

Discussion: Human dendritic cell line models for DC differentiation and clinical DC vaccination studies.

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Abstract

Dendritic cells (DC) are increasingly applied as cellular adjuvants in the immunotherapy of cancer. Since the development of a standardized DC vaccine product is often hampered by the relatively limited availability of DC precursors and the inter- and intra-donor variability, and the preparation of individual vaccines for each and every patient is very labor intensive, it would be preferable to use DC from a readily available and unlimited source, such as cell lines can provide. It has been described that leukemia-derived cell lines are able to differentiate into functional DC, creating possibilities for the development of highly reproducible DC vaccines. This review discusses the different human DC cell line differentiation models described so far. Based on the available data, characteristics that determine the ability of leukemia cells to differentiate along the different precursor stages into functional DC will be formulated. In addition, evidence will be provided that the human CD34+ acute myeloid leukemia cell line MUTZ-3 provides DC that exhibit the functional properties that are crucial for the *in vivo* generation of CTL-mediated immunity, and thus currently represents the most valuable and sustainable model system for both myeloid DC differentiation and clinical DC vaccination studies.

Introduction

Dendritic cells (DC) are professional antigen presenting cells (APC) and are key regulators of the immune system. Located at the boundary between the inside and the outside world, DC provide a bridge between the innate and adaptive immune system. As sentinels throughout the body, DC capture and process antigen, undergo maturation and subsequently migrate to the secondary lymphoid organs, where they present the processed antigens to naive T cells and initiate primary T cell responses. *In vivo*, DC originate from bone marrow-derived CD34+ hematopoietic progenitor cells (HPC). CD34+ HPC can give rise to two classic 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 (1,2). Besides that, CD34+ HPC can also give rise to a non-classical plasmacytoid DC (PDC) subset, which has been described to secrete large amounts of IFN- α/β upon viral encounter, thereby representing the first barrier to intruding viruses (3,4).

Several protocols have been developed to generate human DC *in vitro*. DC can be generated from blood or bone marrow-derived CD34+ HPC or peripheral blood-derived CD14+ monocytes (5-7). Upon culturing in the presence of GM-CSF, TNF- α and TGF- β or IL-4, CD34+ HPC progenitors can be differentiated into LC or IDC, respectively (6,8,9). 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- α/β or IL-15, monocytes are able to develop into IL-4-DC, IFN-DC or IL-15-DC, respectively (10-15). The development of such DC differentiation protocols has been of great importance for studying DC biology, and the subsequent implementation in clinical DC vaccination studies. Unfortunately however, DC differentiation and clinical DC vaccination studies are often hampered by difficulties in the generation of large amounts of immuno-stimulatory DC due to low and varying levels of DC

precursors, inter- and intra-donor variability or to the use of dysfunctional cancer patient-derived DC precursors.

It has been described that tumor cells of the myeloid and lymphoid lineage exhibit the potential to differentiate into DC-like APC. Primary myeloid leukemic blasts are able to differentiate *in vitro* into functional DC, expressing high levels of co-stimulatory and MHC class I and II molecules, and capable of inducing leukemia-specific cytotoxic T cells (16-21). Moreover, this DC differentiating potential has also been shown for leukemia-derived cell lines, especially those that originate from the myelogenous or monocytic lineage, such as THP-1, HL-60, KG-1 and MUTZ-3, creating possibilities for the development of a highly reproducible model for DC differentiation and clinical DC vaccination studies. This review outlines the different human DC cell line differentiation models described so far. In this perspective, we will discuss the DC differentiation properties of the different leukemia cell lines, as well as the differences in functional properties of the different leukemia cell line-derived DC. Moreover, characteristics that determine the ability of leukemia cells to differentiate along the different precursor stages into functional DC will be formulated. We will provide evidence that the myelomonocytic leukemia cell line MUTZ-3 represents the most valuable and sustainable model system for myeloid DC differentiation, exhibiting discrete transitional stages in myeloid DC development to both IDC and LC. Furthermore, MUTZ-3-derived DC display the functional characteristics required for the *in vivo* generation of CTL-mediated immunity and thus represent a suitable candidate for application in clinical tumor vaccination settings.

