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## **Characterization of a human dendritic cell line; a prelude to allogeneic dendritic cell-based tumor vaccination**

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## **1. General introduction: rationale for Immunotherapy**

With an estimated 1.4 and 3.2 million new cancer cases and approximately 600,000 and 1.7 million estimated cancer deaths in the year 2006 in the USA and Europe respectively, cancer is the second leading cause of death in the western world (1,2). Although improvements have been made in surgical techniques, radiotherapy and adjuvant chemotherapy, a large number of patients that have initially been cured, eventually relapse and die of the disease. Therefore, there is an obvious need for additional treatment modalities, targeting residual tumor cells and thereby preventing metastatic spread. Cancer immunotherapy represents a promising approach to eradicate the disseminating tumor cells from the blood circulation, as well as micro-metastases from distant organs. In recent years, exciting new developments have resulted in different immunotherapeutic approaches, targeting either or both the innate and the adaptive immune system.

### **1.1 Innate and adaptive immunity**

The immune system consists of two main components, the innate and the adaptive immune system. The innate or natural immune system provides a first line of defense against microorganisms and is very important for the control of bacterial infections. The innate immune system acts relatively antigen-nonspecific, does not provide immunological memory and is composed of soluble factors like complement and cellular components like natural killer (NK) cells and phagocytic cells, such as neutrophils, granulocytes, monocytes, macrophages and dendritic cells (DC). By sensing pathogens or micro-environmental tissue damage, these components signal the presence of “danger” to the adaptive immune system. The adaptive immune response is mediated by B cells and T cells (3,4) that have undergone rearrangements of their immunoglobulins or T cell receptor genes, and is characterized by exquisite specificity and long-lasting memory development. The adaptive immune system exerts its function through the presentation of antigenic peptides in the context of the Major Histocompatibility Complex (MHC). MHC class I molecules are expressed on all nucleated cells and display antigenic peptides derived from endogenous, cellular proteins. These endogenous proteins are degraded by the proteasome in the cytosol, transported into the endoplasmic reticulum (ER) by the transporter associated with antigen processing (TAP), where they are subsequently trimmed by aminopeptidases to produce 8 – 10 amino acid long MHC class I binding peptides (5-7). Resulting peptides are able to bind to the peptide binding groove of newly synthesized MHC class I molecules and this MHC-peptide complex is subsequently moved via the Golgi apparatus to the cell surface of antigen presenting cells (APC). MHC class II molecules are expressed on professional APC, including B cells, macrophages and DC. Exogenous proteins, acquired via endocytosis or internalization of the plasma membrane, are processed to peptides in the endosomal pathway, loaded onto MHC class II molecules and subsequently transported to the nucleus where they present peptides to CD4+ T cells. In addition, specialized APC, i.e. DC, can also present exogenous proteins in the MHC class I pathway, via a process called cross-presentation. This process facilitates the generation of an MHC class I-restricted immune response against pathogen-infected or cancer cells, which is important to prevent the spreading of these pathogens and cancer cells through the lymphatic system or the blood.

An important bridge between the innate and the acquired immune system is provided by NK cells and DC, in which NK cells are the major source of type-1 polarizing cytokine interferon- $\gamma$  (IFN- $\gamma$ ) and other immune regulatory cytokines like granulocyte macrophage-colony stimulating factor (GM-CSF), transforming growth factor  $\beta$  (TGF- $\beta$ ), tumor necrosis factor (TNF)  $\alpha$  and  $-\beta$ , interleukin-1 (IL-1), IL-2, IL-3, IL-5, IL-8, and IL-10 (8,9). DC, the most powerful professional APC, are also known to be an essential link, since they are the most capable inducers of both primary and secondary immune responses (10). As sentinels throughout the body, DC are able to capture antigen, undergo maturation and subsequently migrate to the draining lymph nodes, where they are able to present the processed antigen to lymphocytes, thereby starting adaptive immunity.

## **1.2 Immunotherapy**

Cancer immunotherapy aims to (re)activate the host's immune system to fight cancer. The rationale for the development of anticancer immunotherapy is based on a more than 100-year old observation by William Coley. Treating sarcoma patients with a mixture of attenuated streptococcal and staphylococcal bacteria, also known as Coley's toxins, resulted in regression of the tumor, indicating that the immune system was capable of recognizing a tumor in the correct immunological context (11). Over the following 100 years, much was learned about the principles of the immune response to tumors. Nowadays, several immunotherapeutic approaches have been developed, which can be classified as either active or passive immunotherapy. Whereas passive immunotherapy aims at the development of short-lived immune responsiveness through the infusion of immunomodulatory agents, like cytokines, or the infusion of immune effector agents like antibodies or lymphocytes, active immunotherapy is designed to induce endogenous, long-lasting tumor-specific immune responses by the use of cancer vaccines.

### **1.2.1 Passive Immunotherapy**

#### *Monoclonal antibodies*

Monoclonal antibodies are increasingly applied as a passive form of specific cancer immunotherapy for several human malignancies (as reviewed by Adams *et al.* (12)). The biological modes of action of these passively infused antibodies are numerous, including interaction with components of the immune system inducing antibody-dependent cellular cytotoxicity (ADCC), or inducing complement-dependent cytotoxicity (CDC), as described for Trastuzumab and Rituximab respectively (13,14). Besides that, antibodies might also change signal transduction within the tumor cell, thereby altering growth rate of the tumor cells or resensitizing the tumor cells to cytotoxic agents. A target antigen for these kinds of antibodies is for example the epidermal growth factor receptor EGFR (15,16). Monoclonal antibodies can also be used to target (pro)drugs, toxins or radioisotopes to directly kill tumor cells. An example of such an antibody is Gemtuzumab, which targets a derivative of the potent cytotoxic DNA-cleaving antibiotic, calicheamicin via CD33 to hematopoietic cells and myeloid leukemia cells (17).

### *Cytokines*

Cytokines are small soluble proteins that are known to be involved in cell growth, differentiation and functional activation. They regulate both the cells of the innate and the adaptive immune system, exerting their effects by binding to their receptors. Various cytokines have been studied for their therapeutic potential in the treatment of cancer. Unfortunately, in most cases the therapeutic effect was only modest. Also, systemic infusion of cytokines has been shown to be associated with significant side-effects (18,19). However, some cytokines, such as IL-2, IFN- $\alpha$  or GM-CSF, showed promising clinical results, either as a single agent and/or as adjuvant therapy (20-23).

