	125, 126
	Amphiphilic lipids	CpG	Lymph nodes through albumin	Vaccine delivery to lymph node for T-cell activation	165
	Lipids	siRNA against CCR2	Monocytes	Inhibit migration of inflammatory monocytes	166
	Lipids	mRNA	B cells	Protein production in B cells	167
	Polystyrene	Antigen	DC	Vaccination	31
	Polystyrene glyco-block copolymers	–	Macrophages	Polarization of macrophages	168
	Poly (propylene sulfide)	Drugs, or antigen (Ova or Tb protein) + CpG	DC	Vaccination	46–49
	Poly(lactic-*co*-glycolic acid)	LIF or IL-6 + CD4	T cells	Inducing specific T-cell activation	64
	Poly(lactic-*co*-glycolic acid)	Rapamycin and peptide antigens	DC	Inducing tolerance	169
	Poly(lactic-*co*-glycolic acid)—phosphatidylserine	–	T cells	Reduce effector T-cell proliferation	170
	Carbosilane dendrimers	–	Macrophages	Repolarization of macrophages	171
	Hyaluronic acid	ICAM-1 ligand	DC	Promoting DC binding and preventing its interaction with T cells	172
	Single walled carbon nanotubes	Antibodies against CD3, or peptide loaded MHC-II	T cells	Artificial antigen presenting cells	88, 89
	Iron-dextran	Tumor antigenic peptide loaded MHC-II and anti-CD28	T cells	Artificial antigen presenting cells	90

Table 1: continued

Biomaterial size	Base material used	Drug or biological substance/s	Immune component targeted	Application	Refs.
	Iron oxide	–	Macrophages	Repolarization of macrophages in tumor microenvironment	173
	Gold and silver	–	Macrophages	Polarization of macrophages	174, 175
Virus-like particles (VLP; sub-micron sized)	Hepatitis B p33 antigen or bacteriophage Qβ	CpG	DC	Vaccination	29
	Bacteriophage Qβ	IL-1α or IL-1β	B cells	Vaccination against IL-1	129–132
	Bovine papillomavirus	TNF-α peptide	Not determined	Antibody production against TNF peptide	176
Sub-micro and micro (~ 100–20,000 nm) particulates	ICMV	Antigen (Ova, pathogen or tumor proteins) + MPLA	DC	Vaccination	44, 45, 177
	Multilamellar lipid particles	IL-15 and IL-21, or Shp1/2 inhibitor	T cells	Activating anti-tumor T cells	62, 63
	Liposome	Peptide loaded MHC-II or antibodies against CD3/CD28	T cells	Artificial antigen presenting cells	82, 83
	Liposome	Albumin or conalbumin	T and B cells	Inducing cell activation and specific antibody production	178–180
	Polystyrene	–	Neutrophil	Modulating neutrophil function	22
	Polystyrene	Antibodies against CD3 and/or CD28	T cells	Artificial antigen presenting cells	78–80
	Polystyrene (Dynal®)	Peptide loaded MHC-II and antibody against FAS	T cells	Killing antigen specific T cells	100
	Polystyrene or poly(lactic-*co*-glycolic acid)	Encephalitogenic myelin peptides	Macrophages	Inducing anergic T cells and regulatory T cells	181
	Polyvinyl pyrrolidone—PEG	Antisense oligonucleotides	DC	Tolerance-generating vaccine	53, 56
	Poly lactic acid—PEG	Tetanus toxoid	–	Antigen delivery	42
	Poly(lactic-*co*-glycolic acid)	Antibodies against DEC205, or CD11c, or P-D2 peptide	DC	Targeted DC delivery	182
	Poly(lactic-*co*-glycolic acid)	Rapamycin	DC	Inducing tolerogenic DC	58
	Poly(lactic-*co*-glycolic acid)	Rapamycin or retinoic acid, and TGF-β or IL-10	DC	Inducing tolerogenic DC	183
	Poly(lactic-*co*-glycolic acid)	Vitamin D3, TGF-β, GM-CSF, insulin B peptide	DC	Inducing tolerogenic DC	184

