CHAPTER 18

Harnessing the Therapeutic Potential of Dendritic Cells

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INTRODUCTION TO THE DENDRITIC CELL

On first sight, a statement like 'Nobel laureate Ralph Steinman's life and death were intricately connected with dendritic cells' may sound trivial or superfluous. However, it isn't! Ralph Steinman and Zanvil Cohn detected dendritic cells (DC) in 1973 (Steinman and Cohn, 1973). It took him and his group at Rockefeller University the following decade to provide evidence that DC are the pivotal and only cell type to activate an immune response against pathogens, i.e., viruses, bacteria, fungi and parasites (Steinman et al., 1983). However, in a special edition of 'Scientific American' published in 1993, DC are only marginally mentioned – macrophages were still described as the principal activators of immunity against pathogens. It took another decade from their initial discovery before DC came into the spotlights of immunology and became a subject of applied biomedical research. In 2007, Steinman published a paper entitled 'Taking dendritic cells into medicine' (Steinman and Banchereau, 2007). In the same year, he was diagnosed with stage IV (metastatic) pancreatic cancer, an aggressively growing type of tumor with dismal prognosis, typically resulting in a median survival of less than a year. Subsequent to and in combination with conventional therapies, he treated himself with several variants of DC-based therapeutic vaccines targeted specifically against his own tumor cells, which were in early clinical development at that time (Grawitz, 2011). When the Nobel Prize Committee called on October 3, 2011, to inform him about being awarded the 'Nobel Prize in Physiology or Medicine', not knowing that he had died 3 days ago, he had survived with pancreatic cancer for more than 4 years. In view of a 5-year survival rate of about 1%, he definitely had a life and death connection with DC!

THE DUAL ROLE OF DENDRITIC CELLS IN THE IMMUNE SYSTEM NETWORK

DC are patrolling the body regions where infectious agents (viruses, bacteria, fungi and parasites) are most likely to enter: the skin, airways, intestines and genitals. They move between and underneath of cells lining the respective organ but are also found

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elsewhere in the body. The cell surface of DC is greatly enlarged through long protrusions of their cell body, which appear like branches of a tree (ancient Greek: Dendron), when looked at in a microscope. Receptors embedded into the 'dendritic' surface of the cell membrane are capable of capturing pathogens and engulf them for destruction if they display fitting ligands – also called 'eat-me signals' – for these types of cellular uptake receptors. Importantly, pathogens are not the only targets of DC. Body cell displaying signals of 'non-normality' on the outside of their cell membrane are detected, engulfed and processed by DC (Wang et al., 2015). Likewise, tumor cells, if dying, decaying or otherwise displaying 'eat-me signals', can be taken up for destruction and further processing by DC as well.

Aside from acting as 'Sentinels' to engulf pathogens or tumor cells, DC contain another diverse group of receptors that act as 'Sensors' of pathogens by their characteristic surface patterns, so-called pathogen-associated molecular patterns (PAMP). PAMP binding to these 'Sensors' (pattern recognition receptors or PRR) on DC leads to their activation. For tumor cells, the patterns recognised by PRR are called damage-associated molecular patterns (DAMPs) (Schaefer, 2014). Once activated by the binding of PAMP or DAMP to the fitting PRR, DC conduct the orchestration of the immune cell network into an efficacious immune response against the respective type of pathogen or tumor cell. Michel Nussenzweig, who worked for more than three decades with Steinman and contributed significantly to all aspects of Steinman's research, said in a Nobel Lecture, that he delivered on behalf of the late Ralph Steinman: 'Dendritic cells are "Sentinels" that capture pathogens, as Metchnikov suggested (nobelprize). They are "Sensors" for infection that use their cell surface pattern recognition receptors to become activated. In addition, once activated, DC become "Conductors" of the immune orchestra, the individual cells of which play harmonious roles to protect and regulate the body's immune system. Dendritic cells link Metchnikov to Ehrlich', the two laureates of the 1908 Nobel Prize in Physiology or Medicine, Ilya Metchnikov and Paul Ehrlich, described 'Innate Immunity' and 'Adaptive Immunity', respectively (Paul, 2011).

DC have a pivotal role for the innate and adaptive immune responses in a dual mode of action (Fig. 18.1). Once activated through their sentinel and sensor functions, DC secrete cytokines of the type I interferon family, which in turn upregulate responder cells to secrete other cytokines and chemokines. In one branch of the dual modes, the genuine signaling pathway upregulates proliferation and activation of innate effector killer cells, i.e., natural killer cells (NK cells) and natural killer T cells (NKT cells). The other branch of the bifurcated pathway leads to the 'maturation' of DC into professional antigen-presenting cells (APC). Antigens are the target structures where the effector cells of the adaptive immune system home in to or its antibodies bind to, if presented on the surface of target cells (Murphy and Weaver, 2017).