### *Adoptive cell transfer (ACT)*

The adoptive transfer of effector cells is a well-studied approach to treat human cancers. In the early 1980s, the focus of ACT was more on the use of cells from the innate immune system, such as NK cells and lymphokine-activated killer (LAK) cells. The idea behind this approach was to activate these NK or LAK cells to recognize and kill tumor cells of all different histological origins in a rather non-specific fashion. Although this approach had been demonstrated to be successful in animal models (24), as well as to some extent in clinical trials (25,26), in the late 1980s, a more specific, antigen targeted approach making use of tumor antigen-specific T cells had been proven to be more effective in its therapeutic potency (27). Whereas the adoptive transfer of tumor antigen-specific CD8+ T cells clones has been demonstrated to lack clinical effectiveness (28,29), probably due to the absence of CD4+ T cell help, the transfer of tumor infiltrating lymphocytes (TIL), containing significant numbers of CD4+ T cells, has been proven to mediate tumor regression (30). Indeed, TIL have been demonstrated to be effective, inducing tumor regression in tumor bearing mice (27,31) and to some extent in patients suffering from melanoma (32-34). However, although objective clinical responses have been observed when treating melanoma patients with TIL and high dose IL-2, responses were often transient and persistence of the transferred T cells was only limited (35). Evidence from murine studies showed that the impact of transferred T cells could be increased by lymphodepletion, either via the elimination of CD4+CD25+ regulatory T cells (36) or via disruption of homeostatic T cell proliferation (37,38). Indeed, as described by Dudley and co-workers, prior lymphodepletion improved persistence and function of transferred T cells in metastatic melanoma patients, resulting in clonal repopulation of the transferred T cells and regressing metastatic lesions (39). Unfortunately, the success rate of the use of TIL as a treatment for patients suffering from cancers other than melanoma has been less effective, possibly due to the absence of TIL with proper tumor recognizing capabilities in these types of tumors (40-42). Transferring tumor-reactive T cell receptor (TCR) genes into cytotoxic T cells or T helper cells might be an attractive strategy to circumvent these problems. Currently, functional capacity and persistence of these TCR-gene transferred T cells is being investigated *in vitro* and *in vivo* (43,44).

### **1.2.2 Active Immunotherapy**

Active immunotherapy aims at the development of a long-lasting anti-tumor immune response. In order to induce a durable and protective immune response *in vivo*, active specific cancer vaccines are

being studied widely. The identification of tumor-associated antigens (TAA), that distinguish normal cells from cancer cells, provided the basis for the development of antigen-specific immunotherapy. Processing and presentation of these TAA either via the host's or via a donor's APC had been shown to be an essential step in the generation of an immunological response against these tumor antigens. Many approaches aiming at eliciting antigen-specific responses, such as peptide-based vaccines, whole tumor cell vaccines, viral vector vaccines or DC-based vaccines, have been developed.

#### *Peptide-based vaccines*

It is generally accepted that T cells recognize antigens in the form of small, 8 to 10 amino acid long peptides, bound to MHC molecules on APC. Peptide-based vaccines aim at loading these peptides onto human leukocyte antigen (HLA) molecules on antigen-presenting cells *in vivo*, thereby initiating an anti-tumor immune response. Feasibility of peptide vaccination has been demonstrated in animal models both in prophylactic (45) and therapeutic settings (46). Nowadays, peptide-based vaccines have also been tested in numerous phase I and II clinical trials, especially for patients suffering from melanoma. In general, vaccinating patients with TAA-derived peptides with or without immune-potentiating adjuvants, such as incomplete Freund's adjuvant (IFA), GM-CSF, IL-2 or IL-12, induced clinical responses in only 0 to 30 % of treated patients (47-49). The limited clinical responsiveness observed in these studies is probably due to the use of relatively weak immunogenic peptides derived from self-proteins, lack of CD4 T cell help and tumor escape mechanisms. In addition, other important drawbacks of this approach are the relatively limited availability of adenocarcinoma-derived tumor antigens and the peptides' HLA allele restriction. Nevertheless, peptide-based vaccination has been shown to be feasible and safe, with moderate successes. Therefore, attempts to improve effectiveness of peptide-based vaccination are currently being undertaken, for example by making use of DC as a vaccine vehicle, by the use of multiple TAA-derived epitopes or by combining peptide vaccination with immune-modulating agents such as CpG or anti-CTLA-4 antibody (50,51). In addition, the use of so-called long peptide vaccines have also been demonstrated to be very promising, since they include both CTL and T helper epitopes. Moreover, because of the length of these peptides, direct binding to MHC molecules cannot take place and these peptides require endocytosis, processing and presentation by professional APC in order to induce T cell responses, thereby circumventing the potential risk of tolerance induction (52,53).

In conclusion, so far numerous T cell-defined TAA epitopes have been described and are available for clinical application. Together with the relatively inexpensive production of clinically approved batches of synthetic peptides, the straightforward administration in a clinical setting and the advantage of working with a well-defined product for immune monitoring purposes, peptide-based vaccination remains a promising approach for clinical anti-tumor immunotherapy.

#### *Whole tumor cell vaccines*

Another anti-tumor vaccination approach that is widely studied, in mice and men, is whole tumor cell vaccination. By making use of tumor cells as a source of antigen, T cells directed against a wide range of known and unknown TAA are targeted to elicit an anti-tumor immune response. As a result,

problems such as HLA allele restriction or tumor escape via loss of antigen can be avoided. Early clinical studies were mainly performed with autologous tumor cell vaccines. In a study conducted by Vermorken and co-workers, active specific immunization (ASI) with irradiated autologous tumor cells and Bacille Calmette-Guérin (BCG) in stage II colon cancer patients resulted in a significant improvement of recurrence-free survival, but not in a prolonged overall survival (54). Using the same approach in melanoma patients, of 40 patients treated, only five showed responses, four complete and one partial response, with a median duration of 10 months (55). Although vaccination with irradiated autologous tumor cells has been shown to be feasible and safe with promising results, response rates are low and improvements are necessary. In addition, one has to take into account that autologous tumor cell vaccination is not applicable for all patients, since not every tumor is easily accessible and the number of consecutive vaccinations is dependent on the size of the tumor and the subsequent *ex vivo* preparation of the vaccine. Consequently, recent investigations have focused on the use of allogeneic tumor cell vaccines. The observation by Huang and co-workers that TAA from allogeneic vaccines are not presented to the immune system by the tumor itself, but efficiently transferred *in vivo* to bone marrow-derived DC, indicates that TAA are cross-presented and that HLA matching of tumor cell vaccines is not a critical step in whole tumor cell vaccination, thereby removing conceptual difficulties in the use of allogeneic cells for vaccination (56). Besides that, allogeneic vaccines have several advantages over the use of autologous vaccines. Tumor cell lines can be extensively characterized *in vitro*, are relatively easily produced under good manufacturing practice (GMP) conditions and combining different tumor cell lines in the vaccine could provide a broad range of antigens shared between the cell line and the patient. Moreover, modification of the tumor cell vaccine by introducing immuno-modulating agents, such as cytokines, can more easily be made. Indeed, such a generalized approach, using inactivated allogeneic tumor cell lines engineered to secrete GM-CSF has been demonstrated to be feasible and safe, with vaccine-induced immune activation and anti-tumor immune responses in several different tumor types, such as advanced metastatic prostate cancer, pancreatic cancer or non-small cell lung cancer (57-59). Moreover, the observation that the use of GM-CSF-secreting allogeneic tumor cell vaccines (also known as GVAX®; Cell Genesys, Inc., San Francisco, CA) in patients with metastatic, hormone-refractory prostate cancer resulted in improved survival, as well as PSA declines in 32% of patients treated in the high-dose group, provided the rationale for the development of the phase 3 trial program that is currently ongoing (reviewed by Simons and co-workers (60)).

#### *Dendritic cell-based immunotherapy*

The recognition of DC as the main orchestrators of the adaptive immune response has been of great interest for the development of anti-tumor immunotherapy. As sentinels throughout the body, DC are designated to capture antigen, undergo maturation and subsequently migrate to secondary lymphoid organs, where they present the processed antigen to lymphocytes, thereby initiating immune responses. Pilot DC vaccination studies have demonstrated the induction of anticancer immune responses with encouraging clinical results (61-63). Many efforts have been made to further develop DC vaccination strategies into a novel therapeutic option for the treatment of cancer. In the next

paragraphs, the basic of DC biology are discussed, followed by an overview of conducted DC vaccination trials, focusing on the different DC subsets, the origin of the DC and providing a rationale for the development of an allogeneic human DC cell line for clinical DC vaccination.