Table 1: continued

Biomaterial size	Base material used	Drug or biological substance/s	Immune component targeted	Application	Refs.
	Poly(lactic-*co*-glycolic acid)	Chemoattractants	DC	DC recruitment for potential vaccine	65
	Poly(lactic-*co*-glycolic acid)	CCL22	Regulatory T cells	Treg recruitment for tolerance	67, 68
	Poly(lactic-*co*-glycolic acid)	Antibodies against CD3, CD28. And IL-2	T cells	Artificial antigen presenting cells	86, 87
	Poly(lactic-*co*-glycolic acid)	Peptide loaded MHC-II + anti-CD28. Antibodies against PD1	T cells	Artificial antigen presenting cells	91
	Poly(lactic-*co*-glycolic acid)	IL-2, TGF-β, and rapamycin	T cells	Inducing Treg from naïve T cells	103
	Poly(lactic-*co*-glycolic acid)	IL-2, TGF-β, and antibody against CD4	T cells	Inducing Treg	104
	Poly(lactic-*co*-glycolic acid)	siRNA against IL-10, CpG	DC	Activating T cells	140
	Poly(lactic-*co*-glycolic acid)	Poly-IC, ovalbumin	Lymph node (DC)	Activating T and B cells	149
	Poly(lactic-*co*-glycolic acid)	EAE antigens	Macrophages and DC	Inducing tolerance	185, 186
	Polyplexes	Etanercept	TNF-α	Blocking TNF activity	136
	Acrylate-based	IL-1Ra	IL-1 receptor	Blocking IL-1 signaling	133
	Alginate	Various chemokines	DC	DC recruitment for potential vaccine	66
	Alginate	IL-10 encoding plasmid	Macrophage	Generating suppressive macrophages	139
	Gelatin	CpG, antigen	DC	Vaccination	30
	Calcium phosphate	Hen egg lysozyme antigen	B cells	B-cell activation	187
Macro (> 20 μm) particulates and scaffolds	Poly(lactic-*co*-glycolic acid)	GM-CSF, CpG and tumor lysates	DC	Vaccination (anti-tumor)	37, 38
	PEG	Peptide functionalized	TNF-α	Capturing TNF and preventing its activity	138
	PEG (or gelatin)	Peptide functionalized	Lymph node	Scaffolds for artificial lymph nodes	147, 148
	Alginate	–	Neutrophils	Assessing limits of neutrophil generation	14, 21
	Alginate modified with peptide, silica microparticles	Antibodies against CD3, CD28 and CD137, IL-15 super-agonist	T cells	Expanding and maintaining tumor-reactive T cells, similar to artificial lymph nodes	188

Table 1: continued

Biomaterial size	Base material used	Drug or biological substance/s	Immune component targeted	Application	Refs.
	Collagen	Stromal cells + DC, or chemokines	T and B cells	Recruitment of cells to form tertiary lymph nodes	144–146
	Gelatin + silicate nanoparticles	Stromal cells expressing CD40L and BAFF	B cells	Ex vivo B cell culture	154, 155
	PEG based with photoinferter	Antibody against FAS	T cells	Inducing T-cell apoptosis	189
	Puramatix (TM) peptide, PLGA	GM-CSF, CpG, Insulin	DC	DC recruitment and tolerogenic vaccine	190

across epithelial barriers to the site of inflammation[10]. More recently, a similar approach was developed by Xue et al. for delivery of paclitaxel to brain tumors (mouse model of glioma) using neutrophils as they efficiently cross the blood brain barrier[11]. While the development of such neutrophil-based drug delivery systems is still in its infancy, they show tremendous promise.

Another reason that neutrophil modulation has not been explored much is that until recently, neutrophils were thought to play a limited role in immune responses to non-pathogenic diseases. A number of studies now show that neutrophils are relatively abundant at sites of autoimmune disease as well as in the tumor microenvironment, and that there are a number of functional subsets of these cells with potentially different functions[12,13]. Of these subsets, a significant proportion are potentially suppressive, and it remains to be seen if they may be exploited to either reduce neutrophil-associated damage[8, 14] or modify the immunosuppressive tumor microenvironment. Alternately, pro-inflammatory neutrophils present at sites of tissue damage may be targeted through drug delivery carriers to inactivate them. One such system has been described by Wang et al. where they use an albumin nanoparticle system to deliver a drug that blocks integrin signaling in neutrophils, thereby neutralizing their ability to enter or bind to inflammatory tissues[15]. Similarly, Vij et al. developed non-steroidal anti-inflammatory drug-loaded poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles, whose surface was modified with antibodies to target neutrophils. In a mouse model of obstructive lung disease, this system showed good efficacy[16]. While in theory targeting of neutrophils (or other immune cells) is possible using antibodies or ligands that bind to cell-surface receptors, in practice the efficiencies are moderate at best (that is, particles are likely going to be taken up by phagocytic cells non-specifically), and there are very few unique and cell-specific (or disease-specific) receptors available for targeting.

Subsets: Immune cells may express a specific set of genes following activation that differentiates them from their non-activated or differently activated counterparts. One classic example is the Th1 and Th2 helper subsets of CD4 expressing helper T cells.

A different approach to engineer neutrophil responses is to modulate their production. While there is ample information on the rate of neutrophil maturation in the bone marrow and their half-life in blood[17], we do not yet know the maximum rate at which these cells may be produced. Increased production of neutrophils (granulopoiesis) is known to occur following systemic infection, which has been studied in detail by Manz and colleagues[18, 19], and others[20]. As the need for neutrophils in circulation or peripheral tissues increases (following inflammatory stress),

Half-Life: In this context, time in which half the number of original cells are removed from circulation.

granulocytic progenitors have been shown to divide more[21], and it has been observed that immature neutrophils are released into circulation from the bone marrow[20]. Theoretically, it would be expected that a maximum rate of neutrophil production would be reached at some level of inflammatory stress; however, the same has not been demonstrated experimentally. Utilizing pathogenic agents to achieve a level of local inflammatory stress that results in neutrophil production maxima may be difficult to achieve, as these agents generally result in systemic issues in the animal model used for such experimentation. To avoid this specific problem, we have shown that a sterile biomaterial implant model may be used to generate local inflammation without systemic side-effects. Additionally, the data suggest that neutrophil numbers at implant-sites rises with increasing implant numbers (a surrogate similar to number of infecting organisms), but eventually a plateau in cell accumulation is reached[14, 21]. Similarly, Fromen et al. observed that injecting a large number of nanoparticles that are phagocytosed by neutrophils resulted in the accumulation of these cells in the liver (possibly for clearance), and thereby reduced accumulation of neutrophils at sites of tissue injury[22]. Put together these reports are preliminary evidence of potential maxima in neutrophil generation. Additional studies are required for confirmation of true maxima in cell production. Further, it remains to be seen if the potential limit to neutrophil production/recruitment/function observed in these studies may be exploited for improving biomaterial compatibility, or in other conditions where neutrophil responses are not desirable.

