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The effects of properties and interactions of surface molecules in antigen presenting cells on T cell activation

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인공 항원제시세포의 표면 분자의 특성 및 상호작용이 T 세포 활성화에 미치는 영향

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Abstract Efficient production of antigen specific cytotoxic T cells is critical for appropriate adoptive immune response. *In vitro* culture and expansion of human T lymphocyte clones are very sophisticated and subtle procedure in immune cell therapy and hard to control. Therefore, many groups devoted their efforts to manipulate artificial antigen presenting cells (aAPCs) that can induce T cell activation and clonal expansion. To mimicking of natural antigen-presenting cells, aAPCs encompass basic signal molecules required for T cell activation: MHC:antigen complexes, co-stimulatory molecules and soluble immune modulating molecules. Orchestrated organization of these molecules is important for efficient T cell activation. Here, we discuss how those molecules have been incorporated in several aAPC models, but also how physical properties od aAPC are important for interaction with T cells.

Key Words : APC, Antigen, T cell, Surface molecules, Immune response

요 약 인체 적응 면역 반응을 일으키는데 중요한 항원 특이적 T 세포를 활용한 면역 세포 치료에서 T 세포를 체외에서 배양하고 클론 확장시키는 과정은 매우 섬세하고 복잡하여 조절하기가 쉽지 않아 T 세포의 활성화와 클론 확장을 유도하면서도 조절 및 취급이 용이한 인공 항원제시세포 개발의 필요성이 대두되고 있다. 인공 항원제시세포는 인체의 항원제시세포의 세포 표면 분자와 작용을 모방하게 되는데, 기본적인 신호 분자인 MHC-항원 복합체, 공동 자극 분자, 그리고 용해성 면역 조절 분자를 필수적으로 발현하여야 한다. 또한 T 세포가 항원과 접촉할 때, 이들 분자들이 잘 조직화되어 작용하는 것이 효과적인 T 세포 활성화에 중요하다. 본 논문에서는 여러 인공 항원제시세포 제작 방법과 세포 표면 분자들의 결합 방법과 물리적인 특성이 T 세포와의 상호작용에 중요함을 고찰하였으며, 효과적인 T 세포 활성화를 유도하며 면역세포치료에 적용 가능한 인공항원제세세포의 제작 방법을 살펴보았다.

주제어 : 항원제시세포, 항원, T 세포, 표면 분자, 면역 반응

1. Background

The immune system constantly protects our body from various pathogens. Key to the success of our immune system lies in its ability to develop immunity to pathogens it has never encountered before. Professional antigen-presenting cells (APCs), such as dendritic cells (DCs), B cells, macrophages, have critical role in adaptive immune response. In addition to their role of

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protecting us from external pathogens, DCs are also required for the generation of anti-tumor immunity[1,2].

When any pathogens manages to break through the skin barrier, it is likely to encounter APC like a dendritic cells. Using process known as phagocytosis, dendritic cells engulf and breaks down the invading pathogens. After that, DCs present parts of the pathogens on its cell surface as antigens bound to membrane protein called Major Histocompatibility Complex (MHC). Thereafter DCs migrate to the nearby lymphoid organs and activate T cells.

Immune system also scans our body for abnormalities in cellular secretion products or debris, which is a potential sign of cancerous growth. When abnormal growth is detected, those cells responsible are eliminated. The generation of such anti-tumor immunity is also mediated by DCs[1,2] and their function as APC has already successfully been exploited. Unfortunately, the high costs of culturing each patients individual cells and processing them into a vaccine under good manufacturing practice (GMP) is a potential difficulties for clinical use[3,4].

The needs for modular, off-the-shelf DC has led researchers to production of artificial antigen presenting cells (aAPCs). Wide range of acellular scaffolds have been manufactured to meet basic requirements for T cell activation[5] and consequently used to activate T cells. Choosing adequate combination of scaffolds and set of T cell activating ligands is critical to induce appropriate T cell response. Controling T cell activation makes aAPCs not only an attractive alternative to DCs in cancer immunotherapy, but also an important way to investigate the factors that influence native T cell activation.

The importance of scaffold properties in intercellular signal transduction has been noticed. Factors like avidity, shape, rigidity and size can influence the outcome of cellular signaling events. There have been increased attempts to use techniques from biochemical techniques to mimic natural signal transduction pathways[6].