### 1.3 Dendritic cells

#### 1.3.1 Dendritic cell biology

##### *DC subsets*

DC were first observed in 1868 by Paul Langerhans as dendritically shaped cells in the human epidermis (64). Since the recognition of DC in mouse spleen by Steinman and co-workers in 1973 (65), much progress has been made in the understanding of their origin and their role in the immune system. The earliest studies on DC biology and functions have mainly been performed on mouse spleen-derived DC; more recently, human DC have also been widely studied. Although the development of DC *in vivo* is not fully understood, it has been described that DC originate from bone marrow-derived CD34+ hematopoietic progenitor cells (HPC). CD34+ HPC can give rise to two classical myeloid DC subsets, i.e. Langerhans cells (LC), which line the epithelial layers of the skin, and interstitial dendritic cells (IDC), which can be found in the dermis and throughout the body (66). Besides that, CD34+ HPC can also give rise to a non-classical plasmacytoid DC (PDC) subset, which have been described to secrete large amounts of IFN- $\alpha/\beta$  upon viral encounter, thereby representing the first barrier to intruding viruses (figure 1) (67,68). The development of culture systems to produce large amounts of DC *in vitro*, have facilitated DC studies tremendously. *In vitro*, myeloid DC can be cultured from blood or bone marrow-derived CD34+ HPC or peripheral blood-derived CD14+ monocytes (69-71). Upon culturing in the presence of GM-CSF, TNF- $\alpha$  and TGF- $\beta$  or IL-4, CD34+ HPC progenitors can be differentiated into LC or IDC, respectively (70,72,73). Besides that, IDC can also be generated from CD14+ monocytes. In the presence of different cytokine combinations, CD14+ monocytes can develop into DC with distinct phenotype and function. As described, upon culturing with GM-CSF and IL-4, IFN- $\alpha/\beta$  or IL-15, monocytes are able to develop into IL-4-DC, IFN-DC or IL-15-DC, respectively (74-79).

##### *Antigen capture, processing and presentation*

*In vivo*, DC precursors migrate from the bone marrow and circulate through the blood to multiple sites in the body, where they reside in an immature state. In this state, they are efficiently and continuously sampling the environment for antigens via several mechanisms (80-84), such as macropinocytosis or receptor-mediated endocytosis using either C-type lectins such as the macrophage mannose receptor (MMR) and DEC-205 or Fc $\gamma$  receptors RI (CD64) and RII (CD32). Besides that, DC can also phagocytose viruses, bacteria, intracellular parasites or apoptotic/necrotic cells. After internalization, the antigen is transported to the endosomal/lysosomal compartment, after which the engulfed proteins can be degraded by proteases, loaded onto MHC class II molecules, and subsequently transported to the plasma membrane and presented to CD4+ T cells.

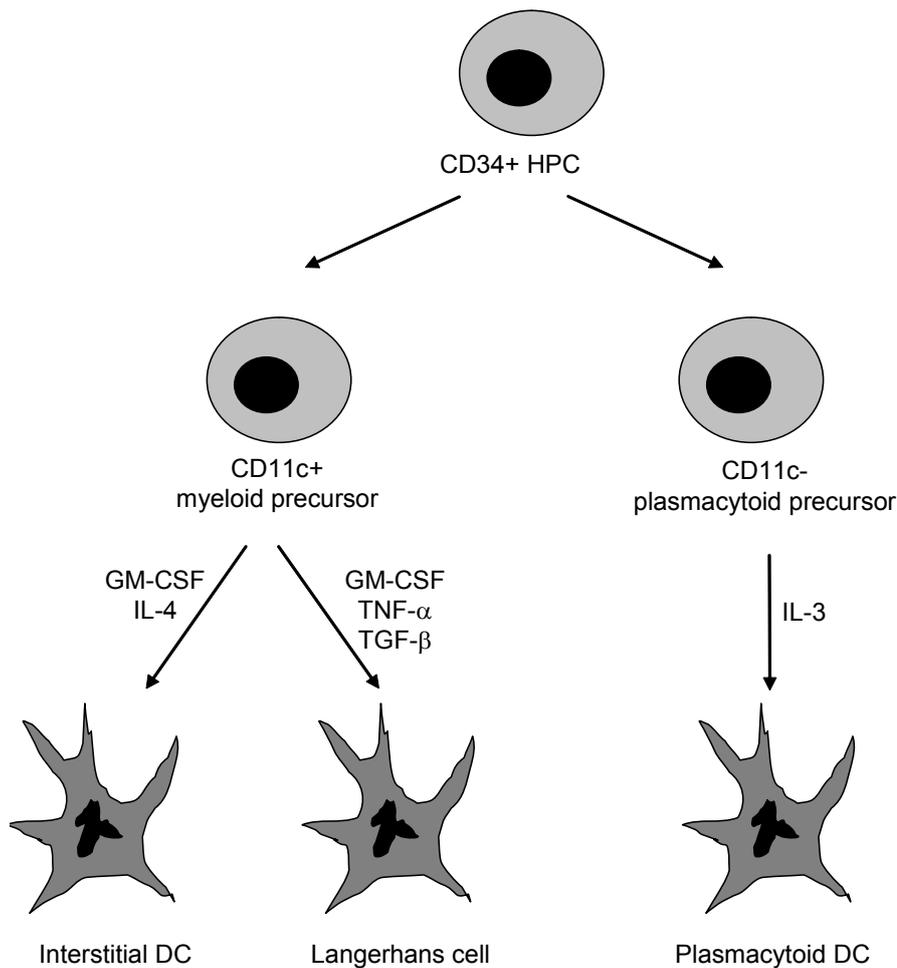


Figure 1. **Subsets of human dendritic cells.** Three subsets of human dendritic cells (DC) can be discerned. Both CD11c<sup>+</sup> myeloid DC precursors and CD11c<sup>-</sup> plasmacytoid DC precursors originate from bone marrow-derived CD34<sup>+</sup> hematopoietic progenitor cells (HPC). CD11c<sup>+</sup> myeloid precursors are able to differentiate into Interstitial DC (IDC) in response to GM-CSF and IL-4, or Langerhans cells (LC) in response to GM-CSF, TNF- $\alpha$  and TGF- $\beta$ . Plasmacytoid DC can be generated from CD11c<sup>-</sup> plasmacytoid DC precursors in response to IL-3. N.B. Additional LC differentiation routes involving alternative cytokines (e.g. IL-15) have recently been identified *in vitro*; their *in vivo* relevance remains to be established.

On the other hand, exogenous proteins can also be presented to CD8<sup>+</sup> T cells via presentation in MHC class I. Although MHC class I was initially described to display antigenic peptides derived from endogenous, cellular proteins that have been degraded by proteosomal cleavage, translocated from the cytosol to the endoplasmic reticulum (ER) and loaded onto newly synthesized MHC class I molecules (5,7), it has since been described that exogenous proteins that do not have access to the cytosol, can still be presented in an MHC class I restricted manner (85). This phenomenon, called cross-presentation, is specific for DC and thought to be involved in the generation of CTL responses against pathogens, transplantation antigens and tumors, as well as in the generation of tolerance to self antigens by the deletion of auto-reactive CD8<sup>+</sup> T cells (86). Two main intracellular pathways have

been described for cross-presentation. Peptides derived from exogenous proteins can be loaded onto MHC class I molecules via peptide exchange in recycling endosomes (87), or via TAP-dependent loading in the ER after passing through a phagosome-to-cytosol pathway (88).