Leukemic DC differentiation

Myeloid leukemic blasts represent malignant, myeloid-lineage committed hematopoietic progenitor cells, that have been described to be able to differentiate *in vitro* along the DC pathway and develop into APC with phenotypic and functional DC characteristics (16-18,22). Although culturing primary leukemic blasts with various combinations of cytokines has been demonstrated to yield functional DC, not all leukemic blast are responsive to cytokine treatment (20,23,24), possibly due to the lack of specific receptors or components of the proximal signal transduction pathways. By making use of agents that bypass the receptor/ligand signaling required for DC differentiation, this cytokine unresponsiveness could be circumvented. Examples of such agents are calcium ionophores (CI), which have been described to signal via increasing calcium mobilization and subsequent activation of the calmodulin/calcineurin/calmodulin-dependent kinase signaling axis (25,26), or phorbol esters like phorbol 12-myristate 13-acetate (PMA) and 12-O-tetradecanoylphorbol 13-acetate (TPA), which exert their effect via protein kinase C (PKC) and downstream signaling molecules such as RelB and other members of the NF- κ B transcription factor family (27,28). Indeed, CI or phorbol ester-treated DC precursors rapidly display characteristics of activated DC, including down-regulation of CD14, up-regulation of co-stimulatory and MHC molecules, *de novo* expression of CD83 and the acquisition of dendritic cell morphology (25,27,28). Of note, although the use of CI/phorbol esters results in a more mature DC phenotype, these agents also lead to instant maturation, thereby bypassing the immature stage, as well as to lower viability as compared to cytokine-induced DC differentiation (20,28).

THP-1

The THP-1 cell line was first described by Tsuchiya and co-workers as a human leukemia cell line with distinct monocytic characteristics such as lysozyme production and phagocytosis capacity (29). Although THP-1 cells have been demonstrated to acquire DC properties upon stimulation with cytokines, the DC differentiation capacity of THP-1 cells is relatively low, since generally less than 5% of THP-1 cells express the classic myeloid DC marker CD1a after differentiation (Table I and (23,24,30)). Moreover, THP-1 DC failed to acquire DC-like functions, as indicated by the absence of increased allogeneic T cell stimulatory capacity of the immature THP-1 DC compared to the THP-1 precursors (30). In contrast, the inclusion of CI resulted in complete differentiation and instant maturation of the THP-1 cells, expressing high levels of CD80, CD86, CD40 and CD83, displaying increased allogeneic T cell stimulatory capacity, and markedly decreased receptor-mediated endocytosis capacity within 24 hours (30).

Of note, upon stimulation with PMA, THP-1 cells could also display macrophage-like characteristics, expressing the acetylated low density lipoprotein (LDL) receptor, apolipoprotein E (ApoE) and lipoprotein lipase (LPL) (31-33).

Table I. Comparison of DC differentiation capacity of different leukemia cell lines

Cell Line	% Positive cells	
	CD1a ^a	CD1a/ CD83 ^a
THP-1	5	5
KG-1	10	10
HL-60	0	0
U-937	0	0
K562	0	0
MUTZ-3	60-90 %	0

^a percentage CD1a-positive and CD1a/CD83-positive cells after 7 days stimulation with 100 ng/ml GM-CSF, 1000 U/ml IL-4 and 2.5 ng/ml TNF- α

KG-1

KG-1 is a cytokine-responsive CD34+ myelomonocytic cell line derived from a patient with erythroleukemia undergoing myeloblastic relapse (34). Upon culturing with phorbol esters, KG-1 develop pseudopodia and display phagocytosis, nonspecific esterase and lysozyme-secreting activity, all features of the monocyte/macrophage lineage (35,36). Recently, KG-1 cells have also been described to acquire DC-like properties upon stimulation with either cytokines or PMA +/- CI. However, as also observed for THP-1 cells, the percentage of KG-1 cells that express CD1a after differentiation is only around 10 %, demonstrating the low DC differentiation capacity of KG-1 cells (Table I and (23,24,27,37)). In addition, differentiation was accompanied by distinct expression of the DC maturation marker CD83, indicating instant maturation induction. Nevertheless, stimulation with either cytokines or PMA +/- CI does lead to some degree of DC differentiation, as demonstrated by the

expression of intermediate to high levels of CD86, CD83 and HLA-DR, and the enhancement of the allogeneic T cell stimulatory capacity of KG-1 DC compared to un-stimulated KG-1 cells (27,30). Yet, differentiation/maturation of the KG-1 DC has been shown to be incomplete, since maturation was not associated with decreased antigen uptake and the acquisition of LN-migration capacity (27,38).