This article explores the role of scaffold properties in cellular interactions and signaling procedures. Focus will be on the biomimicry of APCs in the context of cancer immunotherapy using artificial scaffolds.

2. Adoptive Immunity and APCs

An important lineage from which many different cell types associated with the adaptive immune system arise is the common lymphoid progenitor. Collectively known as lymphocytes, these cells are mainly found in the blood and the lymphatic system. Most lymphocytes are antigen-specific cells, meaning that they can respond to one antigen only. The only exception comes from the natural killer cell (NK cell), which is specialized in killing tumor or virus infected cells.

The remainder of the lymphocyte subtypes are antigen-specific. Because of the vast number of pathogens that our body might encounter during our lifetime, a huge repertoire of different antigen-specific cells exists in our body. This is possible through the modular way in which an antigen receptor is produced. By continuous recombination of the genes that code for the antigen receptor, our body can produce up to a billion different lymphocytes. Only when a lymphocyte is presented with the antigen specific to its antigen receptor, it goes from a naive to an activated state. In the activated state, lymphocytes are called effector cells and perform various immunological functions in the body.

There are two types of lymphocytes, the B cells and the T cells. B cells are most popularly

known as the source for antibodies. After their antigen receptor recognizes an antigen, B cells transform into plasma cells or effector B cells. These cells produce and secrete large amounts of immunoglobulin (Ig) proteins, which is a soluble form of the antigen receptor on their cell membrane. This protein, also known as an antibody, helps the immune system to recognize a specific pathogen, thereby promoting its removal from the body.

Although T cells also recognize antigens through an antigen receptor (the T cell receptor, or TCR), their effector functions are different from B cells. T cells can differentiate into several different effector subtypes, which can be categorized as cytotoxic (specialized in killing), helper (stimulates other T cells and B cells) or regulatory (controls/suppresses other T cells).

T cells can be found in the blood and the lymph. They differentiate from the common lymphoid precursor in the bone marrow, a primary lymphoid organ. From there, they migrate to the thymus where they mature. They then go on to secondary lymphoid organs such as the lymph nodes and the spleen where fully matured naive T cells wait to be stimulated by antigen-presenting cells.

When lymphocytes enter the thymus, they lack a functional T cell receptor nor do they express cluster of differentiation 4 (CD4) or CD8, both TCR co-receptors. At this stage, they are called double negative (DN) cells. As the DN cells proliferate, they start to express low levels of CD4, CD8 and the TCR, which consists of an α and β chain.

The incredible diversity of TCRs comes from the somatic recombination of the genes that code for the α and β chains respectively. This process, known as V(D)J recombination, is the origin of TCR diversity. After translation of the genes that code for the TCR, the full α:β heterodimer is formed at the surface of the cells, which are now called double positive (DP) cells because they express both the CD4 and CD8 coreceptor.

Cells that engage with TCRs, such as DCs, do this through antigens bound to MHC molecules on their surface. There are two classes of MHC molecules; those that bind endogenous antigens (MHC class I) and those that bind extracellular antigens (MHC class II).

To ensure that the newly formed TCRs function properly, they need to be able to bind to antigen/MHC complexes but avoid strong binding to self-antigens since that may lead to auto-immunity.

The desired binding properties of TCRs is determined by positive and negative selection in the thymus. Positive selection ensures that the newly formed TCR has at least a weak affinity for an MHC complex. Double positive T lymphocytes that fail to bind an MHC complex through their TCR during positive selection die within 3-4 days. DP cells that survive positive selection will eventually become single positive (CD4 or CD8) T lymphocytes. During negative selection, apoptosis is induced in cells that bind too strongly to self-antigen/MHC complexes. Less than 5% of all DP cells survive this process, but such strict selection ensures that all single positive T lymphocytes that leave the thymus can recognize MHC and are self-tolerant. These matured cells, now called single positive (CD4 or CD8) naive T lymphocytes, move to the periphery and end up in secondary lymphoid organs where they are maintained in low numbers until they meet “their” antigen and rapidly expand in numbers.

Mature, naive T cells that circulate the blood and the lymphatic system need to be activated by antigen-presenting cells before they are fully functional. Once activated, T cells become

effector T cells and perform various immunological tasks.