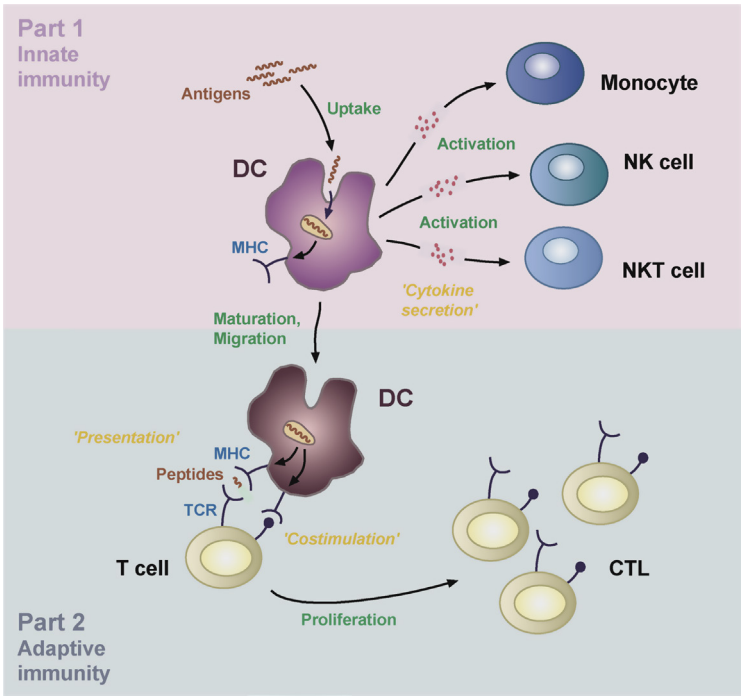

Figure 18.1 The dendritic cell (DC) dual mode of action. (1) After recognition of relevant signals, DCs initiate innate immune activation; (2) antigen loading and presentation triggers the adaptive immune system for antigen-specific responses. *CTL*, cytotoxic T lymphocyte; *MHC*, major histocompatibility complex; *NK cell*, natural killer cell; *NKT cell*, natural killer T cell; *TCR*, T cell receptor.

ANTIGENS ARE MADE FOR PRESENTATION

For the purpose of antigen presentation, almost all cells of the human body carry members of an extremely diverse protein family on their cell surfaces, named 'major histocompatibility complex 1' (MHC-I), a term that is reminiscent of its use to identify immunologically matching organs for transplantation (Murphy and Weaver, 2017; Mosaad, 2015). MHC-I cannot present whole protein molecules as antigens, but only small fragments of proteins called peptides. To generate these peptides, a small percentage of all cellular proteins are, following biosynthesis, not used as enzymes, structural proteins, receptors, ligands, signals, or the like, but chopped up into peptides ranging in length from 8 to 13 amino acids. Peptides, fitting into the complementary binding groves of MHC-I molecules, will be loaded onto MHC-I in specialised intracellular compartments of the endoplasmic reticulum. Each MHC-I with a bound peptide will be transported to and inserted into the cell membrane, with the MHC-I bound peptide facing the outside of the respective cell (Paul, 2011; Murphy and Weaver, 2017). This way, a representative 'peptide picture' of all the proteins of a cell is shown on its outside.

These external identifiers of a cell's internal protein content are continuously inspected by the patrolling effector cells (CTL, cytotoxic T lymphocytes, or CD8+ T cells). CTL use their highly diverse genuine surface receptor family, named T cell receptor (TCR), for inspecting MHC-I bound peptides (Murphy and Weaver, 2017; Mosaad, 2015). If their TCR does not fit the complex of a peptide bound by MHC-I, the inspecting CTL patrols on and leaves the respective cell unharmed. In the cases where the CTL has a TCR fitting the peptide:MHC-I complex, the CTL binds the target cell by its TCR and additional costimulatory proteins and destroys it (Fig. 18.1) (Palucka and Banchereau, 2012; Constantino et al., 2017).

But which peptide signatures are recognised by the CTL and which are not? This key question of immunology gave 'adaptive immunity' its name. Essentially, 'adaptive' means that the patrolling CTL 'know' the peptide signatures to all regular (in a sense of healthy, nonmutated) cellular proteins of an individual and will leave those cells unharmed. 'Adapted' means that there is no fitting TCR on any of the CTL to bind peptides from regular proteins presented by MHC-I on the outside of body-own cells. Adaptation happens after birth and takes place mainly in the thymus gland residing behind the breastbone; the letter T in CTL refers to their site of adaptation, i.e., the thymus gland.