HL-60

The acute promyelocytic leukemia cell line HL-60 is a multipotent cell line capable of differentiating along the granulocytic, monocyte-macrophage or eosinophilic pathway. Upon culturing with chemicals or physiological agents, such as dimethyl sulfoxide (DMSO) and retinoic acid, HL-60 develop into mature granulocytes (39,40), whereas when exposed to 1,25-dihydroxy-vitamin D3 or phorbol esters, HL-60 cells develop into monocyte-macrophage-like cells (41-44). Moreover, HL-60 cells are also able to differentiate into eosinophilic granulocytes when cultured under mild alkaline conditions (45).

The extraordinary multipotency of HL-60 cells suggested the possible sensitivity of the HL-60 cell line for differentiation along the myeloid DC pathway. Whereas HL-60 cells have been demonstrated to be insensitive to cytokine-driven DC differentiation (Table I and (22,46)), inclusion of calcium mobilization treatment resulted in DC differentiation (46). Upon CI-treatment, HL-60 cells rapidly (within 20 hours) up-regulated CD86 and demonstrated *de novo* expression of CD83, CD80 and CD54. Expression of CD40, and more importantly CD1a only became apparent after 72-96 hours, whereas CD83 expression, which had been shown to be transient with a peak expression at 20 hours, was undetectable at 96 hours. In addition, CI treatment also resulted in a marked increase in APC function, as determined by enhanced allogeneic T cell stimulation capacity. However, the observation that HL-60 cells failed to express MHC class II molecules and down-regulated MHC class I molecules upon CI treatment, suggests that antigen-specific T cells stimulatory capacity might be low (46).

Monomac-6

The human acute monocytic leukemia cell line monomac-6 exhibits a well-differentiated monocyte phenotype, with phagocytosis activity, the expression of NaF sensitive nonspecific esterases and the expression of mature monocyte marker CD14 (47). Besides that, monomac-6 cells are able to produce IL-1 β , IL-6 and TNF- α in response to LPS and are capable of migration towards β -chemokines such as MIP-1 α and β , RANTES and MCP-1, as also described for human blood monocytes (48-51). However, although monomac-6 cells closely resemble human blood monocytes, they are not able to differentiate into DC (24,30).

U-937

The histiocytic lymphoma cell line U-937 also exhibits monocytic characteristics, displaying monoblast morphology, lysozyme production and esterase activity (52). In contrast to monomac-6 cells, U-937 cells do not display phagocytosis activity, indicating that they are arrested at a relative early stage of monoblast development (47). Yet, U-937 cells are able to acquire mature monocyte-like

morphologic and phenotypic characteristics upon stimulation with PMA (53,54). Nevertheless, despite its monocyte-like characteristics, the U-937 cell line is unable to differentiate into DC (24,30).

K562

The chronic myelogenous leukemia (CML) cell line K562 is a multipotent cell line, capable of differentiating along the megakaryocytic, erythroid and monocytic pathway (55-57). In addition, upon stimulation with PMA +/- TNF- α , K562 cells develop DC-like cytoplasmic projections, but expression of typical DC markers such as CD86, CD40 and CD83 remains low. Moreover, DC differentiation was not terminal, since removal of the PMA/TNF- α resulted in a reversal of the DC phenotype and function. In addition, K562 cells were also shown to be unresponsive to cytokine-induced DC differentiation (Table I and (58)). Taken together, although PKC signaling does induce some level of DC differentiation, K562 cells are not very potent in developing along the DC pathway.