2.2 Dendritic Cells, B Cells and T Cells

Dendritic cells (DC) are professional antigen presenting cells (APC) that link the innate and adaptive arms of the immune system. Their primary function is to process biomolecular components from pathogens (non-self) or apoptotic cells (self), and present them to lymphocytes in an immunogenic context leading to the initiation of an adaptive immune response[23]. B and T cells are the cellular arm of the adaptive immune system, whose main goal is to eliminate pathogens or cells displaying molecules that are non-self, while inducing tolerance to cells presenting self-molecules. Together, these three cells control a significant proportion of immune response that the body generates, and engineering their function would be useful for the treatment of a wide variety of disorders. Concepts related to engineering adaptive immunity are discussed in three sub-sections below.

FDA: Food and Drug Administration of the Govt. of the United States of America.

2.2.1 Immuno-Activation

The knowledge that the immune system is capable of 'remembering' past encounters with pathogens and preventing repeat infections resulted in the human endeavor to develop safe methods to generate memory immune responses against dangerous pathogens. The primary approach to achieve the same was to actively expose individuals to specific quantities of either the whole pathogen or components of it, which did not result in a full blown infection but was sufficient to generate protective immune response[24]. This process, termed as inoculation, gave rise to today's vaccination strategies. Vaccines are composed primarily of two components; an antigenic component (live, attenuated or killed pathogen, or a mixture of proteins from the pathogen) and an adjuvant (which activates the antigen presenting cell). However, a simple combination of these components is often either insufficient to generate strong and protective immunity, or results in toxic side-effects[25, 26]. A number of factors influence the efficacy of vaccination. First, both antigen and adjuvant must be co-delivered to the same APC to induce strong and specific response[27, 28]. It has been shown that delivery of adjuvant in the absence of antigen could result in unintended acute inflammation[29, 30]. Second, both components may need to be transported to precise tissue compartments, such as the lymph nodes, to induce effective responses[31, 32]. Third, protein antigens (not associated with the entire pathogen) may need to be displayed in a specific geometry for the immune system to recognize it as a pathogen-associated molecular pattern resulting in robust responses[25]. Fourth the kinetics as well as the amount of antigen play a crucial role in determining the type of immune response[33, 34].

Defined synthetic nano- and microparticles (with specific size, shape and surface properties) have enabled the development of vaccines that are capable of overcoming many of the aforementioned problems. Virus-like particles (VLPs) are possibly the most developed system, with a number of FDA-approved and commercially available vaccines utilizing them for antigen-adjuvant delivery (reviewed by Bachmann and Jennings[25], and Zeltins[35]). VLP-based vaccines are primarily against viral pathogens, may be expensive to formulate, and not all viral proteins self-assemble to

form particles. Hence, numerous other biomaterial systems are simultaneously being explored for vaccine applications[36]. One such system has been developed by Mooney and colleagues is a PLGA scaffold loaded with DC recruiting chemokine (GM-CSF), activating adjuvant (CpG), and antigens (tumor lysates) that generates robust anti-tumor immunity[37, 38]. This system is currently being tested in a Phase I clinical trial for the treatment of melanoma. The same system has also been used to identify specific components of the immune system that respond to vaccinations[39]. While showing high efficacy, such depot systems involving DC recruitment may be expensive to manufacture and use clinically.

Particle systems loaded with antigens and adjuvants, which actively or passively target APC may be used as an alternative. Numerous particle-based vaccine strategies that involve DC targeting for modulation of function have been developed (reviewed by Singh et al.[40]), and over a dozen are currently being tested in clinical trials[41]. Many others are still under development in laboratories, and show tremendous promise. Among these, a significant proportion is being developed for mucosal delivery due to the ease of administration using this route. Some of the early work in this area was done by Alonso and colleagues, where they demonstrated that polyethylene glycol (PEG)-coated poly-lactic acid particles were capable of carrying antigen across the nasal mucosa with localization in lymph nodes[42, 43]. In these studies, while immunoglobulin production suggested effective activation of adaptive immunity through DC, targeted delivery to these cells was not shown. More recently, Irvine and colleagues have developed an antigen-encapsulated multi-lamellar lipid vesicle that presents lipid-based adjuvants on its surface resulting in strong T cell and antibody responses[44]. Further, the same group describes the use of this system for effective nasal delivery of antigens, and elegantly showed that lung APC is targeted to generate memory T-cell responses[45]. Similarly, Swartz, Hubbell and colleagues have developed an appropriately sized polymeric nanoparticle capable of delivering antigen to DCs in the lymph nodes. Their studies show that a 20-nm poly (propylene sulfide) particles loaded with antigen preferentially (when compared to 100-nm particles) localizes in the lymphatics, is taken up by lymph node resident DCs, remains there for up to 5 days, and generates strong immune responses against the antigen[46, 47]. Further, this system may be delivered through intradermal or pulmonary routes, activates DC and results in robust cytotoxic T-cell responses[48, 49]. Progress on any of these or one of the many other systems not discussed here holds promise for the development of particle-based vaccines against many human pathogens.