If a body cell is infected, though, by a pathogen or has become a tumor cell, peptides from irregular (in a sense of not healthy, mutated) pathogen- or tumor-derived proteins will be presented on MHC-I. Because the CTL had not been adapted to such irregular peptides, there will be almost certainly (defined in terms of statistical probability) a CTL clone with a fitting TCR to bind cells tagged with these peptides and destroy them (Murphy and Weaver, 2017). Conversely, the absence or impairment of a fitting CTL clone allows an infection to spread, or cancer cells to proliferate.

INITIATION OF INNATE IMMUNITY: FIRST PART OF DENDRITIC CELL DUAL FUNCTION

To quantitatively remove pathogens, like macrophages and other phagocytes do, is not the primary goal behind the sentinel function of DC. DC rather engulf infectious pathogens and decaying tumor cells for further inspection through their repertoire of PRR. For this purpose, cells need to be dismantled to make PAMP and DAMP, often hidden in large molecular complexes accessible for PRR binding. With pathogens, these are typically their nucleic acids (RNA and DNA). In tumor cells, proteins, nucleic acids or membrane components are frequently displaced with respect to their cognate cell compartments. For example, proteins normally belonging to the cytoplasm are found in the nucleus and vice versa, membrane components are flipped outside in or inside out and nuclear DNA and mitochondrial DNA are found leaked into the cytoplasm. For this purpose, DC are equipped with a limited set of so-called scavenger receptors to detect a wide variety of 'eat-me signals' (Wang et al., 2015; Poon et al., 2014).

The scavenger receptors engulf pathogens or chunks from decaying tumor cells by receptor-mediated endocytosis. Once the receptor is activated by bound targets, the cell membrane in the region where the ligand:receptor complex is located becomes dented. The resulting dent subsequently forms a membrane vesicle with the ligand:receptor complex residing inside, dissociates from the cell membrane and floats inside the DC. Such internalise membrane vesicles containing the payload from receptor-mediated endocytosis are called 'early endosomes'. Depending on the internalised material and cellular signaling, their contents may become acidified resulting in the 'early endosomes' to become 'late endosomes' or lysosomes. The acidic pH in lysosomes activates a battery of degradative enzymes including proteases, lipases and glycosidases, which together are able to dismantle the internalised biological structures by fragmentation of their proteins, lipids and carbohydrate units. These processes eventually make the pathogen's or tumor cell's PAMP or DAMP accessible for binding to PRR inside the endosomes or lysosomes (Naslavsky and Caplan, 1918).

The activation of PRR by danger signals, either on infection of cells by pathogens (PAMP) or through other forms of cell damage (DAMP), in principle leads to similar initial response pathways in DC. Activation by either PAMP or DAMP is therefore best summarised by the term 'stressed cell response' (SCR). SCR, mediated by the sentinel function of DC, is the starting signal for innate immunity (Gasteiger et al., 2017). DCs respond to SCR by secretion of type I interferons (IFN-alpha, IFN-beta), thereby initiating the acute inflammation pathway and the activation pathways for effector cells of innate immunity, i.e., monocytes, NK cells and NKT cells. Activation and proliferation of innate effector cells requires a complex interplay of various cytokines and chemokines under the control of interferon gamma (IFN-gamma), a type II interferon (Murphy and Weaver, 2017; Sokol and Luster, 2015).

BRIDGING BETWEEN INNATE AND ADAPTIVE IMMUNITY: SECOND PART OF DENDRITIC CELL DUAL FUNCTION

The bridging function of DC between initiation of innate immunity and relaying the presence of PAMP and DAMP to the adaptive immune system depends on the antigen-presentation capabilities of DC. This is why DC are called professional APC. Like almost all body cells, DC also operate the MHC-I antigen-presentation system. As explained above, the MHC-I system displays a 'snapshot' of all proteins within a cell as a peptide pattern at the cell's outside for inspection by the effector cells of adaptive immunity (CTL, CD8+ T cells) (Murphy and Weaver, 2017).

As professional APC, DC operate a second antigen-presentation system. This system, MHC-II, is adapted to slightly longer peptides composed of 12–20 amino acids, compared with the peptides presented by MHC-I (8–13 amino acids) (Murphy and Weaver, 2017). In contrast to MHC-I, the MHC-II system presents antigens that were processed

by DC in their function as sentinels from engulfed pathogens or stressed cells. Thus, MHC-II presents mainly 'foreign' or 'irregular' (from SCR) antigens and generates signals messaging '*I just ate pathogens or tumor cell fragments; there are stressed cells in my neighborhood*'.