MUTZ-3

Phenotypic characterization

The MUTZ-3 cell line is derived from the peripheral blood of a patient with acute myelomonocytic leukemia, and exhibits morphological and phenotypical characteristics of monocytes such as the expression of monocyte specific esterase and myeloperoxidase enzymes and the expression of monocytic marker CD14 (59). Different from the above mentioned leukemia cell lines, the MUTZ-3 cell line has been described recently to be cytokine-dependent for its proliferation and survival (59,60). Whereas many leukemia cell lines are often refractory to cytokine treatment and require pharmacological agents to induce DC differentiation, thereby bypassing important stages in the differentiation of DC, the MUTZ-3 cell line has been shown to down-regulate CD14 in response to GM-CSF and IL-4 and to exhibit characteristics of CD34-derived DC precursors (24). As described by us, the MUTZ-3 cell line consists of three subpopulations, a proliferating pool of small CD34⁺/CD11b⁻ progenitors, which can differentiate through a CD34⁻/CD14⁻/CD11b⁺ intermediate stage into a more differentiated, non-proliferating CD14⁺/CD11b⁺ DC precursor population (see also figure 1). Over the course of differentiation (CD34⁺→CD34⁻/CD14⁻→CD14⁺), the MUTZ-3 precursors acquire a more myeloid DC precursor phenotype, with up-regulated expression of the differentiation markers CD1c, CD11b, CD11c, CD13 and CD45RO. Moreover, cytokine receptors that are associated with DC precursor proliferation such as IL-3R, IL-6R and SCFR are down-regulated, whereas cytokine receptors that are associated with DC differentiation such as GM-CSF-R, TNF- α -RI and RII are up-regulated over the course of differentiation (figure 2). Indeed, MUTZ-3 precursors have been demonstrated to be sensitive to cytokine-driven DC differentiation, with subsequent transitional DC differentiation stages. By inducing DC differentiation with GM-CSF, TNF- α and IL-4 or TGF- β , immature CD1a⁺/DC-SIGN⁺ IDC or CD1a⁺/Langerin⁺, Birbeck-granule expressing LC could be obtained respectively, from both CD14⁺ DC precursors and total MUTZ-3 progenitor cells, as described (24,61). In contrast to other AML-derived cell lines, DC differentiation capacity of the MUTZ-3 cells is relatively high, since CD1a expression after DC differentiation ranges between 60 and 90 % for MUTZ-3 IDC and LC, respectively (Santegoets et al, unpublished data and (61)). Besides that,

MUTZ-3-generated IDC and LC also express intermediate to high levels of co-stimulatory, adhesion and MHC class I and II molecules, indicating that MUTZ-3 IDC and LC exhibit true DC phenotype. In addition, MUTZ-3 IDC and LC exhibit characteristic DC morphology, a.o. cytoplasmic protrusions (figure 3A and B), cytoplasmic MHC class II compartments (MIIC), and/or Birbeck granules (figure 3C and D). DC differentiating capacity was also confirmed by transcriptional profiling (Santegoets et al, unpublished data, Table II and (23)). Similar to skin DDC and LC, MUTZ-3 LC express significantly higher levels of CD1a transcripts and protein as compared to MUTZ-3 IDC. Moreover, MUTZ-3 IDC exhibited true IDC characteristics expressing the macrophage mannose receptor (MMR), DC-SIGN, the scavenger receptor CD36, chemokine receptor CCR5 and coagulation factor XIIIa (Table II), as also described for skin DDC and MoDC (62-66). On the other hand, MUTZ-3 LC had been shown to resemble skin LC, expressing Langerin, membrane adenosine triphosphatase (ATPase) and CCR6, all hallmarks of the LC lineage (67-70).