2.2.2 Tolerance

As our understanding of the role of APC in activating adaptive immune responses improved, it became clear that the same interactions could be used to suppress immunity or generate tolerance. APC activate T cells through three signals: MHC–TCR interaction (signal 1), co-stimulation (signal 2), and cytokines (signal 3)[23] [also discussed by Chandele in this issue]. Alteration or absence of signal 2 and/or 3 results in the generation of antigen-specific T cells that are either anergic (non-responsive), exhausted (unable to initiate immune action against antigen-expressing pathogen/cell), suppressive or regulatory in nature. This concept may be utilized to engineer tolerance-generating DC[50], which may be desired in the context of disorders involving an overactive immune system (autoimmunity, allergies, etc.), or when immune activity is not desired (transplantation or foreign body implantation).

Tolerogenic DC have been produced ex vivo through a variety of methods: (i) by Steinman and colleagues through ex vivo culture in the presence IL-4 and GM-CSF resulting in immature DC[51, 52]; (ii) by Giannoukakis, Trucco and colleagues through treatment of DC with antisense RNA that reduces levels of co-stimulatory molecules[53]; and (iii) through the use small molecules (reviewed by Thomson and colleagues[54, 55]). However, approaches involving ex vivo cell modulation are expensive, and require facilities that are beyond the reach of many. Alternatives to these approaches are the use of particulate systems capable of delivering the same agents to DC in vivo. Indeed, Giannoukakis, Trucco and colleagues have shown that a microsphere-based approach may be used to deliver their combination of therapies to DC for inducing tolerance[56, 57]. We have also shown that delivery of the small molecule rapamycin in ~ 3-μm sized PLGA particles to DC results in immunosuppressive cells[58], and suggested that a similar strategy may be used for combinatorial delivery of different agents to induce tolerogenic DC[59]. A number of similar approaches utilizing particles and scaffolds have been developed by Roy and colleagues, Shea and colleagues, and Keselowsky and colleagues among others, which have been recently reviewed here[60, 61].

Tumor Lysates: Broken down and possibly enzymatically digested components of tumor cells/tissue. Usually implies a combination of peptides and proteins.

Mucosa: The lining of body cavities (such as oral, nasal, gastrointestinal etc.)

Tolerogenic: Combination of the words tolerance and generating.

2.2.3 Direct Modulation of T-Cell Function

Modulating T-cell activity through changes in DC function normally ensures antigen-specificity, which is otherwise cumbersome to achieve. Nevertheless, bypassing DCs to engineer T-cell function directly may be desirous in many circumstances. A classic example involves developing methods to improve activity of tumor antigen-specific T cells, which do not function effectively in the immunosuppressive tumor microenvironment. While small molecules and protein-based therapeutics to enhance activity or block suppression of T cells are well known, delivering them to antigen-specific T cells is a challenge. Irvine and colleagues developed a method of overcoming this problem through ex vivo alterations to T cells that could help them function in immunosuppressive environments. They showed that the efficacy of tumor antigen-specific T cells may be greatly improved through ex vivo attachment of synthetic nanoparticles containing the cytokines IL-21 and a super-agonist version of IL-15 (for activating T cells in an immunosuppressive tumor microenvironment) to the surface of these cells. Such a modification resulted in robust proliferation of the tumor (melanoma) antigen-specific T cells, elimination of tumor cells, and the establishment of a memory T-cell population[62]. In addition, the same strategy was used to provide drugs that block suppression of T-cell activation resulting in anti-tumor responses in a mouse model for prostate cancer[63]. Fahmy and colleagues used a similar strategy of attaching nanoparticles containing the immunomodulatory cytokine LIF (leukemia inhibitory factor) onto helper T cells using an antibody against the surface protein CD4 (expressed exclusively on helper T cells), resulting in effector T cell suppression and Treg generation, leading to prolonged survival of heart transplants[64].

Ligands: In this context, a molecule (carbohydrate, peptide or protein) that binds to a specific larger biomolecule (usually a protein).

Another example of directly affecting T cell function is recruiting specific sub-classes of cells to tissue sites where they may normally not be present. It is well known that cells move up a chemoattractant gradient, and controlled release technology may be used to established artificial gradients for cell recruitment[65, 66]. Utilizing this technology, we developed a degradable microparticle system for releasing a regulatory T cell (Treg)-specific chemokine, CCL22, and demonstrated in vivo recruitment of these cells[67]. Such a system was able to suppress inflammation associated with periodontitis, as shown in a mouse and canine model of the disease[68].

Chemoattractant: A chemical molecule, in this case proteins, that attracts motile cells.

Alternately, T cells may be utilized for delivering therapies as shown by Vile and colleagues. Their strategy involved utilizing tumor antigen-specific T cells to deliver therapeutic viral vectors. The authors observed that viral vectors passively adsorb onto T cell surfaces, which can be used to modify tumor antigen-specific T cell surfaces before adoptively transferring them into mice-bearing B16 melanoma cells. The viral vectors that 'hitchhiked' on T cells were able to transfect tumor cells and enhance anti-tumor responses[69]. Such 'hitchhiking' strategies have now been used by many others to deliver drugs to a variety of tissues in vivo.