Most nucleated cells display MHC molecules on their cell surface, but only DCs are capable of activating T cells. DCs continuously sample their surroundings for pathogens and depending on the way a pathogen enters the dendritic cell, the antigen fragments derived from the pathogens are loaded onto different types of MHC molecules. Extracellular pathogens that enter the cell through either receptor-mediated phagocytosis or macropinocytosis are loaded onto MHC class II receptors and pathogens that are located directly in the cytosol (such as most viruses) are loaded onto MHC class I molecules after proteolytic degradation. However, some exogenous proteins internalized by DCs end up on MHC class I complexes, a phenomenon referred to as cross presentation. The distinction between peptide-MHC I (pMHC I) and pMHC II complexes on the APC surface is important because it determines the type of T cell that gets activated.

After DCs capture an antigen, they move from the peripheral tissues like the skin to draining lymph nodes via lymphatic vesicles and mature[10]. In the lymph nodes, antigen presentation to naive T cells takes place[7].

Activation of naive T cells by APCs requires three signal, as illustrated in figure 6. The first signal in T cell activation is the recognition of a pMHC complex by a TCR on the T cell surface. The bond between a single pMHC:TCR is rather weak, but it can be stabilized by either CD4 or CD8, both co receptors of the TCR on the T cell surface. The pMHC II:TCR complex is stabilized by CD4 and the pMHC I:TCR complex is stabilized by CD8. Since single positive, naive T cells express only one of the two co receptors, this gives rise to two different populations of effector T cells ($CD4^+$ or $CD8^+$) after activation.

A second signal is provided by co stimulatory molecules on the APC, such as CD80 (B7.1) or CD86 (B7.2). These surface molecules interact with CD28 on naive T cells. This second signal is needed for further activation of the T cell. Stimulation solely through the TCR, without co stimulatory signals, can lead to a state of anergy in the T cell. In this state, T cells are selectively non-responsive when presented with an antigen later on[8]. Adhesion molecules, such as the integrin LFA-1 on the T cell which interacts with ICAM-1 on the APC surface can be seen as another co stimulatory signal, since they serve to prolong and stabilize APC:T cell contact. A third signal comes from soluble signaling molecules, collectively known as cytokines. This signal influences to which effector cell a naive T cell will differentiate.

The nature of the T cell response is strongly related to the nature of the presentation of the different activation signals. Activation of CD8+ T cells occurs through TCR recognition of a pMHC I complex on the APC and co stimulation through CD28 on the T cell surface. The effector cells of the CD8+ subtype are called cytotoxic T cells (CTLs) and these cells are capable of killing cells in an antigen-selective fashion. For example, when a CTL encounters a virus-infected cell presenting a viral peptide derived antigen on its MHC I complex, it will kill that cell.

3. Cancer Immunotherapy

Cancer cells often have altered protein expression levels compared to normal cells, which can result in the display of antigens that are associated with tumors. In order to establish antitumor immunity, the immune system has to complete several steps, illustrated in Fig. 1. First, a tumor associated antigen must be taken up by dendritic cells in an inflammatory context to ensure full maturation of the DCs. Next,

these antigen loaded DCs must mature and find their way to the lymph nodes where they can interact with naive T cells. The exact composition of T cell subtypes required to establish antitumor immunity is not known, but it is certain that it must include CD8+ cytotoxic T cells (CTLs) [9]. In the presence of an inflammatory signal, such as IL-12, these CD8+ T cells develop into a memory subtype which persists long after activation[10]. Although most of the work on T cell mediated cancer immunotherapy has focused on the generation of CTLs, the $CD4^+$ T cell subtype has an important role in sustaining an active immune response by secreting the stimulatory cytokine IL-2[11]. After the establishment of an activated repertoire of tumor-antigen specific T cells, the CTLs have to reach the tumor site where they can execute their cytotoxic function. Several factors hamper the proper functioning of CTLs in the tumor microenvironment, such as limited expression of tumor-antigen presenting MHC I molecules by the cancer cells and the presence of immunosuppressive cytokines.