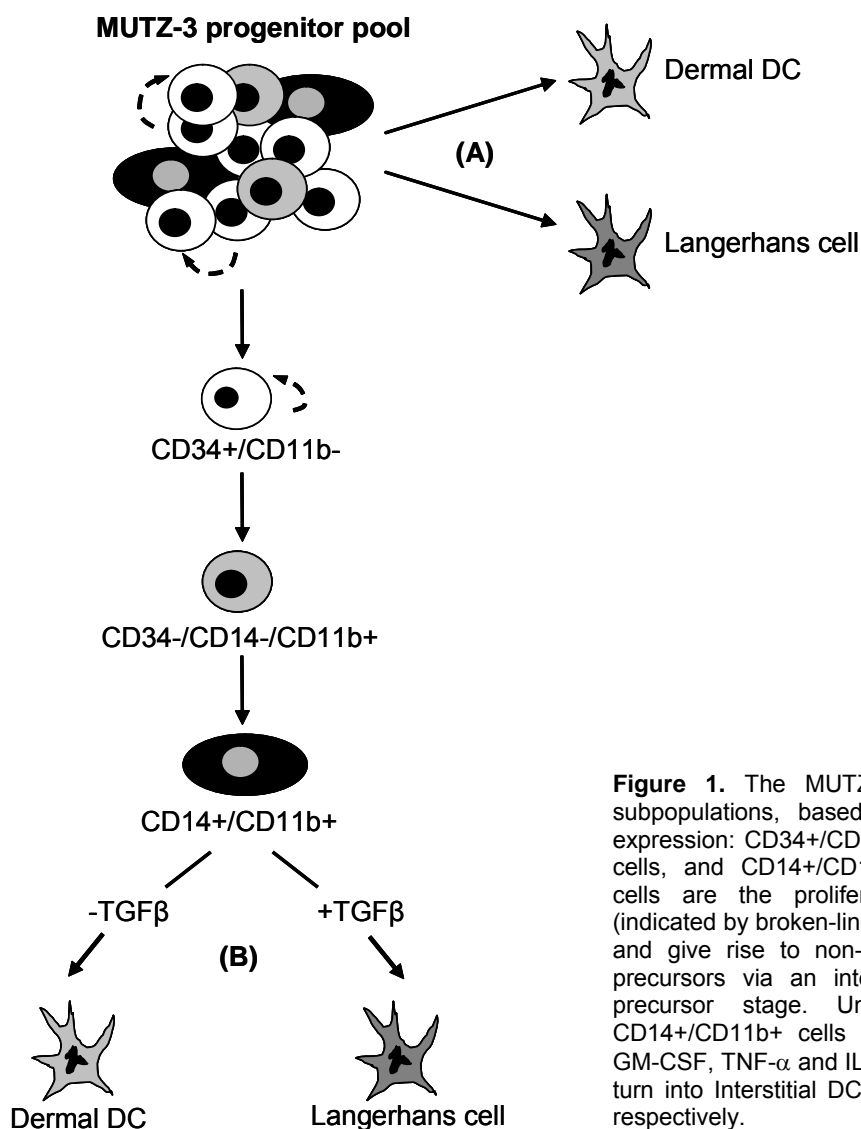


Figure 1. The MUTZ-3 cell line consists of three subpopulations, based on CD34, CD14 and CD11b expression: CD34+/CD11b- cells, CD34-/CD14-/CD11b+ cells, and CD14+/CD11b+ cells. The CD34+/CD11b- cells are the proliferating, self-replenishing fraction (indicated by broken-lined arrows) of the MUTZ-3 cell line and give rise to non-proliferating CD14+/CD11b+ DC precursors via an intermediate CD34-/CD14-/CD11b+ precursor stage. Un-separated (A) and isolated CD14+/CD11b+ cells (B) cultured in the presence of GM-CSF, TNF- α and IL-4 or GM-CSF, TNF- α and TGF- β turn into Interstitial DC (IDC) or Langerhans cells (LC), respectively.

Table II. Differential characteristics of MUTZ-3 derived IDC and LC.

Gene	mRNA expression profile		Literature	
	MUTZ-3		IDC [†]	LC [†]
CD1a	++	+++	+	+
CD205, DEC-205	+	+	+	+
CD206, MMR	+++	++	+	-
CD207, Langerin	++	+++	-	+
CD209, DC-SIGN	++	-	+	-
CD36	++	++	+	-
Factor XIIIa	+	-	+	-
CCR5	+	+	+	-
CCR6	-	++	-	+
ATPase	-	+	-	+
E-cadherin	-	-	-	+

[†] Analyzed by using high density microarray; Signal intensity levels: - <200, + 200 - 500, ++ 500-5000, +++ >5000)

* From literature; expression on either human CD34-IDC, CD34-LC, CD14+ monocyte-derived IDC or skin-derived IDC and LC

Importantly, MUTZ-3-derived IDC and LC could also be further matured under the influence of cytokines or CD40 ligation, resulting in up-regulation of co-stimulatory and adhesion molecules CD80, CD86, CD40, CD54 and HLA-DR and *de novo* expression of CD83 (figure 4 and (61,71)), supporting the validity of this model for primary myeloid DC differentiation. On the other hand, lipopolysaccharide (LPS) did not induce maturation of MUTZ-3-derived DC, most likely due to reduced TLR4 receptor signaling and subsequent phosphorylation of p38-MAPK, ERK1 and ERK2, as demonstrated by Kim and co-workers (71).