#### *Maturation and migration*

Immature DC are particularly well-equipped to capture and process exogenous antigen, while mature DC are specialized in antigen presentation and T cell activation. Maturation of DC is a complex process that leads to terminal differentiation, transforming DC into highly immunogenic cells that are very potent in T cell stimulation. This maturation is accompanied by down-regulation of endocytic/phagocytic capacity, morphological changes with subsequent formation of characteristic cytoplasmic veils, acquisition of high cellular motility, migration towards lymphoid organs and the up-regulation of receptors involved in migration, antigen presentation and T cell stimulation. Maturation is induced by stimuli (also called “danger signals”) that alert the immature/resting DC to the presence of pathogens, inflammation or tissue damage (4). These maturation signals originate either from host-derived molecules like CD40L (89), inflammatory cytokines like TNF- $\alpha$  or IL-1 $\beta$  (90), or from agents such as lipopolysaccharides (LPS), bacterial DNA (91) and double-stranded RNA (92). The latter signal via toll-like receptors (TLR), a class of receptors that are able to recognize pathogen-associated molecular patterns (i.e. PAMPs) and upon recognition activate intracellular signaling pathways, resulting in induction of pro-inflammatory cytokines, chemokines and type I interferons (IFN- $\alpha$  or - $\beta$ ) (93,94).

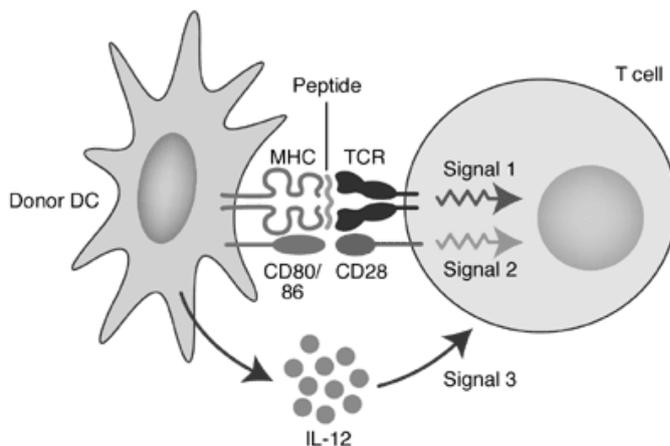
As mentioned above, maturation transforms DC into highly immunogenic cells, with excellent antigen presentation and T cell activation properties. Three main processes are involved in this transformation. First, maturation induces up-regulation of peptide-MHC class I and II complexes by increased expression of TAP and immunoproteasome components (95,96), by activation of lysosomal hydrolases and up-regulation of transport of p-MHC complexes to the cell surface (97). Second, maturation induces an increased expression of adhesion molecules and co-stimulatory molecules (CD80, CD86 and CD40), which have been described to interact with their receptors on T cells and are known to be important for T cell activation and the formation of the immunological synapse. Third, maturation is also known to induce the secretion of cytokines that are involved in the generation of T helper and CTL responses, such as IL-12 (89).

DC maturation is tightly linked to migration from the periphery to the T cell areas of the draining lymph nodes, where the actual T cell stimulation takes place. This migration involves the coordinated action of several chemokines and their receptors. DC maturation results in the down-regulation of receptors that are involved in attracting immature DC to the inflammatory environment, such as CCR1, CCR5 and CCR6, thereby turning off the sensitivity to their ligands macrophage inflammatory protein (MIP) -1 $\alpha$  and  $\beta$ , Regulated on Activation Normal T cell Expressed and Secreted (RANTES) and MIP-3 $\alpha$  (98). In addition, maturation induces the up-regulation of chemokine receptor CCR7, which results in the responsiveness of DC to MIP-3 $\beta$  and 6Ckine. Consequently, maturing DC will enter the lymph stream in response to 6Ckine, which is expressed by lymphatic vessels (99), and upon arrival in the draining lymph node migrate to the paracortical areas of the draining lymph nodes in response to MIP-

3 $\beta$  and 6Ckine (100). Importantly, MIP-3 $\beta$  and 6Ckine (CCL19 and CCL21) are also involved in attracting naïve T lymphocytes, indicating that these chemokines play a key role in DC – T cell interactions in the lymph nodes.

### *T cell activation*

The ability to activate naïve T cells is a unique function of DC. After antigen uptake, maturation and migration to the secondary lymphoid organs, DC have evolved into specialized antigen presenting cells with proper T cell activating properties. Recently however, DC have also been described to be involved in the induction of peripheral tolerance. Under steady-state conditions and in the absence of microbial stimulation, i.e. in the absence of proper DC activation, DC have been shown to be involved in inducing tolerance by either apoptosis of activated T cells (86), anergy (101) or the induction of regulatory T cells (102,103). Whereas improper DC activation is important for the induction of tolerance against self-antigens, thereby controlling auto-immunity, this is detrimental to the development of T cell immunity against for instance pathogens and tumors. Consequently, generating T cell immunity against these harmful pathogens/tumors not only depends on signaling via MHC-peptide complex and the T cell receptor (TCR; signal 1) but also requires additional signals (see figure 2). DC – T cell clustering is stabilized by integrins and adhesion molecules like CD2 (LFA-2), CD58 (LFA-3), CD50 (ICAM-2) and CD54 (ICAM-1) (104). A crucial factor in DC – T cell interaction is the interaction between co-stimulatory molecules CD80/CD86 on DC and CD28 on T cells, also known as “signal 2”. This interaction is important in sustaining T cell activation and activation of T cells in the absence of this interaction leads to the induction of T cell anergy (105,106). Additional co-stimulatory signals, provided by the TNFR/TNFligand family members 4-1BB-4-1BBL, OX40-OX40L and CD27-CD70, are involved in sustaining, diversifying and/or amplifying the immune response (107). Whereas the interaction of CD80/CD86 with CD28 on T cells plays a major role in activation and expansion of effector and T helper cell subsets, ligation of CD80/CD86 with CTLA-4 on T cells, a counter-receptor that is up-regulated upon activation, negatively regulates T cell activation, and thus plays an important role in T cell homeostasis and maintaining peripheral tolerance (108,109).



**Figure 2. The interaction between dendritic cells and T cells involves three signals.** Signal 1 follows the interaction between major histocompatibility complex (MHC) molecules containing peptides on the DC and the T-cell receptor (TCR) on the T cell. Signal 2 follows the interaction between co-stimulatory molecules (e.g. CD80/86) on the surface of the DC with CD28 on the T-cell surface. Signal 3 follows the secretion of bioactive IL-12 by the DC. Taken from expert reviews in molecular medicine, 2002. Cambridge University Press.

Besides signal 1 and 2, T cell activation also requires the production of inflammatory cytokines such as IL-12. The production of IL-12 can be initiated by signaling via TLRs, or by ligation of CD40 by CD40-ligand-expressed on CD4<sup>+</sup> T helper cells. The requirement of such a third signal has been demonstrated by Curtsinger and co-workers (110). Upon stimulation with antigen-bearing DC in the absence of IL-12, naïve CD8<sup>+</sup> T cells will undergo clonal expansion, but will be less potent in developing effector function (111). Indeed, although these signal 3 tolerant CD8<sup>+</sup> T cells are able to recognize targets cells, they are not able to mediate cytolysis due to lack of granzyme B in their lysosomal granules (112).