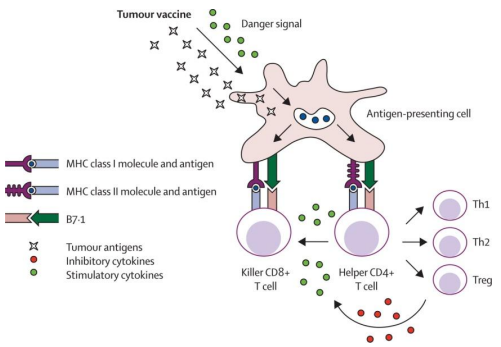

Fig. 1. Antigen capture and presentation by antigen presenting cells. Tumor-associated antigens are exogenously delivered to provide a source of antigen. Antigens can then be taken up by antigen-presenting cells, expressing co stimulatory molecules such as CD80, to be processed and presented to T cells. Phenotypes of CD4+ T cells can either enhance (eg, Th1) or inhibit (eg, Treg) the immune response[12].

To help the immune system to generate an effective immune response against cancer, two fundamentally different promising approaches have evolved. One approach is aimed at the delivery of (exogenous) tumor antigen to dendritic cells to enhance antigen presentation to T cells, while the other focuses on ex vivo activation and expansion of patient-derived naive T cells, followed by reinfusion of these cells. The latter process is also known as adoptive cells transfer, or ACT. Both approaches are discussed in more detail below, as they form the therapeutic context for the development of aAPCs.

4. Methods for mimicking immunomodulation

4.1 Physical properties: size

At least when it comes to the way in which micro- and nanoparticles interact with their surroundings. The profound effect of particle size on its interaction with its surroundings has been observed at the cellular level in terms of endocytosis and signal transduction, but also in body biodistribution and in vivo motility.

All cells are capable of engulfing extracellular material through processes collectively known as endocytosis. Endocytosis provides a means for cells to absorb nutrients and probe their surroundings. There are some size restrictions to the different endocytotic pathways; while most cells are only capable of ingesting sub-micron sized particles by pinocytosis, only phagocytotic cells readily take up particles that are over one micron in size[13]. However, endocytosis of particles larger than 1 μm has been shown for non-phagocytotic cells. Gratton et al. showed uptake of solid microparticles up to 3 μm in size after incubation with cervical cancer cells (HeLa)[14]. Particle size is well

known to influence cellular uptake, which is illustrated by the rate of phagocytosis of polystyrene microparticles by macrophages[15]. This was shown to be size-dependant and maximum phagocytosis was observed at a particle diameter of 2 to 3 μm, which interestingly coincides with the typical diameter of bacteria. For nanoparticles in the range of 30-100 nm, with ζ-potentials ranging from -23 mV to +9 mV, size is the major determinant for non-specific uptake by macrophages, where 100 nm particles are taken up fastest[16]. The rate of particle uptake is of mportance in intercellular signaling, since slower uptake usually more time for the particle to reach its target.

At the cellular level, particle size may also influence signal transduction when the particle is coated with signaling molecules, something that is evident from T cell stimulation with artificial antigen-presenting cells. Microparticles are better at activating T cells than nanoparticles coated with the same stimulatory ligands[17,18]. This may be explained by the requirement of a large, continuous surface for the formation of a mature immune synapse, a structure several microns in diameter.

The lymphatic system is an important size-dependant targeted organ for immunomodulation. After intradermal administration, nanoparticles are known to reach the draining lymph nodes in a size dependant manner through the interstitial fluid, with an upper size limit of around 100 nm dependant on tissue and injection pressure. In DC targeted immunotherapy, passive migration of sub-100 nm nanoparticles to draining lymph nodes is often used to deliver antigen to dendritic cells[19,20,21]. Although particles up to 180 nm can also reach the draining lymph nodes after subcutaneous administration, this can take up to several days[22]. Recently, Hubbell and coworkers showed that OVA-antigen coupled to a solid core, 30 nm nanoparticle via a reduction-sensitive linker elicited a CD8+ T cell response whereas OVA-antigen encapsulated in 125 nm polymeric vesicles (polymersomes) preferentially elicited a CD4+ T cell response after subcutaneous administration[21].

4.2 Physical properties: shape

Although it has long been known that viral and bacterial shape influences in vivo behavior, the systematic study of shape-effects on the behavior of micro- and nanoparticles has only recently gained attention with the advent of techniques that allow for the synthesis of non-spherical particles[23,24].