Functional characterization

DC have been described as professional APC and key regulators of the immune system, displaying an extraordinary capacity for T cell stimulation and the initiation of immune responses. Besides phenotypic requirements for proper T cell activation, DC should also meet functional requirements in order to act as fully immunostimulatory DC. DC should not only be fully mature, but also exhibit high T cell stimulatory capacity (allogeneic and antigen specific), be able to migrate in response to lymph node-produced chemokines and produce cytokines that are involved in T helper and cytotoxic T cell function (2,72,73).

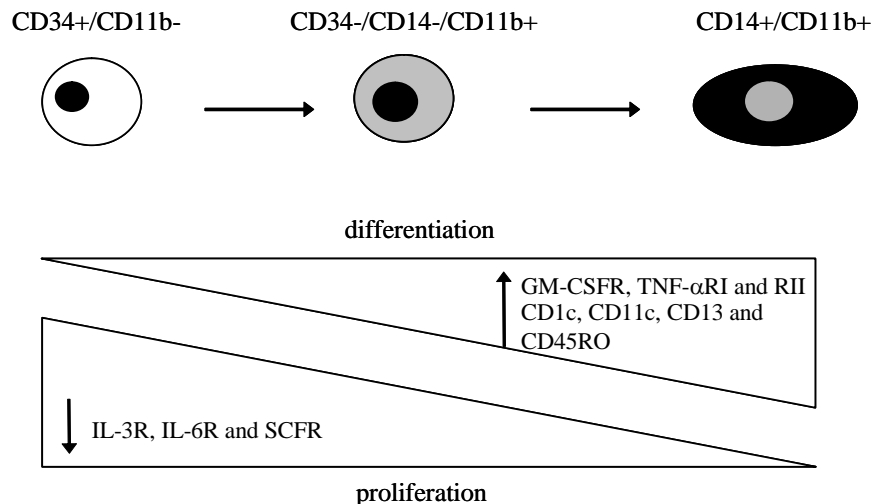


Figure 2. Over the course of differentiation (CD34+/CD11b-→CD34-/CD14/CD11b+→CD14+/CD11b+) the MUTZ-3 progenitors down-regulate cytokine receptors associated with proliferation such as IL-3R, IL-6R and SCFR, and up-regulate differentiation markers such as CD1c, CD11c, CD13 and CD45RO and cytokine receptors associated with DC differentiation such as GM-CSFR, TNF-αRI and RII.

Central to their role as professional APC, DC should be able to prime naïve T cells. As described, immature MUTZ-3 IDC displayed a three-fold enhancement of allogeneic T cell stimulatory capacity compared to MUTZ-3 progenitors, and this was further up-regulated upon maturation (24,61). Of note, MUTZ-3 LC generally displayed higher levels of co-stimulatory molecules, which translated into a superior allogeneic T cell stimulatory capacity of MUTZ-3 LC as compared to MUTZ-3 IDC (figure 4 and (61)). Besides allogeneic T cell stimulatory capabilities, DC also should be able to induce and activate CD8+ T cells directed against pathogen- or tumor-associated antigens. Indeed, we were able to show that both MUTZ-3 IDC and LC can prime tumor antigen-specific T cells *in vitro* across a range of allogeneic backgrounds. While the priming capacity of MUTZ-3 IDC had been demonstrated to be equally efficient to that of autologous monocyte-derived DC (MoDC) (74), MUTZ-3 LC supported the induction of tumor antigen-specific CD8+ T cells at an overall lower efficiency compared to MUTZ-3 IDC (Santegoets *et al*, submitted). The latter might be related to the low level or absence of pro-inflammatory (and T cell stimulatory) cytokine production (such as IL-12p70, IL-15 and IL-23) by MUTZ-3 IDC and LC, respectively. Indeed, transduction with IL-12p70 significantly improved priming efficiency of IDC and LC, as well as tumor recognizing and lytic capabilities of primed T cells (Santegoets *et al*, submitted), indicating that pro-inflammatory cytokines are required to ensure optimal T cell stimulation. Of note, the low level or absence of pro-inflammatory cytokine production by MUTZ-3 IDC and LC could be explained by the leukemic origin of the MUTZ-3 cell line, since AML-blast-derived DC are described to produce only low amounts of IL-12 (75). Yet, the observation that MUTZ-3 IDC and LC also do not produce any IL-10 (Santegoets *et al*, submitted) argues against a possible immunosuppressive phenotype.