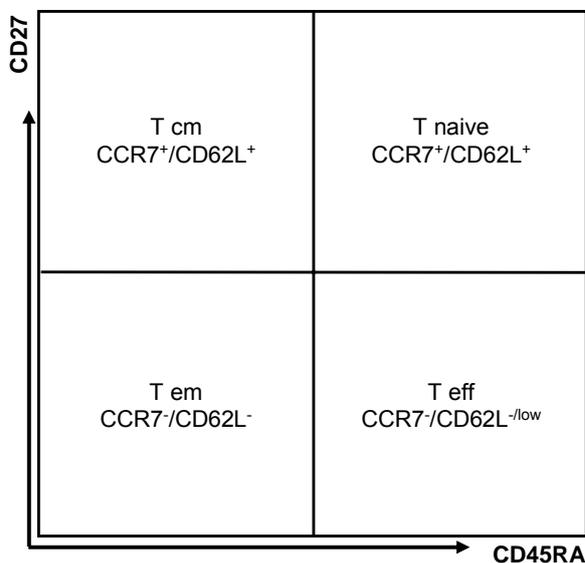
### *T cell polarization*

An effective defense against various types of dangers such as pathogens or tumors requires specialized classes of immune responses. To provide these, distinct subsets of CD4<sup>+</sup> T helper cells need to be generated with different effector functions. DC are known to be involved in the differentiation towards these distinct subsets. This T cell polarizing signal (also known as signal three) is mainly dependent on the cytokine profile and maturation stimulus of the DC, and can skew CD4<sup>+</sup> T cells towards T helper 1 (Th1) cells, T helper 2 (Th2) cells, T regulatory (Treg) cells or the recently described Th17 cells. Type 1 polarizing cytokines, such as IL-12 and IL-27, drive naïve T cells to produce IFN- $\gamma$  and TNF- $\beta$ , thereby strongly promoting a Th1 response (113). This Th1 response is involved in the induction of cell-mediated immunity (supporting CD8<sup>+</sup> T cells) against intracellular pathogens such as viruses, but also against tumor cells. MCP-1, IL-4 and OX40L are well known type 2 polarizing factors that drive naïve CD4<sup>+</sup> T cells to become Th2 cells, producing large amounts of IL-4, IL-5 and IL-13 (114). These cytokines are important for the defense against helminths and stimulate B cells to produce antibodies. Besides promoting effector Th1/Th2 responses, DC have also been described to play a role in the induction of regulatory T cells. By producing the anti-inflammatory cytokine IL-10, DC can either directly induce the development of Treg cells (115), or indirectly, via the inhibition of full DC maturation (116). DC-derived cytokines such as TGF- $\beta$ , IL-6 and IL-23 have been demonstrated to be involved in the development and/or maintenance of Th17 cells (117-120). This novel subset of effector T cells, has been described recently to be involved in the pathogenesis of inflammatory and autoimmune diseases such as rheumatoid arthritis, experimental autoimmune encephalomyelitis and inflammatory bowel disease (121-123). Although Th17 cells were demonstrated to be present in peripheral blood and tumor tissues of advanced carcinoma patients, the nature of these Th17 cells in the context of tumor immunology is still unknown (124).

### *T cell differentiation*

Once activated, naïve T cells will undergo several phenotypic and functional changes, and differentiate into effector cells. During this differentiation process, they will undergo a chain of events that include activation of multiple signal transduction pathways, structural reorganization of the cytoskeleton, alterations in cell adhesion and migration and the induction of cell division, to develop into functional effector T cells. After executing their effector function, the T cell response will undergo contraction (i.e. effector cells die by apoptosis). A small portion of the T cells will persist as circulating

memory cells. These memory T cells are long-lived, and will respond more rapidly upon re-encounter of the antigen. The nature of the development of this immunological memory is still unknown. Exploring the mechanism behind the development of memory T cells has resulted in several proposed mechanisms. In the classical linear differentiation model, long-lived memory T cells develop from effector T cells after contraction of the immune response (125), whereas in the divergent differentiation model, memory T cells develop directly from activation of naïve T cells and represent a developmental pathway distinct and independent from that of effector T cells (126). Recently, a revised model has been proposed, i.e. the 'intersecting pathway model', in which memory T cell development can be driven through both antigen-dependent activation (i.e. via naïve-to-effector-to-memory differentiation) and antigen-independent homeostatic factors such as IL-7 (i.e. naïve-to-memory differentiation) (127). Furthermore, it has also been suggested recently that effector and memory T cells are simultaneously generated from the division (mitosis) of a T cell via asymmetric cell division (i.e. "the one cell, two fates model" (128). In addition to the initial programming event, memory T cells also need to be maintained during the interval between primary and subsequent exposure to antigen. Factors involved in the homeostasis of these memory T cells are cytokines such as IL-7 and IL-15 (129), and TNFR family members such as 4-1BBL, CD30L and OX40L. Although mice lacking 4-1BBL showed a normal primary expansion and contraction of the CD8+ T cell response to influenza virus, they showed decreased T cell numbers 3-5 weeks post-infection (130,131).



**Figure 3. Expression of CD45RA and CD27 on different T cell subsets.** The different T cell subsets can be defined by the expression of CD27, CD45RA, CD62L and CCR7. Naïve T cells (Tnaïve) are defined as CD27<sup>+</sup>/CD45RA<sup>+</sup>/CCR7<sup>+</sup>/CD62L<sup>+</sup>, effector T cells (Teff) as CD27<sup>-</sup>/CD45RA<sup>+</sup>/CCR7<sup>-</sup>/CD62L<sup>-low</sup>, central memory T cells (Tcm) as CD27<sup>+</sup>/CD45RA<sup>-</sup>/CCR7<sup>+</sup>/CD62L<sup>+</sup> and effector memory T cells (Tem) as CD27<sup>-</sup>/CD45RA<sup>-</sup>/CCR7<sup>-</sup>/CD62L<sup>-</sup>.

Several studies have reported phenotypic and functional differences between the different T cell subsets based on the expression of CD62L (adhesion molecule involved in leukocyte rolling on high endothelial venules), CCR7 (chemokine receptor involved in lymph node homing), CD27 (co-stimulatory receptor for T cell survival and growth), CD45 (figure 3) and effector molecules such as IFN- $\gamma$ , IL-5 and IL-4 (CD4<sup>+</sup> T helper cells) or IFN- $\gamma$ , TNF- $\alpha$ , perforin and granzyme B (CD8<sup>+</sup> cytotoxic T cells). Naïve CD4<sup>+</sup> and CD8<sup>+</sup> T cells are defined as CD27<sup>+</sup>/CD45RA<sup>+</sup>/CCR7<sup>+</sup>/CD62L<sup>+</sup> and IFN- $\gamma$ , TNF- $\alpha$ , perforin

and granzyme B negative. Effector T cells are defined as CD27-/CD45RA+/CCR7-/CD62L<sup>- or low</sup> and IFN- $\gamma$ , TNF- $\alpha$ , perforin and granzyme B positive. Further more, distinct subsets of memory T cells can be distinguished: central memory T ( $T_{cm}$ ) cells and effector memory T ( $T_{em}$ ) cells. Central memory T cells can be defined as CD27+/CD45RA-/CCR7+/CD62L+. These  $T_{cm}$  exhibit lymph node-homing properties, lack effector functions, and upon re-challenge generate a new wave of effector T cells. Effector memory T cells on the other hand, can be defined as CD27-/CD45RA-/CCR7-/CD62L- and IFN- $\gamma$ ,+/TNF- $\alpha$ /perforin+/granzyme B+, representing a readily available pool of antigen-primed T cells that can enter peripheral tissues to mediate an inflammatory response instantly (132-134).