2.2.4 Artificial Antigen Presenting Cells

An alternate approach to engineer DC function is replacing them entirely with an artificial system, termed as artificial antigen presenting cells (aAPC). Cellular systems that function as aAPC have been in widespread use for over two decades now. Cells from the fruit-fly (Drosophila) were among the first to be modified to function as aAPC by Sprent and colleagues[70, 71], and subsequently both mouse[72, 73] and human[74, 75]-derived cell lines have been established for the same purpose. While the cell-based systems are effective, they lack in vivo translatability, and it is difficult to control the number and type of molecules (signals) presented by these cells (reviewed by Kim et al.[76], and Turtle and Riddell[77]).

Acellular systems that are capable of overcoming these drawbacks have also been developed simultaneously. One of the first such systems was described by Curtsinger et al.[78], where they used polystyrene particles coated with ligands and antibodies that would bind to and initiate signaling through proteins on the surface of T cells. The ligands and antibodies were specifically those that were involved in APC-T cell immunological synapse formation (molecules that provide signal 1 and 2). Similarly, June and colleagues utilized antibodies against CD3 and CD28 coated on Dynal® magnetic beads (developed by John Ugelstad and colleagues) to induce T-cell activation and expansion[79, 80]. Induction of antigen-specific T-cell proliferation was also possible using the same system through a MHC-peptide tetramer in place of the antibody against CD3 (reviewed by Turtle and Riddell[77]). Nevertheless, they are associated with two major drawbacks—the surface of this system was rigid, which prevented ligand/antibody movement on the particle surface (as would occur on DC), and it was unable to provide signal 3 (cytokines) for appropriate activation of T cells.

The first problem was addressed around the same time the rigid systems were being developed by Dustin and colleagues, who used supported lipid bilayers bearing molecules that provide signal 1 and 2 to show that the immunological synapse is dynamic[81]. Soon after, Prakken et al. described the development of liposome system incorporating MHC-II-peptide to mimic an APC[82]. A similar liposome-based approach having three pre-clustered antibodies to interact with T cells was developed by Zappasodi et al.[83]. Likewise, it has been shown that exosomes (liposomal vesicles released by cells) from DCs and B cells that have MHC-II molecules as well as co-stimulatory receptors could be used as aAPC[84, 85]. The advantage of liposome and exosome systems is their ability to not only mimic the biochemical signals, but also provide biomechanical cues that are similar to a natural APC.

The second problem of the absence of signal 3 was addressed by Fahmy and colleagues, where they encapsulated IL-2 in PLGA particles and subsequently coated with antibodies against CD3 and CD28, to provide all three signals to T cells[86, 87]. The same group has developed carbon nanotube-based systems for similar applications[88, 89]. Similar work has been performed by Schneck and colleagues on developing synthetic systems that replicate APC function[90, 91], and more recently on addressing the issue of minimum aAPC particle size[92]. A number of other aAPC systems have been developed over the past two decades for understanding the biology of the immunological synapse and for inducing T-cell activation (reviewed by Irvine and Doh[93], Delcassian et al.[94]).

Another group of aAPC systems are self-assembled biomaterials that are able to present high densities of antigen in a spatially organized manner to induce B- and T-cell responses. A repetitive and ordered arrangement of proteins on the surface of pathogens results in direct B-cell activation through antigen-specific B-cell receptors, and it has been shown that the number of antigens displayed dictates the type and strength of the immune response[95]. Collier and colleagues utilized this feature to develop peptide-based self-assembled systems that directly engage with adaptive immune cells. They have shown that incorporating antigenic epitopes into a peptide assembly results in the robust production of antibodies directed against the epitopes in the absence of any additional adjuvant[96], and that such a system could be used to develop a vaccine-like therapy against malaria[97]. Further development of the same system, resulted in control over the dose of antigen displayed and capability of simultaneous display of multiple proteins, thereby enabling strong multi-antigenic immune responses[98]. Importantly, in the absence of the antigenic epitopes, the self-assembled peptide was not immunogenic[96, 99]. Others have also developed self-assembled systems for activating immune responses, which are discussed in greater detail by Webber and colleagues in another article of this issue.

aAPC may also be used to generate tolerance, which is achieved by presenting signals that induce T-cell death or act as inhibitors/modulators of T-cell activation. An example of the former approach, Schütz and colleagues have developed a killer aAPC by attaching a HLA-A2-Ig dimer molecule (that may be loaded with peptides) and an antibody against FAS (that interacts with FAS expressed on T cells) on epoxy beads, which induces apoptosis in antigen-specific T cells[100, 101]. An example of the latter is our work on inducing Treg by activating naïve T cells in the presence of immunomodulatory small molecules and cytokines. We show that a combination of IL-2, TGF-β and rapamycin is able to stably induce Treg in the presence of artificial activation signals[102], and that controlled release of these molecules from PLGA particles[103] could potentially be used as a method to generate tolerance. A similar approach was utilized by McHugh et al. to deliver IL-2 and TGF-β to T cells using nanoparticles whose surface was modified with an antibody against CD4[104].