Receptor-mediated endocytosis (as explained above) of pathogens or stressed cells, first results in maturation of the DC by activating the necessary MHC-II antigen processing and presentation machinery. In a second step, those DC with antigens loaded on MHC-II start moving into lymphatic vessels, to reach the nearest neighboring ('draining') lymph node (LN). As an example, if a DC engulfed a pathogen or stressed cell somewhere in the left lower arm, it will move through the ubiquitous lymphatic vessels to the LN in the left armpit. Within a defined region of the LN, MHC-II presentation of antigens is then monitored by helper T cells (T_H cells or CD4+ T cells) using their respective TCR (Murphy and Weaver, 2017). A TCR of a T_H cells fitting the antigen:MHC-II complex constitutes 'signal one' of the adaptive immune response. 'Signal two' is established by so-called costimulatory ligands and their receptors, and 'signal three' is provided through cytokine/chemokine secretion. Only if all three signals are appropriately set, the adaptive immune response against a pathogen or tumor cell becomes effective (Murphy and Weaver, 2017).

An efficacious adaptive immune response is capable of generating the effector cells of adaptive immunity, i.e., CTL (CD8+ T cells) but also B cells which maturate into antibody-producing plasma cells. Whether the response is quantitatively dominated by CTL or by B cells depends on the way the antigens were presented (MHC-I or MHC-II) and on the cytokine milieu during presentation. CTL will destroy target cells if an MHC-I presented antigen of a target cells fits and binds to the TCR of a CTL. Antibodies bind to and tag their specific antigen targets on the surface of pathogens or tumor cells. Such tagged targets are then destroyed by effector cells of the innate immune system (NK cells and NKT cells) which had been activated by DC in the corresponding innate immune response (Murphy and Weaver, 2017).

CROSS-PRESENTATION AND "WHAT IT IS GOOD FOR"

The above described uptake of PAMP and DAMP, their processing into antigens and 'regular' presentation by MHC-II ultimately leads to tagged pathogens and tagged tumor cells to flag their respective destruction by effector cells of the innate immune system, i.e., NK cells and NKT cells. Nonetheless, experimental and clinical tumor immunology and immunotherapy clearly teach that CD8+ T cells (CTL) targeting antigens from DAMP are key components for the efficacious elimination of tumor cells and to achieve a durable antitumor response (Sharma and Allison, 2015). However, as described above, CTL activation against DAMP requires antigen presentation by MHC-I for binding to the fitting TCR and activation of CTL.

How then could MHC-I presentation be accomplished for antigenic sources, such as tumor cells, taken up by DC through their scavenger receptors – a process that would regularly lead to MHC-II presentation and generation of antibodies instead of CTL?

The solution to the problem is an immune response pathway by the name of 'cross-presentation'. To enter the cross-presentation pathway, DAMP taken up by scavenger receptors, are not processed into MHC-II-presentable antigens but transferred to the cellular compartment for MHC-I processing and subsequently loaded onto MHC-I. MHC-I loaded with DAMP-derived antigens will then move to the cell surface of DC and present their antigens to CTL.

However, the cross-presentation pathway, which was detected only recently, is only enabled in a small subpopulation of DC. In humans such DC are characterised by the expression of BDCA3 (a transmembrane protein termed CD103 in mice) and of Batf3 (a transcription factor) (Broz et al., 2014; Spranger et al., 2017). In summary, successful immunotherapy of tumors depends on the activation of tumor-targeting CTL through cross-presentation of DAMP-derived antigens by this specialised subpopulation of DC.

THERAPEUTIC STRATEGIES USING THE DC UNIVERSE

Their vast potential of recognizing cancer cells through the SCR, to present relevant tumor antigens, and to raise strong and specific immune responses against the cells expressing these antigens, particularly tumor cells, render DC ideal targets for immunotherapeutic approaches in oncology. Two main therapeutic strategies can be delineated: First, the preparation and modification of DC outside of the patient's body (ex vivo) and second, techniques to target the DC patrolling inside the patient (in vivo) (Fig. 18.2).

The ex vivo modification of DC aims at optimizing their antigen-presentation abilities. The general process starts with harvesting the patient's own cells ('autologous') and generating mature DC followed by 'loading' with appropriate tumor antigens and subsequently injecting these modified DC back into the patient.

Harnessing the DC's capabilities via in vivo targeting can be done by antigen loading, maturation and activation. These techniques require no harvest of cells but modification inside the body, which is achieved through injection of antigens, antigen complexes or certain stimuli of the respective DC receptors.

As a result of both strategies, the activated and modified DCs should unfold their potential to initiate a strong and targeted immune response against the tumor. In the following paragraphs, each approach will be described using prominent examples which mostly have already been evaluated in immunotherapeutic clinical trials in cancer patients.