#### *Dendritic cell interactions with other lymphocytes.*

DC have been described to play a key role in the regulation of both innate and adaptive immunity. Besides activating T cells, DC can also activate NK cells, natural killer T (NKT) cells and B lymphocytes.

NK cells are effector cells of the innate immune system. Although the CTL killing machinery is shared between NK cells and CD8+ T cells, NK cell recognition of targets cells occurs via MHC independent inhibitory and activating receptors such as CD16, NKp30, NKp44, NKp46 and NKG2D. NK cells can be activated by DC (135-138). This activation might occur either directly via cell-cell contact, for example through the interaction of MHC class I-related protein A/B on DC and NKG2D on NK cells (135) or indirectly via the production of soluble factors such as IL-12. Interestingly however, it has been described recently that the DC – NK cell interactions function bi-directionally, indicating that DC are not only able to activate NK cells, but NK cells are also able to activate DC. This NK cell-directed DC activation resulted in the maturation of the DC, as shown by up-regulation of CD86 and the production of IL-12 (139,140). The DC maturation was shown to be mainly dependent on cell-cell contact, although NK-derived TNF- $\alpha$  and IFN- $\gamma$  also contributed to maturation. Importantly, NK-mediated DC maturation also induced the up-regulation of MHC class I molecules, making the DC more resistant to NK cell mediated lysis and thereby selecting for a more immunogenic DC during the initiation of the immune response.

NKT cells bridge the adaptive and innate immune system. NKT cells represent a population of lymphocytes that share characteristics with both NK cells and T cells. They are known to exhibit a biased TCR gene usage, recognize glycolipids presented via the MHC class I homologue CD1d and produce high levels of cytokines, particularly IL-4 and IFN- $\gamma$ , thereby co-determining the type of immune response (141). They have been shown to be involved in controlling auto-immunity (142), in resistance to tumors (143) and in the protection against infectious agents (144). DC presenting the synthetic glycolipid  $\alpha$ -galactosylceramide via CD1d are able to activate NKT cells to produce IFN- $\gamma$  and thereby promote the resistance to tumors. Vice versa, DC can also become fully mature upon interaction with activated NKT cells, demonstrating a reciprocal activation interaction between DC and NKT cells (145,146).

DC have also been described to play a crucial role in the initiation of humoral immune responses. Indirectly, via DC-mediated activation of naïve T cells in the T cell area of the secondary lymphoid organs, and subsequent expression of CD40L, B cell survival and proliferation will be promoted (147),

as well as B cell differentiation and isotype switching (148,149). B cell activation can also be regulated by DC directly. DC enhanced the differentiation of activated memory B cells towards IgG-secreting cells and activated naïve B cells towards plasma cells, through the production of IL-12 and soluble IL-6R $\alpha$  (150,151). In addition, B cell proliferation and immunoglobulin secretion can also be initiated by the interaction between the B cell activating factor belonging to the TNF family (BAFF) on DC and BAFF receptor on B cells (152).

### 1.3.2 DC in tumor immunology

Early data on the possible role of DC in tumor defense were based on studies in experimental animals, as well as on human studies in which DC infiltration of the primary tumor was found to be associated with prolonged survival and a better prognosis (153,154). However, in most cancer patients, functional DC, expressing appropriate levels of CD80 and CD86, are often absent in tumors, resulting in a reduced T cell stimulatory capacity and the induction of tolerance, as also described by Chau and co-workers (155). In addition, tumor-produced immunosuppressive cytokines such as IL-10, TGF- $\beta$  and vascular endothelial growth factor (VEGF) might be responsible for reduced DC development and function. Likewise, DC dysfunction is not only observed at the tumor site, but also in peripheral blood of patients (156).

The recognition of DC as key players in controlling immunity, have made DC promising targets for the development of anti-cancer immunotherapy. *In vivo* modulation of the tumor environment by the activation and differentiation of DC might shift the balance from tolerance induction to the induction of an anti-tumor immune response. A promising approach to achieve this is DC vaccination. By making use of DC as a vehicle for tumor antigen delivery, protective immunity against tumors and a reduction of the size of established tumors has been observed in animal models (157-159). Indeed, pilot DC vaccination studies in humans have also yielded promising results. Initially, DC vaccination studies were performed with DC directly isolated from peripheral blood (160,161). Major disadvantages of this approach were the low yields of DC (161), the variety of peripheral blood DC subsets (162) and the varying percentages of these DC subsets in cancer patients. The development of methods to generate DC from hematopoietic progenitor cells *in vitro* has facilitated DC vaccination studies tremendously. The main sources of DC progenitors that have been described to yield sufficient amounts of DC are blood or bone marrow-derived CD34<sup>+</sup> HPC and monocytes.

In the last years, DC are increasingly applied as adjuvants for the immunotherapy of cancer. Although early clinical trials indicate that DC vaccination is feasible and safe, and can initiate immune responses in cancer patients (163-166), it has become clear that immunological and clinical outcome are dependent on the quality of the DC vaccine. Because of the tight balance between induction of tolerance or anti-tumor immunity, it is of great importance to prepare an appropriate immunostimulatory vaccine. Therefore, a number of challenges in DC preparation need to be addressed, including antigen loading, the route of delivery and administration schedule, the activation status and the source of DC (167,168).

### *Antigen loading of DC*

The use of DC as vehicle for tumor antigen delivery and subsequent TAA-specific T cell activation requires loading of the DC with tumor-associated antigens. DC can be loaded with small 8-10 amino acid long peptides, whole protein, tumor lysates, transfected with mRNA, DNA, transduced with TAA-encoding viral vectors, or fused with tumor cells.

DC can be very efficiently loaded with TAA-derived MHC class I and II restricted peptides (169,170). However, some important limitations to the use of this approach have been described including HLA restriction of the peptides, limitations of the response to only the used TAA, the relatively limited availability of adeno-carcinoma associated tumor antigens and the rapid turn-over of exogenous peptide-MHC complexes.

Part of these problems could be overcome by utilizing full-length recombinant proteins or tumor lysates (61). Full-length proteins or tumor lysates provide not only MHC class I restricted TAA presentation, thereby activating CD8<sup>+</sup> T cells (i.e. cross-priming), but also provide MHC class II restricted TAA presentation leading to T helper cell activation. In addition, these approaches bypass HLA restriction. Importantly, tumor cell lysates provide a broad range of tumor antigens, either known and unknown, of which some might be uniquely mutated. A disadvantage is however that these tumor lysates also include an unknown number of auto-antigens that would normally be ignored by the host, but under these circumstances might cause autoimmunity. Besides that, cross-presentation of the exogenous proteins is required in order to get MHC class I presentation and subsequent CD8<sup>+</sup> T cells activation. The need for this rather inefficient process can be overcome by making use of gene transfer methods that result in direct antigen processing in the MHC class I pathway, including transporter peptides linked to full-length proteins (for example HIV *tat*) (171), tumor RNA transfections (172) or the use of TAA-encoding recombinant viral vectors (173). The latter may also provide additional immuno-stimulatory signals to the DC via exposition to viral components.