While many of the aforementioned systems are being used in research laboratories, the clinical translation of aAPC for in vivo use is yet to occur. A major challenge with translation is the number of unique molecules or entities that are required to form a fully functional aAPC. For example, a simple acellular aAPC system has at least four distinct components—base material (lipid, metal, polymer, etc.), linker to attach antibodies on material surface, an antibody or ligand mimicking MHC–TCR interaction, and another antibody or ligand providing co-stimulation. Understanding toxicity, compatibility and degradation of all of these materials individually as well as in combination may prove to be a major challenge.

2.3 Other Immune Cells

2.3.1 Monocytes and Macrophages

Monocytes and macrophages play important and well-documented roles in protecting our body

Liposome: An enclosed biomolecular entity whose surface is lined with phospholipids, which separate the components inside the enclosure from the outside.

Immunological Synapse: A junction between DC and T cells, where the two cells are communicating through direct contact and/or secreted molecules.

Translation: In this context, the process of developing basic research performed (or technology developed) in a laboratory to a product that is used in the clinic, following tests for safety and efficacy.

against pathogens, maintaining tissue homeostasis, and assisting with the repair and regeneration of injured tissues[105–107]. Simultaneously, these cells are also known to contribute to or be the cause of many pathologies, including promoting an immunosuppressive microenvironment in tumors[105, 108]. Thus, it is believed that engineering monocyte/macrophage function has the potential to treat many diseases. Modulating their activity may be achieved through methods and technologies described above for neutrophils and DC, and will not be discussed here as they have been reviewed recently[106, 107, 109]. In addition to these approaches, macrophages may be used to study biological processes occurring at various tissue sites due to their ubiquitous presence in the body. A number of nanoparticle-based materials for in vivo imaging of macrophages have been developed (reviewed by Weissleder et al.[110]), and examples of their utilization are recent studies on detecting macrophages in atherosclerotic plaques or imaging tumor-associated macrophages[111] to characterize efficacy of anti-tumor treatments[112]. Similar studies on in vivo macrophage function at diseased tissue sites promise to provide better mechanistic understanding of the disease itself.

A number of other immune cell types, such as NK cells, eosinophils, basophils, mast cells, and innate lymphoid cells, have diverse roles in limiting pathogenic infections as well as maintaining homeostasis. However, studies on engineering their activity are sparse, possibly because a lot remains to be understood about their biology.

3 Proteins (Extracellular) of the Immune System

One of the cornerstones of modern immunological research is the study of non-cellular factors (proteins) present in blood that helps in the fight against infections. In fact, the first immunotherapies developed (last decade of the 19th century) were mixtures of proteins present in the blood (primarily antibodies), then termed as antitoxins or antiserum[113]. Since then a number of extracellular peptides and proteins have been identified, which have active roles in immune responses against pathogenic organisms as well as for the maintenance of tissue homeostasis. Possibly the most well-studied, engineered, and utilized among these proteins are immunoglobulins, also referred to as antibodies. Antibodies are examined in detail in another article in this issue (Dhar and Das), and hence will not be discussed here.

3.1 Complement

Another group of well-studied proteins are those that belong to the complement system. The complement system consists of multiple circulating proteins, which upon activation assist in establishing an inflammatory immune response by recruiting various cells, enable clearance of antibody-bound infectious agents (classical pathway), and could result in direct killing of pathogens or host cells through the creation of pores in the membrane (alternate pathway)[23, 114]. Their activity is important for neutralization of pathogens as well as the opsonization of antibody-bound cells (which could be foreign cells, pathogen-infected cells or aberrant cells). However, their activity is also known to create problems for biomaterial implants as well as biomedical devices that come in contact with blood[115]. With respect to biomaterials being implanted in vivo, complement proteins along with a host of other serum proteins reversibly bind and form a coating on implant surfaces[116]. The protein layer leads to the activation and adhesion of immune cells such as monocytes[117–119], which usually results in fibrosis of the implant. Similarly, binding of complement proteins, among others, to blood contact devices results in activation of coagulation cascades as well as secretion of inflammatory mediators[120]. Avoiding these complications, engineering the surfaces of implants and devices has been one of the major research topics in the field of biomaterials, and has been reviewed by Ekdahl et al.[121].

Independent of the research on avoiding complement binding to biomaterial surfaces, a significant amount of work has focused on strategies to improve complement binding to tumor cell surfaces. Host cells unlike pathogens, express a variety of membrane-bound proteins that regulate complement binding to cell surface, and hence are not killed. Tumors exploit this strategy by overexpressing complement regulatory proteins (CRP) to prevent complement-mediated cell cytotoxicity following administration of tumor antigen-specific antibodies[122]. To overcome this problem, Kirschfink and colleagues (among many other groups) have shown that antisense oligonucleotides against the CRPs CD46 and CD55[123], or siRNA against CD46, CD55 and CD59[124] may be used to increase sensitivity of tumors to complement attack. Additionally, the same group has shown that siRNA against CRPs may be encapsulated into liposomal particles for increased delivery to tumors[125], which can also be actively targeted using transferrin molecules coupled to the liposome[126]. These strategies are

Complement: The word to describe these proteins was introduced by Paul Ehlrich, to emphasize their role in 'complementing' the activity of antitoxins (antibodies) in killing pathogenic organisms.

Overexpression: In this context, increased production of a specific protein.

able to increase complement-dependent tumor cell cytotoxicity either by themselves or when used in combination with tumor antigen-specific antibodies and macrophages. Nevertheless, following the observation that mice lacking specific complement proteins show increased tumor growth, number of reports have suggested that complement proteins may assist in carcinogenesis through recruitment of immunosuppressive cells, or by promoting cancer cell metastasis (reviewed by Pio et al.[127]). Hence, the strategy of modulating complement regulatory protein expression for the treatment of tumors is being reconsidered.