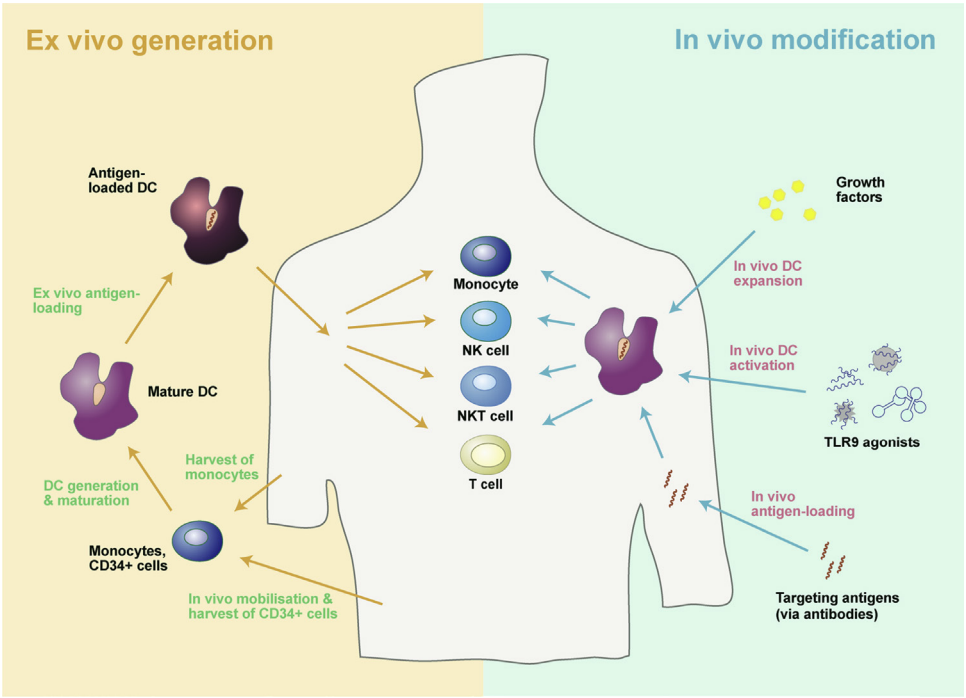

Figure 18.2 Strategies to employ activated dendritic cell (DC) for immunotherapy. (1) Generation of loaded DC outside of the body via harvesting of immune cells, maturation and loading of DC and reintroduction into the body; (2) In vivo approach to activate and/or load DC (can be combined with immunogenic therapies for release of tumor antigens: e.g., radiotherapy, chemotherapy). *NK cell*, natural killer cell; *NKT cell,* natural killer T cell.

EX VIVO APC GENERATION – FROM THE OUTSIDE IN

The generation of potent DC (professional APC) outside of the body requires manipulation of a pool of precursor cells in cell culture and providing them with tumor-derived antigens ('antigen loading') to obtain DC ready to serve as 'therapeutic cancer vaccines'. With help of the presented tumor antigens, the DC vaccines should initiate a specific immune response against the tumor cells and consequently a long-lasting immunity. Thus, it is important to choose the respective antigens carefully because only correct presentation of the ideal antigen can elicit the desired antitumor response (Coulie et al., 2014).

Generation of Potent DC

There are two methods for generating DC ex vivo – differentiation from peripheral mononuclear cells (PBMC) or from CD34+ hematopoietic precursor cells. PBMC can easily be drawn from the patient's body and the monocyte fraction will be cultured in the presence of GM-CSF (a stimulation factor) and interleukin 4 to obtain immature

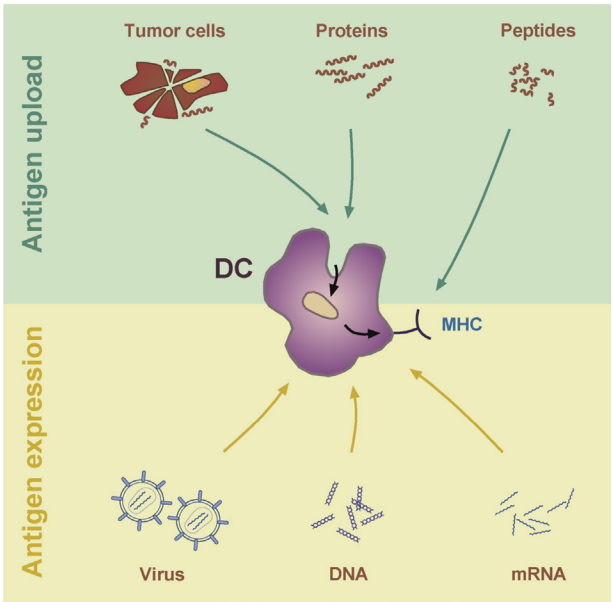

Figure 18.3 Techniques of antigen loading. (1) Upload of antigens via electroporation/pulsing of tumor cells (intact or lysates), antigenic proteins or direct loading of peptides onto major histocompatibility complex (MHC) molecules; (2) introducing antigens for internal expression using vector systems (virus modified for antigen expression, DNA-based antigen expression constructs or messenger RNA coding for antigens). *DC*, dendritic cell.