The shape of micro- and nanoparticles can have a profound effect on uptake by phagocytotic[25-28] and non-phagocytotic cells[14,29]. As a rule of thumb, particles with a higher aspect ratio show slower uptake, independent on particle size[28] or cell type[29]. As a high aspect ratio particle is more likely to approach a cell with one of its low curvature sides, extensive actin remodeling, an energy dependent process, is required to internalize the particle. This can help to explain the observations made by Sharma et al., who found that ellipsoid polystyrene microparticles attached more readily to macrophage surfaces than their spherical counterparts, but are taken up less efficiently[25]. The size-independent, aspect ratio-dependent uptake of micro- and nanoparticles by macrophages.

Particle shape also significantly influences the circulation time in vivo, an effect which can be observed from the disk shaped red blood cell (RBC), which can stay in circulation up to 120 days. Although the prolonged circulation time

observed with RBCs is not only due to its shape, prolonged circulation of synthetic microparticles that mimic the dimensions close to those of RBCs has been observed[30]. Geng et al. found that elongated micelles (filomicelles) show circulation times of up to a week, which was significantly longer than their spherical counterparts[31]. A tempting explanation for the prolonged circulation time of disks and filomicelles is their ability to align with the blood stream, which would minimize contact with the cell wall and phagocytotic cells present in the blood[31].

4.3 Presentation of surface molecules

In intercellular communication processes, such as immune recognition or cellular adhesion, multiple surface bound ligands are often presented to enhance signal strength. The combined strength of multiple binding interactions is known as avidity, not to be mistaken with the affinity associated with a single ligand-receptor interaction. An excellent example of the power of multivalent binding is found in the significant (100-fold) increase in affinity of TCR:pMHC binding in situ vs TCR:pMHC binding in solution[32]. In fact, the interaction between a single TCR and peptide-MHC complex is very weak, with a dissociation constant (Kd) in the order of 1-100 μM resulting in the requirement of multiple pMHC complexes to stimulate T cells[33]. The power of multivalent binding has led to the development of scaffolds that can display multiple ligands[34]. Such scaffolds can be anything from proteins to dendrimeric polymers or solid particles.

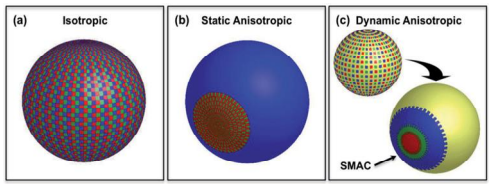

Fig. 2. Three ligands (recognition (red), costimulatory (green), and adhesion (blue)) that could be presented on the surface of artificial antigen presenting cells (aAPCs) in various ways. (A) Isotropic surface presentation of randomly distributed ligands. All three ligands are presented uniformly over the particle surface. (B) Anisotropic presentation of ligands in a patch pattern on the surface of a particle. Recognition and costimulatory ligands are randomly distributed in the patch, with a surrounding field of adhesion ligands. (C) Dynamic anisotropic presentation of ligands on a fluid supported lipid bilayer (SLB; yellow) [6].

Ligands can be bound to a scaffold in a random fixed (isotropic),site selective fixed (anisotropic) or dynamic fashion, illustrated in Fig. 2. The ability of ligands to move in response to a binding event can be crucial for signal transduction, and this dynamic remodeling of surface bound ligands is increasingly recognized as an important aspect of cellular communication[35]. Although working with solid micro- and nanoparticles is more appealing than liposomes from an engineering point of view due to the poor stability of liposomes[36], solid particles only allow for the attachment of fixed surface bound ligands. An elegant solution for this problem is to coat solid particles with a lipid bilayer, effectively mimicking the fluid nature of natural cell membranes. Ashley et al. coated silica nanoparticles with a lipid membrane that was targeted to hepatocellular carcinoma[37]. A combination of targeting and fusogenic peptides bound to lipids in the bilayer ensured rapid uptake and release of the drug-loaded silica core inside the targeted cells. The influence of the fluidity of the spherical supported lipid bilayer (SLB) on binding to Hep3B cells was investigated using

either a fluid DOPC or an non-fluid DPPC lipid for the construction of the SLB. At similar targeting peptide concentrations (~6 per particle), a 100-fold increase in binding affinity was observed when the fluid DOPC was used to construct the SLB. This dramatic increase in binding was attributed to the ability of ligands in the fluid SLB to reorganize, as shown in Fig. 3. Similarly, lipid coated silica microparticles that were designed to capture viral particles through multiple ligand-receptor interactions showed a reduction in captured viral particles at a lower temperature due to reduced lateral mobility of the ligands in the SLB[38]. The ability of ligands to dynamically reorganize in response to a binding event has been studied for leukocyte mimics, or leuko-polymersomes (bilayer vesicles constructed of amphiphilic block copolymers)[39].