3.2 Cytokines

Typically associated with cellular immunity, cytokines are being discussed here in their role as extracellular mediators of immune responses. Cytokines collectively refers to a large number of relatively low molecular weight proteins that are involved in diverse functions such as activation of the immune system (both inflammatory as well as regulatory activation), promoting cellular proliferation, recruitment of immune cells, and even in developmental processes. Their aberrant function has been linked to many disorders, and numerous therapeutic strategies are being developed to modulate their activity. While a few antibody-based approaches to limit cytokine activity are already available in the market (reviewed by Dhar and Das), altering cytokine activity using particulate systems has been a major focus of biomaterials research over the last decade. A few of these strategies that affect activities of specific cells have been discussed in previous sections. Here, approaches that affect a plethora of immune functions through modulation of single cytokine activity will be elaborated.

Two specific cytokines belonging to the interleukin 1 family, IL-1α and IL-1β, are among the most studied and targeted for therapeutic purposes. Both cytokines are pro-inflammatory and major players in a number of inflammatory and autoimmune diseases. Suppressing their activity is achieved through an endogenous molecule, IL-1 receptor antagonist (IL-1Ra), which has been developed and FDA approved [recombinant version of IL-1Ra, named Anakinra first developed by Amgen (USA) and now licensed by Swedish Orphan Biovitrum AB (Sweden)] as a therapeutic for rheumatoid arthritis. While showing high efficacy, IL-1Ra needs to be administered at frequent and high doses, and increases susceptibility to infections and allergic responses[128]. To overcome the first problem, a team of researchers from Cytos Biotechnology Ltd. (now Kuros Biosciences, Switzerland) developed a vaccine against IL-1α and IL-1β using bacteriophage VLP, which upon administration resulted in reduced cartilage damage in a collagen-induced arthritis model in mice[129]. The same group has shown that their IL-1α vaccine may be used for protection against atherosclerosis[130] and the IL-1β vaccine shows high efficacy in a mouse model for type-2 diabetes[131] and safety in humans[132]. While the safety data in humans is promising, approaches that involve vaccination against self-proteins can carry the risk of developing autoimmune responses over the long-term, and suppressed responses against infectious agents.

An alternate approach to solve the issues associated with systemic delivery of IL-1Ra or inducing systemic immune responses against the IL-1 cytokine family is the use of particulate systems for the local delivery of IL-1Ra. García and colleagues have developed an acrylate-based block co-polymer that encapsulated IL-1Ra in self-assembled nanoparticles, which upon injection into intraarticular spaces of a rat model showed increased local retention time and efficacy in inhibiting IL-1-mediated signaling[133]. Using a slightly modified polymer backbone to develop nanoparticles of larger size, the same group then showed that IL-1Ra could be delivered to the joint tissue for up to 14 days[134] (compared to 3 days in the previous study) and these particulates blocked IL-1 β signaling in vitro[135]. It remains to be seen if such strategies will show efficacy in preclinical animal models and can be developed into therapeutics for clinical use.

Another cytokine that is targeted for therapeutic purposes is TNF-α. Both monoclonal antibodies (Adalimumab) and fusion proteins that are TNF-α inhibitors (Etanercept) are currently being used in the clinic, and function by blocking the activity of TNF. To improve the biological stability and bioavailability of etanercept, methods to encapsulate it in particle formulations have been developed by Jung et al.[136] and Ferreira et al.[137]. A different approach used by Lin et al. is the fabrication of a PEG hydrogel containing a peptide agonist to TNF-α, which was capable of sequestering the soluble TNF-α and preventing its harmful activity on cells encapsulated in the hydrogels[138]. Similar single cytokine modulating strategies have been used for altering IL-10 amounts. Jain et al. delivered IL-10 coding plasmid DNA using alginate nanoparticles in a rat model of arthritis resulting in increased IL-10 presence and repolarization of macrophages to the M2 phenotype[139]. Contrastingly, Pradhan

Endogenous: Found naturally in the body.

et al. delivered IL-10 siRNA in combination with oligonucleotide CpG adjuvant to DC, resulting in enhanced Th1 to Th2 cytokine ratio[140]. While many of these approaches are promising, pleiotropy and redundancy is known to be high among cytokines and their receptors[141], which suggests that targeting any one molecule may not result in the efficacy required for clinical application.