DC. These will be subsequently stimulated with a cytokine cocktail to achieve DC maturation (Johansson et al., 2007; Wołkow et al., 2018).

The second technique uses CD34+ hematopoietic precursor cells, which initially have to be mobilised from the bone marrow with the help of GM-CSF administration to the patient. After a leukapheresis to collect the mobilised CD34+ cells from the patient's bloodstream, an in vitro expansion with help of a GM-CSF, Flt3L and TNFalpha cocktail takes place. After about a week, the resulting cell culture contains a mixture of monocyte-derived DC (moDC), Langerhans-like DC – which have been shown to be very potent CTL stimulators – and a proportion of DC with various differentiation stages (Ramadan et al., 2001; Yui Harada et al., 2011). A third method, the in vivo expansion of DC, will be described later.

Loading-Up With Antigens

The most common approach for generating antigen-loaded DC is pulsing the cells with peptides, whole proteins or tumor cells (O'Neill et al., 2004) (Fig. 18.3). Peptides are directly loaded onto the MHC-I and MHC-II molecules on the DC's surface. However, a careful selection of these peptides is necessary so that the respective MHC-I and MHC-II molecules are able to bind and present the peptides. Proteins and tumor cells

(intact or as tumor cell lysates) must be internally processed to be presented. However, antigens being taken up by DC will be presented by MHC-II and not by MHC-I, as needed for efficacious generation of antitumor CTL. Thus, the small subpopulation of cross-presenting DC (BDCA3- and Batf3-positive) in total DC has to be either preferentially targeted or enriched before pulsing with proteins or tumor cells.

A different strategy to load the DC with antigens is internal expression of genes encoding for tumor antigens resulting in a 'natural' processing and presentation on the DC surface. This is currently achieved through transfection with DNA expression constructs, viral vectors (common: lentivirus) or mRNA, which both have been shown to generate tumor antigen-loaded DC eliciting CD4+ and CD8+ immune responses (Morandi et al., 2006; Cafri et al., 2015; He et al., 2005). This may be combined with other stimuli for maturation of the DC.

IN VIVO MODIFICATION – TARGETING PATROLLING DCs AND RELEASING THEIR NATURAL POTENTIAL

Various techniques have been developed to activate the DC and optimise their antigen presentation and thus eliciting specific CTL responses against tumor cells. Besides effective antigen loading, taking place directly inside the body, the use of ligands or agonists of the immune system's PRR or expansion of the low number of potent circulating DC is described.

In vivo Expansion of DCs

Because of the low abundance of circulating DC in the peripheral blood (less than 1% of all PBMC), the expansion of this population may be beneficial. Administration of hematopoietic growth factors like Flt3L results in increased numbers of circulating DC and showed induction of T cell responses, augmented maturation marker and cytokine production on stimulation (He et al., 2005; Sabado and Bhardwaj, 2010).

In vivo Antigen Loading of DCs

To avoid the time-consuming and labor-extensive process of ex vivo DC modification, techniques for in vivo antigen loading of DC are under evaluation. One approach is to deliver antigens selectively to DC in vivo, via monoclonal antibodies targeting specific surface molecules on DC resulting in enhance CTL and humoral immune responses (Tacken et al., 2006; Shortman et al., 2009; Caminschi et al., 2009).

Targeting Toll-Like Receptor Agonists

In addition, PRR recognizing PAMP and DAMP are used to activate DC by providing their respective ligands, e.g., mimicking an intrusion by pathogens or presence of stressed cells, like tumor cells. Here, the Toll-like receptors (TLR) play an important role in

immunotherapy. Within the TLR molecular family, nucleic acid–sensing TLR are located in endosomes and lysosomes, where TLR3 senses double-stranded RNA, TLR7/8 single-stranded RNA and TLR9 either double- or single-stranded DNA. DNA is an activating ligand for TLR9 if containing CG sequence motifs with nonmethylated C-residues. Methylation status of C-residues in CG motifs discriminate DNA from bacteria, many viruses and mitochondrial DNA of vertebrate cells (usually nonmethylated), from the nuclear DNA of vertebrates, where the C-residue in CG motifs is typically methylated (Majer et al., 2018).