Surface bound ligands can be attached to particles via different synthetic strategies and an optimal ligation strategy most be chosen for each type of ligand-particle pair. A discussion of these strategies is outside the scope of this thesis, but the reader is referred to a recent review on the topic[40].

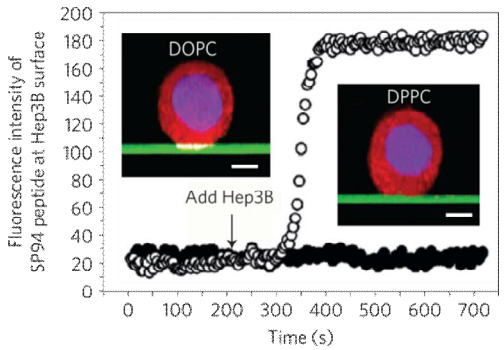

Fig. 3. Recruitment of Alexa Fluor 647-labelled SP94 peptides (white) to the surface of a Hep3B cell when peptides are displayed on a nitrobenzoxadiazole-labelled SLB (green) composed of DOPC (open circles) or DPPC (closed circles). These data were collected at 4°C. Hep3B cells were labeled with CellTracker Red CMTPX (red) and Hoechst 33342 (blue). Inset scale bars=5 μm [37].

5. Artificial antigen-presenting cells

Antigen presentation by natural APCs can lead to variety of T cell responses, depending on which signals are transmitted. Therefore, control over the signals incorporated into artificial antigen-presenting cells improves control over the therapeutic outcome[41]. The information that is transmitted by an APC to activate, expand and differentiate a naive T cell is classically divided into three signals, as illustrated in Fig. 4.

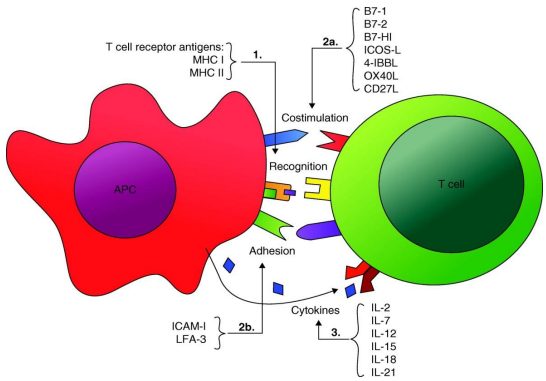

Fig. 4. Schematic of signal classes presented by an antigen presenting cell (APC). Three signals are essential for optimal T cell stimulation: 1. Recognition signals that ligate the T cell antigen receptor, pMHC complexes or antibodies cross-linking the T cell receptor (TCR), 2. Costimulatory molecules of the CD80/86 or TNF family and adhesive molecules that strengthen interactions between cells, 3. Cytokines secreted by APC or other immune cells that bind to receptors on the T cell surface [5]

The first signal, recognition, takes place when a T cell receptor (TCR) on a T cell recognizes a peptide-MHC (pMHC) complex on an APC surface. For artificial antigen presentation, either an pMHC class I (for expanding CD4+ T cells) or pMHC II (for expanding CD8+ T cells) can be used as recognition signal. Often, an MHC I/II non-specific antibody, anti-CD3, is used as an alternative recognition signal on surface of

aAPCs. The use of an antibody as opposed to the more biosimilar pMHC I/II should come as no surprise; antibodies are more easily produced in large quantities and only one aAPC is required for the activation and expansion of a diverse repertoire of T cells.

Costimulation through the interaction of CD80/86 receptors on the APC and CD28 on T cells is known to enhance the strength of the antigen-specific T cell response. Many aAPCs therefore present either CD80/86 or anti-CD28 on their surface as a second signal, although stimulation with aCD28 may only lead to T cell proliferation but not differentiation[123]. In addition to costimulatory ligands, adhesive interactions though ICAM-1 on the APC surface with LFA-1 on T cells may serve to enhance affinity and prolong APC:T cell interaction. As such, anti-LFA-1 has been used in artificial APC systems[42].