### *Route of delivery and administration schedule*

The route of delivery and the administration schedule of the DC have also been described to be very important in generating a potent anti-tumor immune response *in vivo*. As reviewed by Figdor and co-workers (174), DC vaccines are administered via various delivery routes, including intravenous, intradermal or intranodal injections. In mice and humans, subcutaneous (mice) or intradermal (human) injections have been proven to be more effective than intravenous injections (175,176). However, as described by de Vries *et al* (177), less than 5% of intradermally injected mature DC generally end up in the draining lymph nodes, indicating that intradermally injected DC show a poor migratory capacity *in vivo*. On the other hand, lymph node migration of intranodally injected DC has been demonstrated to be much more effective. Unfortunately, this procedure is complex and the tumor and its draining lymph nodes are not always easily accessible.

Besides route of delivery and subsequent migration towards the lymph nodes, the administration schedule of DC is also very important for the induction of a potent anti-tumor immune response. Until now, administration schedules are mostly derived from experience with animal models and vaccination studies in infectious diseases. Vaccinations against infectious agents have shown that prime-boost

regimens with a 4-6 week interval provide optimal responses. However, recent studies have demonstrated that DC immunization stimulates accelerated memory CD8<sup>+</sup> T cell induction that shortens the time required for booster amplifications (178). Still, some caution is necessary, since over-vaccination might cause immune exhaustion. As described by Serody and co-workers, weekly administration of a peptide-loaded DC vaccine for six weeks resulted in a diminished CTL activity, whereas this was not observed when the DC vaccine was administered three-weekly for six treatments (179). In humans, weekly injections with monthly boosting or monthly injection schedules are currently being investigated (167).

#### *Activation status of DC*

Recent DC vaccination studies in humans have demonstrated that mature, but not immature DC, induce an effective anti-tumor immune response (180,181). As described by Dhodapkar and co-workers, injection of immature antigen-bearing DC led to tolerance induction and negatively affected antigen-specific immunity by the inhibition of effector function of pre-existent effector memory T cells. The loss of effector function, demonstrated by a diminished IFN- $\gamma$  production and lytic potential, was not the result of a loss of circulating antigen-specific T cells. Rather, the decline in IFN- $\gamma$ -producing antigen-specific CD8<sup>+</sup> T cells was associated with the appearance of IL-10 producing antigen-specific T cells, indicating that vaccinating with immature DC led to the induction of T cells with regulatory properties (180). Thus, the effective induction of an anti-tumor CTL response requires fully mature DC that express high levels of MHC and co-stimulatory molecules, produce high levels of IL-12p70, express the chemokine receptor CCR7 and show responsiveness to lymph node homing chemokines. Currently, the most commonly used mature DC preparations in clinical trials, also referred to as the 'gold standard' DC, are prepared by maturation with TNF- $\alpha$ , IL-6, IL-1 $\beta$  and prostaglandin E2 (PGE2). PGE2 has been described to be a key factor in inducing full maturation of DC and subsequent induction of migratory responsiveness towards lymph node-homing chemokines (182,183). A major disadvantage of these 'gold standard' DC is the PGE2-related low level or absence of IL-12 production (184). Therefore, novel DC-activation protocols are currently being investigated. As described by Mailliard and co-workers, PGE2 dependence for DC migration could be overcome by making use of a cytokine cocktail combining the classical type-1 polarizing cytokines such as TNF- $\alpha$ , IL-1 $\beta$  and IFN- $\gamma$  with IFN- $\alpha$  and polyinosinic:polycytidylic acid (p-I:C). These  $\alpha$ -type-1 polarized DC ( $\alpha$ DC1) showed a fully mature phenotype with high migratory potential and strongly elevated IL-12p70 production *in vitro*. In addition, these  $\alpha$ DC1 showed superior T cell stimulatory capacity compared to standard DC (185). Still, *in vivo* effectiveness of  $\alpha$ DC1 needs to be investigated in comparative clinical studies.

#### *Source of DC*

The discovery that large amounts of human DC could be generated *in vitro* has accelerated their characterization, and facilitated subsequent implementation in clinical DC vaccination studies. The main sources of DC progenitors that have been described to yield sufficient amounts of DC are blood or bone marrow-derived CD34<sup>+</sup> HPC and monocytes. *In vitro*, CD34<sup>+</sup> HPC progenitors can be differentiated into interstitial/dermal DC (IDC) in the presence of GM-CSF, IL-4 and TNF- $\alpha$  (70,72), or

into Langerhans cells (LC) in the presence of GM-CSF, TGF- $\beta$  and TNF- $\alpha$  (73). Besides that, IDC can also be generated from CD14<sup>+</sup> monocytes by culturing with GM-CSF and IL-4 (74,75). So far, in the majority of clinical trials MoDC have been used for vaccination, as reviewed by Davis *et al.* and Figdor *et al.* (167,186). MoDC have been demonstrated to be very potent in inducing antigen-specific immune responses in healthy volunteers and in cancer patients. A single injection of mature, keyhole limpet hemocyanin (KLH), tetanus toxoid (TT) and flu matrix (flu-M)-peptide-pulsed MoDC already resulted in the induction of KLH-specific Th1 cells and flu-M-specific CD8<sup>+</sup> T cells (187). Intranodal injections of autologous MoDC loaded with tumor peptides or tumor lysates resulted in clinical responses in 5 of 16 melanoma patients treated, including two complete remissions that lasted for over 15 months (188). Besides MoDC, CD34-derived DC have also been used for vaccination. In patients suffering from melanoma and multiple myeloma, these CD34-DC have been demonstrated to be safe and well-tolerated, and to be capable of inducing both antigen-specific responses and clinical responses (165,166).

Interestingly, it has been suggested recently, that CD34-DC are more effective as compared to MoDC in clinical DC vaccination studies (165), as well as in *in vitro* antigen-specific T cell stimulation (189). It was hypothesized that this superior functional activity might be due to the presence of “contaminating” LC in the CD34-DC preparations. This superior antigen-specific T cells stimulatory capacity of CD34-LC could also be demonstrated *in vitro* (190). However, for clinical DC vaccination purposes, functional differences between IDC and LC need to be characterized more extensively.

To date, most DC vaccination studies have been performed with autologous DC. However, difficulties in the generation of functional DC from cancer patient-derived DC precursors are a major drawback of this approach. As described, these DC precursors might exhibit defective differentiation and maturation capacities, resulting in DC that display lower T cell stimulatory and migratory potential (191-193). By making use of allogeneic healthy donor-derived DC, problems with the generation of such dysfunctional, tolerizing DC could be overcome. However, the use of allogeneic DC might create HLA incompatibility, thereby inducing an allogeneic T cell response, which might interfere with proper tumor antigen presentation and recognition. Nevertheless, the induction of an allogeneic response might also be beneficial in the generation of anti-tumor immunity. The recognition of intact allogeneic MHC molecules induces the activation of high-frequency alloreactive T cells, of which some might also display specificity for tumor peptides that are presented on self-MHC. This phenomenon, called cross-reactivity, has been demonstrated for virus-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells (194,195). In addition, induction of a strong allogeneic CD4<sup>+</sup> T cell response might provide T cell help for the self-restricted T responses directed against tumor peptides (196,197).