When activated, all TLR use a common system of adaptor protein molecules (MyD88, IRAK, TRAF) to finally activate transcription factors of the interferon regulatory factor (IRF) family, i.e., IRF3 and IRF7. On activation, they relocate to the nucleus, where they induce the expression of type I interferon genes (interferon alpha, interferon beta) to exhibit antitumor potential. In addition, the inhibition of a transcription factor system known as nuclear factor kappa B (NFkB) is abolished, allowing NFkB to recruit to the nucleus, where it activates expression of a large family of genes driving the inflammatory response. Thus, TLR activation not only upregulates type I interferons but also initiates inflammation through a bifurcated signaling pathway (O'Neill et al., 2013; Vidya et al., 2018).

In a therapeutic setting, TLR ligation may provide cross-presented tumor antigens with an immunogenic context to generate an antitumor T cell response. A pioneering example of this approach is Coley's toxin. In the late 1800s, William Coley used a mixture of heat-killed bacteria resulting in a potent antitumor effect which may be attributed to bacterial DNA, containing unmethylated CpG motifs. This points to TLR9 as a promising target for immunotherapeutic approaches, especially, because the TLR9 is predominately expressed on plasmacytoid DC (pDC) (Hanagata, 2012).

In fact, TLR9 agonists are currently being evaluated in immuno-oncology trials. The TLR9 agonists in use can be divided into three groups based on their composition: (1) chemically modified, single-stranded oligodeoxynucleotides (CpG-ODN), (2) complexed CpG-ODN (bound to virus-like particles (VLP) or spherically arranged on nanoparticles) and (3) natural covalently closed (dumbbell-shaped) DNA constructs (Wang et al., 2016, 2017; Bichat et al., 2017; Li et al., 2018a; Wittig et al., 2015). While chemically modified TLR9 agonists are potent activators, their modification results in off-target effects limiting their therapeutic window (Heikenwalder et al., 2004; Manegold et al., 2012). Use of VLP to bind CpG-ODN does not require chemical modification of the DNA backbone and the size of the complex may help with the DC uptake by scavenger receptors (Mammadov et al., 2015). However, recognition and activation of VLP by other PRR could blur or dominate the TLR9 effect and may add to clinical toxicities or adverse events. The safest way is to avoid such modification and providing potent TLR9 agonists containing natural ODN. This was shown in comparison with chemically modified TLR9 agonists (Wittig et al., 2015; Schmidt et al., 2015).

This is an interesting development, because immunomodulators initially have been used solely as adjuvants to enhance the effect of vaccines and because the potential application of TLR9 agonists, with pDC as their primary target, has been expanded – for instance, taking advantage of released tumor-derived antigens after immunogenic cell death (e.g., due to chemo- or radiotherapy) as 'body's own vaccine'.

Targeting Cytoplasmic Sensors

Nucleic acids in the cytoplasmic compartment are sensed by two different families of cytoplasmic sensors: (1) cell-own DNA not kept in the nucleus and DNA escaped from mitochondria or (2) RNA different from mRNA, tRNA or miRNA not belonging to the cytoplasm. However, these cytoplasmic sensors or PRRs are not a specialty of DC and therefore selective targeting, if necessary, could cause problems. Nonetheless, both cytoplasmic DNA- and RNA-sensors are applied in immunotherapies with DC-based or innate immunity components.

For cytoplasmic DNA, the sensor is a combined receptor and enzyme by the name of cGAS (cyclic GMP-AMP synthase), which binds longer double-stranded DNA or short DNA fragments forming peculiar structures. On binding to its ligand, the enzyme cGAS synthesises cGAMP (cyclic GMP-AMP) which is the ligand for 'stimulator of interferon genes' (STING). STING activation will eventually lead to the phosphorylation of 'interferon regulatory factor 3' (IRF3) to induce the expression of type I interferon genes (see above) and strong NFkB-driven activation of inflammasomes to process prointerleukin-1beta to interleukin-1beta (IL-1b) (Ablasser et al., 2013; Anna-Maria Herzner et al., 2015; Li et al., 2018b).

For 'foreign' RNA, the sensor is RIG-I (retinoic acid–inducible gene I), a member of a family of combined PRR and double-stranded RNA helicases, to which also the sensors MDA5 (melanoma differentiation antigen 5) and LGP2 (laboratory of genetics and physiology 2) belong. RIG-I senses single- and double-stranded RNA with a 5′-triphosphate that are typical products of viral RNA polymerases or cellular RNA-polymerase III. On binding of RIG-I to its target, multimeric RIG-I forms and binds through its CARD (caspase activating and recruitment domain) to MAVS (mitochondrial antiviral signal) to activate the expression of type I interferons (Barik, 2016; Kopitar-Jerala, 2017). As described for STING activation, these RNA sensors also generate a strong inflammatory response.