Lastly, an important factor in the rapid expansion and differentiation of T cells comes from cytokines, which are either released by the APC or by neighboring activated T cells. Cytokines are extensively used for the ex vivo culture of T cells (in adoptive transfer for example) or for direct in vivo administration as a form of immunotherapy. Cytokine release has only recently been mimicked in local delivery strategies, mainly in biodegradable PLGA particles[43] or anchored to liposomes through an Fc-fragment[44].

It is perhaps the modular, systematic description of T cell stimulation that has attracted (bio)chemical engineers to the field of artificial antigen presentation[5,6,45]. The type of T cell response can be precisely tuned, depending on the signals provided by the aAPC. Controlled display of information to T cells does not only help to increase our fundamental understanding of the nature of T cell activation, but can eventually also lead to well defined immunotherapies.

6. Conclusions and Discussion

Work with dendritic cell-based vaccines has proven that clinically relevant results can be obtained by harnessing the power of the immune system to fight cancer. However, such APC-based therapies are often laborious and costly, limiting their widespread application. This, in addition to the unpredictable outcome of DC manipulation, has led to the development of modular, tunable systems for T cell activation known as artificial antigen-presenting cells.

As the name suggests, artificial antigen-presenting cells should mimic antigen-presentation by professional antigen-presenting cells. The activation of naive T cells by DCs (a professional APC) has been studied extensively, from the molecular to the systemic level. Naive T cells are primarily activated in the lymph nodes, where they scan their surroundings at high speeds until they encounter their cognate pMHC complex on the surface of a DC. Only a few (<10) pMHC complexes are required for the T cell to bind, but once the T cell is bound, it can stay bound for hours. Initial contact leads to the formation of an immunological synapse (IS) between the DC and the T cell. The macromolecular structure of the IS influences T cell activation, through the formation of multiple, spatially separated ligand-receptor interactions. Although the precise role of the overall IS structure on T cell activation is not known, it seems to be essential that TCRs are preclustered, preferably together with costimulatory receptors such as CD28, on the T cell surface. In addition, stimulatory cytokines, such as IL-2, need to be present to assure proliferation of the activated T cells.

The stimulatory capabilities of an APC have

successfully been mimicked by artificial antigen-presenting platforms, varying from liposomal to solid polymeric constructs. Especially in vitro, such systems have proven their value as modular platforms for the stimulation of T cells. However, some essential characteristics of natural T cell stimulation, such as dynamic remodeling of surface bound ligands or the paracrine delivery of cytokines, remain hard to mimic. Another point of concern related to the possible therapeutic use of aAPCs is related to the large size (5-10 μm) that seems to be required for optimal T cell stimulation. Although ex vivo cultured T cells put no restriction on aAPC size, the intravenous delivery of particles larger than a few micrometers may lead to embolism. For this reason, it may be of interest to develop nano-sized aAPCs for the in vivo stimulation of T cells.

The chow signals are presented by the aAPC. An ideal aAPC is structurally stable and allows for a well defined ligand decoration, while preferably being biocompatible/biodegradablehoice of the right material for the construction of an aAPC is definitely not trivial, as basic scaffold material properties partially determine as well. Since the scaffolds reported thus far have primarily been selected based on availability of the scaffold, development of novel scaffolds could open up the possibility to systematically study unexplored biomimetic factors such as shape or surface fluidity.

For the development of new artificial antigen-presenting cells, the ultimate application of such aAPCs should be kept in mind. When used as a model system for the stimulation of T cells in vitro, the choice of scaffold is almost unrestricted. However, when such aAPCs are expected to be used in active vaccination strategies, the scaffold must be biocompatible and small enough to prevent embolism.

Artificial antigen-presenting cells can provide a powerful tool for unraveling the molecular details of T cell activation and show potential use for cancer immunotherapy. The artificial antigen-presenting platforms presented in the literature up to date show that it is possible to stimulate T cells in a biomimetic way using simplified constructs. Although not all requirements for T cell activation, and particularly differentiation, are known, these constructs provide a solid basis from which new artificial antigen-presenting cells can be constructed.

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