The applicability of allogeneic DC vaccines has already been tested in clinical trials, in which patients suffering from B-cell chronic lymphocytic leukemia (198) and renal cell carcinoma (199) were vaccinated with tumor lysate-pulsed allogeneic DC. Allogeneic MoDC vaccination has been shown to be feasible and well tolerated, and of 18 renal cell carcinoma patients that completed the vaccination protocol, two patients showed a mixed response and three showed disease stabilization. However, compared to prior studies in the same department with autologous MoDC, responses generated were relatively poor (199,200), indicating that allogeneic DC vaccination needs to be further optimized.

Fabre and co-workers suggested that for an optimal result in allogeneic DC vaccination, it would be favorable to use DC that were at least in part matched for class I and II alleles. A promising approach to achieve this is the generation of allogeneic DC-tumor cell (TC) fusions. By making use of DC and tumor cells that each express different HLA alleles, one can provide a vaccine that matches a wide range of HLA alleles. Immunogenicity of such vaccines has been proven. As described by Trefzer and co-workers, eight of 17 stage III and IV metastatic melanoma patients treated with 4-weekly intradermal injections of an allogeneic DC-autologous TC hybrid vaccine showed clinical responses, of which one patient showed complete regression of all metastatic lesions, one showed a partial response and six patients showed a stabilization of progressive disease for the duration of 6-15 months (201). In addition, allogeneic DC-allogeneic TC hybrids have also been demonstrated to be well-tolerated, and clinically efficacious in metastatic renal cell carcinoma patients. Of note, no differences in clinical outcome were observed when compared to patients treated with allogeneic DC-autologous TC hybrids (202).

### 1.3.3 Rationale for a DC cell line for vaccination purposes

Although DC biology has been studied extensively throughout the last 30 years, and DC have been described as the main orchestrators of the immune response, so far implementation of DC as vaccine for the immunotherapy of cancer did not live up to the expectations. Clinical DC vaccination has been proven feasible and safe, but clinical results were variable. In order to exploit the full potential of these cells, preparation of the DC vaccine must be standardized and studies must be adequately designed and interpreted. Major obstacles in the generation of a standardized DC vaccine are the relatively limited availability of DC precursors and the inter- and intra-donor variability. Besides that, the preparation of an individual vaccine for each and every patient is very labor intensive and expensive. Therefore, it would be preferable to use DC from a readily available, unlimited source.

#### *MUTZ-3*

It has been described that cell lines derived from tumors of the myeloid lineage, exhibit the potential to differentiate into DC-like APC. Monocytic and myelogenous leukemia cell lines, such as THP-1, KG-1 and HL-60 are able to rapidly differentiate into mature DC-like cells, displaying morphologic, phenotypic and functional characteristics of DC (203-205). However, these leukemia cell lines are relatively insensitive to cytokine treatment, resulting in low percentages of CD1a expressing DC. Pharmacological agents like calcium ionophores are often more successful in inducing a DC-like phenotype, however, this method results in instant maturation and thus bypasses the immature stage.

In earlier studies from our group, the acute myeloid leukemia (AML) cell line MUTZ-3 was found to be cytokine-dependent for its proliferation and survival. In addition, the MUTZ-3 cell line was shown to down-regulate CD14 in response to cytokines, and exhibit characteristics of CD34-derived DC precursors (206-208). In response to GM-CSF, TNF- $\alpha$  and IL-4 or TGF- $\beta$ , MUTZ-3 progenitors acquire an interstitial-like DC or Langerhans-like LC phenotype, respectively (208). These data suggest that the MUTZ-3 cell line may provide easily standardized allogeneic DC from a readily available and unlimited source.

## 1.4 Outline of this thesis

In this thesis, we tested the hypothesis that the human myelomonocytic cell line MUTZ-3 is a rapid and reproducible model for IDC and LC differentiation. Related to that, we also hypothesized that MUTZ-3 and similar cell lines might have therapeutic utility in clinical DC vaccination protocols. To this end, this thesis is divided into two parts, concerned with the following questions:

**Part 1:** Can the CD34+ acute myeloid leukemia cell line MUTZ-3 serve a human interstitial DC (IDC) or Langerhans cell (LC) cell line model? Are the generated MUTZ-3 IDC and LC phenotypically and functionally representative for their *in vivo* counterparts?

**Part 2:** Can the MUTZ-3-derived IDC and LC be used for clinical DC-based anti-tumor immunotherapy? Do the MUTZ-3 IDC and LC exhibit all the required phenotypic and functional characteristics necessary for clinical DC vaccination? Which DC subset is most promising for clinical DC vaccination? Can we develop strategies to further improve the DC vaccine?

### **Part 1: Characterization of the acute myeloid leukemia cell line MUTZ-3 as a human IDC/LC cell line model.**

In **chapter 2**, skin-resident CD1a+ dermal DC and CD1a+ epidermal LC are characterized by transcriptional analysis. By performing global transcriptional profiling of CD1a+ dermis-derived DDC and CD1a+ epidermis-derived LC, using high-density microarray analysis, we tried to gain further insight in the kinship between skin-resident CD1a+ DDC and LC. Besides that, we aimed to find markers that might be related to their *in vivo* function.

In **chapter 3**, the human CD34+ acute myeloid leukemia cell line MUTZ-3 is introduced as a human cell line model of myeloid DC differentiation.

In **chapter 4**, we tried to confirm the validity of MUTZ-3 as a myeloid DC and LC model by analyzing MUTZ-3-derived IDC and LC using high-density microarray analysis. Furthermore, by comparing differentially expressed transcripts of MUTZ-3-derived IDC and LC to differentially expressed transcripts of skin-derived CD1a<sup>+</sup> Dermal DC (DDC) and skin LC, we aimed to find novel subset-specific transcripts and find additional clues for the utility of MUTZ-3 IDC and LC in the study of differential functions between the two DC subsets.

### **Part-2: Development of MUTZ-3-derived IDC/LC for tumor immunotherapy**

In **chapters 5a and b**, the applicability of allogeneic MUTZ-3-derived DC to generate tumor-specific cytotoxic T lymphocytes is analyzed and compared to autologous MoDC. For this, several adenocarcinoma associated, HLA-A2 restricted TAA-derived peptides were used. One of these peptides is derived from the newly described colon tumor antigen Ebp1. Identification and characterization of this novel antigen, and its derived HLA-A2-restricted peptide, is described in **chapter 5b**.

In **chapter 6**, one way to improve DC induced T cell reactivity is described. By making use of a novel bispecific  $\alpha$ CD40/ $\alpha$ CD28 diabody, we aimed at increasing the strength and duration of T cell/DC interactions and thus increasing T cell responsiveness to tumor antigens.

In **chapter 7**, functional properties crucial for the *in vivo* generation of CTL-mediated immunity of MUTZ-3 IDC and LC are analyzed in order to reveal the more suitable candidate for use as a clinical vehicle of tumor vaccines.

Finally, in the concluding **chapter 8**, the different human DC differentiation models described so far are being discussed. Characteristics that determine the ability of leukemia cells to differentiate along the different precursor stages into functional DC will be formulated. Evidence will be provided that the human CD34+ acute myeloid leukemia cell line MUTZ-3 currently represents the most valuable and sustainable model system for both myeloid DC differentiation and clinical DC vaccination studies.

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