4 Organs of the Immune Systems and Cell Production

Most immunological reactions involve a combination of multiple soluble factors and cells, and modulating any one may not sufficiently affect an immune response. This understanding has led to an interest in altering multiple immune components simultaneously, and one strategy to achieve this is through fabrication of engineered secondary lymphoid (spleen, lymph nodes and Peyer's patches) organs. The idea of creating a lymphoid tissue stems from the observations that new lymphoid-like structures (tertiary lymphoid organs) are formed during infections, transplant rejection, and in autoimmune disease (reviewed by Drayton et al.[142] and Aloisi and Pujol-Borrell[143]). Artificially creating tertiary lymphoid organs holds promise for inducing a coordinated and specific immune response. Suematsu and Watanbe were among the first to fabricate a tissue-engineered lymphoid structure using sponge-like collagen scaffold. These scaffolds were loaded with a mouse stromal cell line expressing the chemokine LT-α and containing DC, which resulted in the recruitment of a large number of T and B cells, and the formation of compartmentalized structures similar to a natural lymph node[144]. Additionally, Watanbe and colleagues showed that transplantation of explanted artificial lymph node (generated in mice) to naïve wild-type mice resulted in the production of secondary immune responses, and transplantation to SCID mice resulted in the production of memory B and T cells[145]. More recently, the same group has modified these scaffolds by replacing the stromal cells with chemokine-releasing gelatin hydrogels, which also leads to the establishment functional lymph nodes[146]. Around the same time as the original publication by Suematsu and Watanbe, Irvine and colleagues formulated a porous PEG hydrogel loaded with DC and T cells that could be used for creating new lymphoid structures[147]. They were also able to modify the gels using collagen to increase the number of immune cells present in the scaffold and functionalize them with polysaccharides for chemokine and cytokine binding[148]. However, the establishment of an artificial lymph node alone is not sufficient to obtain desired immune responses. Additional modulation, possibly in the form of sustained release of adjuvants as shown by Jewell et al.[149], or presentation of specific antigens as demonstrated by Mooney and colleagues (reviewed by Gu and Mooney[26]), or creating an immunosuppressive environment as shown by us[103], would generally be necessary. Further, questions regarding the provision for single specific or multiple antigenic stimuli to ensure that the stimulated T and B cells do not cross-react with self-antigens, and the immuno-compatibility as well as degradability of the components used to make the artificial lymph nodes need to be answered prior to the translation of such technology into the clinic.

Aside from modulating function, engineering tools may also be used to produce immune cells. One of the first advances in this area was brought about by Scadden and colleagues, who constructed thymus (a primary lymphoid organ) mimicking scaffolds that brought together mouse thymic stromal cells and human hematopoietic progenitor cells to induce the production of T cells bearing a diverse set of T cell receptors (capable of recognizing different ligands)[150]. Similarly Krupnick et al. used crushed bones mixed with type-I collagen to produce a scaffold that developed both new bone and bone marrow following implantation in mouse small bowel mesentery[151]. Using biomaterial-based matrices and microfluidic chips, many comparable approaches have since been developed for ex vivo and in vivo culture of bone marrow cells and immune cell production[152, 153]. Recently, Singh and colleagues developed a novel method for ex vivo generation of lymphoid tissue, which they term as B-cell organoids[154, 155]. In this approach, engineered stromal cells were co-cultured with naïve B cells in a hydrogel scaffold made of gelatin reinforced with silicate nanoparticles, which resulted in robust B-cell proliferation and activation. Such systems enable fundamental studies on immune cells, help in the establishment of in vitro models for research, and develop a method to generate human or human-derived cells for therapeutic purposes.

5 Perspectives

Over the past few decades, a diverse array of biomaterials have been developed or are under development in laboratories across the world. Each new biomaterial differs from others in its

material composition or physical properties (such as shape and size). While it is believed that the availability of a large pool of chemically and physically diverse biomaterial tools may enable better and faster development of therapeutic technologies, there is a risk of focusing excessively on developing new biomaterials and diverting resources away from the eventual goal of clinical translation. With regard to this issue, two suggestions are put forth: (i) increased attention towards studies that assess safety of previously developed immuno-modulatory biomaterials (especially the effects of non-specific interactions, which are usually much more than expected), such that the same base material may be used for other applications; and (ii) an emphasis on academic–industry collaborations to ensure development of biomaterials that show good efficacy in laboratory studies.

Of the numerous biomaterials for modulating immunity (Table 1), only a few are in clinical trials[41], and a significant majority of those are particulate- and scaffold-based vaccines. This trend is possibly due to the prominence of immunological studies on infectious diseases and the generation of memory responses against pathogens, over the last half century. With recent focus on understanding immune involvement in tissue homeostasis, tumor development, and generation of tolerance, there is an expectation that biomaterial technologies will have an increased role to play in future therapeutics for the treatment of cancer and autoimmune diseases. However, research on these fronts has also led us to recognize that the immune system is more than the sum of its individual components. While there are a few examples of pathological conditions where an individual molecule or a specific pathway in a cell is affected, more often the condition is a result of the actions (or misactions) of a number of different immune components. Hence, greater emphasis needs to be placed on systems approaches to study immunity (an example is the systems immunity program for infectious diseases[156]), and develop technologies that modulate multiple immune pathways simultaneously.

Finally, a large number of technologies which work well in the laboratory and in preclinical animal models (primarily rodent) of research do not translate to humans. One of the reasons for the lack of translation is the difference in mouse and human biology. These differences are especially true with respect to the immune system[157, 158], and while a few of the differences have been identified a lot remains to be discerned[159]. Immunologists and clinicians working on the immune system have recognized this discrepancy, and have initiated efforts to correct it[160–162]. These efforts range from a greater emphasis on studying the human immune system to using "dirty" (harboring or having been exposed to normal pathogens) mice[158, 163] and humanized mouse models[164]. Biomaterial engineers and scientists, however, continue to primarily rely on historic rodent models of research. A shift towards testing biomaterials developed to engineer immunity on human immune cells as well as in rodent models that more accurately replicate human systems is required.

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