A LOOK IN THE NOT-SO-DISTANT FUTURE – COMBINATIONS

Because the DC-based immunotherapeutic approaches – in particular DC vaccines – generally have been shown to be well tolerated and to induce only minimal toxicity, the evaluation of combination therapies is an obvious next step. Besides the release of tumor antigens by chemotherapy or radiotherapy (via immunogenic cell death), the recent

evolvement of checkpoint inhibitors in the immuno-oncology landscape presents an easy opportunity. One example is the programmed death-1 (PD-1)/PD-L1 receptor/ligand system which was described to inhibit antitumor T cell responses. Because the PD-L1 is not only (over)expressed on tumor cells but also on DC, inhibitory anti-PD-1 or anti-PD-L1 may synergise with DC-based therapies by 'releasing the break' regarding effective T cell activation (Tong Seng Lim et al., 2016; Hu et al., 2018).

PERSPECTIVES

Using DC as the key mediator of protective immunity is the basic concept for the development of prophylactic vaccines. Without DC it would not have been possible to develop vaccines against infectious diseases such as polio, tetanus or small-pox and others. These vaccines typically contain an agent that resembles a disease-causing microorganism and is often made from weakened or killed forms of the microbe, its toxins or one of its surface proteins in form of full-length proteins or smaller peptides. After administration, these compounds are recognised by DC and the full cascade from uptake of the antigenic compounds to the activation of specific CTL is started and leads to a specific, protective immune response against the infectious organism. The WHO reports that licensed vaccines are currently available for 26 different preventable infections (World Health Organization). The worldwide market for prophylactic vaccines is expected to reach USD 49.27 billion by 2022 from USD 34.30 billion in 2017 according to a *Markets and Markets* report on vaccines (MarketsandMarkets).

On the contrary, the concept of utilizing DC as a therapeutic target for the treatment of cancer had to deal with a series of failures where a strong immune response against tumor antigens was shown but this did not translate into a meaningful clinical efficacy. Various strategies for developing therapeutic vaccines were used, based on the same principle for the development of prophylactic vaccines. It remained unclear for years why a strong generation of CTL did not lead to an efficacious anticancer effect. It seemed to be impossible to translate the promising work in mouse cancer models into clinical efficacy. A hype on immunotherapy against cancer and in particular therapeutic vaccination in the late 1990s and early 2000s followed a huge depression thereafter.

Although the successful utilization of DC for the development of prophylactic vaccines is established use for decades, it took almost four decades from the detection of DC by Ralph Steinman and Zanvil Cohn in 1973 to FDA approval of the first DC-based therapeutic vaccine (Sipuleucel-T or Provenge) developed by Dendreon in 2010 for the treatment of prostate cancer (Plosker, 2011).

This was regarded as the breakthrough of therapeutic cancer vaccination and raised hopes that the high expectations in the concept of therapeutic cancer vaccination finally would come true. Unfortunately, this hope again was premature. On the one side, the commercialization of a personalised DC-based cancer vaccine turned out to be a huge

challenge in terms of manufacturing, cost of goods and profitability. On the other side, results of clinical trials on therapeutic vaccination still did not meet expectations.

Another company, Argos Therapeutic, focused on DC-based vaccination against cancer had to report negative interim results and subsequent discontinuation of a clinical trial with a DC-based vaccine against renal cell carcinoma in April 2018 (Argos Therapeutics, 2018). Numerous other clinical trials also reported negative data even though an immune response against the target antigens was observed.

Thus, the question remains whether therapeutic vaccination against cancer will ever be an integral part of cancer therapy. But there is hope.

All these clinical trials reporting negative data have one thing in common: their trial design dates back to the time when there was little knowledge of the immune escape mechanisms a tumor develops to overcome a specific immune response. Even when a strong CTL response is launched by a therapeutic vaccination utilizing DC, these CTL might not be able to elicit their cytotoxic activity inside the tumor due to the escape mechanism of the tumor cells.

This situation has changed since ipilimumab was approved by the FDA in 2012 (Pollack, 2015). Ipilimumab is a so-called immune checkpoint inhibitor and the first compound in this class followed by other agents blocking the PD-1/PD-L1 pathway.

The field of cancer immunotherapy is experiencing a renaissance spearheaded by these immune checkpoint inhibitors. This class of drugs led to a deeper understanding of the immune escape mechanisms inside the tumor microenvironment and spurred interest in upgrading existing immunotherapies and, in particular, DC vaccines that previously experienced only sporadic success (Gard et al., 2017). The chances are good that this will lead to more efficacious DC-based therapies in the future (Pulendran et al., 2000).

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