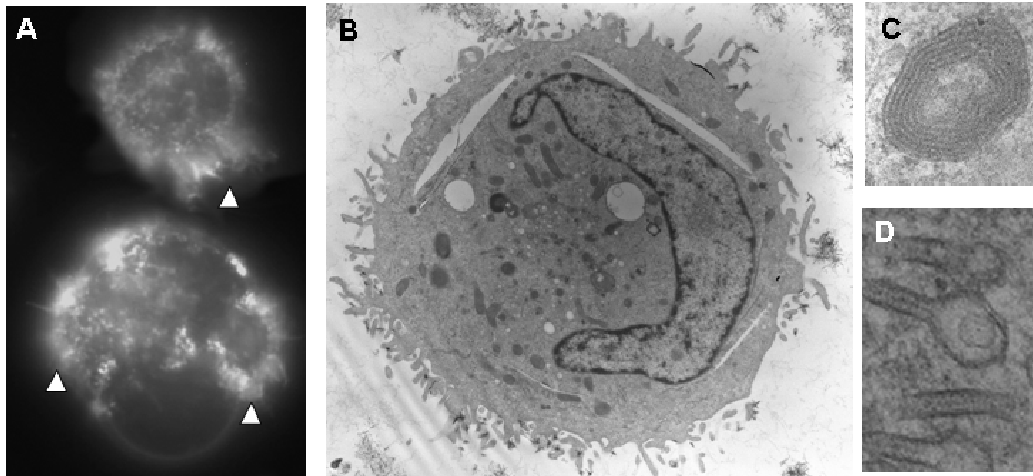


Figure 3. (A) Fluorescence microscopy (400x; HLA-DR staining) reveals veil-like structures (indicated by arrow heads) on the surface of MUTZ-3 IDC. (B) EM (Uranyl acetate and lead citrate staining) also reveals that immature MUTZ-3 LC display characteristic cytoplasmic protrusions and express multilamellar cytoplasmic MHC class II compartments (MIIC) (C) and cytoplasmic Birbeck granules (D).

A pivotal ability of fully mature, immunostimulatory DC is migration to the secondary lymphoid organs. Mature MUTZ-3 IDC and LC express the chemokine receptor CCR7 and are capable of migrating towards lymph node-homing chemokines CCL19 and CCL21. Different to previous observations for MoDC, the *in vitro* migration of MUTZ-3 IDC and LC is independent / less dependent on prostaglandin E2 (PGE2; Santegoets *et al*, submitted). The latter characteristic might be beneficial in clinical DC vaccination settings. Although PGE2 has been demonstrated to be required for effective migration of DC towards lymph node homing chemokines (76,77), the use of this inflammatory mediator with a Th2 driving role also has major disadvantages. As described, maturation of DC in the presence of PGE2 results in impaired IL-12 production and promotes the development of T helper cells that produce high levels of type 2 cytokines such as IL-4 and IL-5 (78,79).

In summary, the human CD34+ AML cell line MUTZ-3 represents a valuable and sustainable model system for myeloid DC differentiation, and provides us with DC that exhibit functional properties that are essential for the *in vivo* generation of CTL mediated immunity.

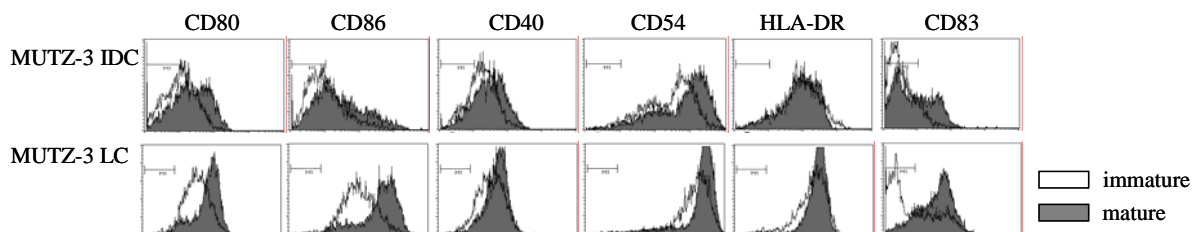


Figure 4. Phenotypic analysis of immature and mature MUTZ-3-derived IDC and LC. Expression levels of co-stimulatory and adhesion molecules CD80, CD86, CD40 and HLA-DR and maturation marker CD83 were analyzed by flow cytometry. The markers in the FACS histograms denote fluorescence intensities of isotype control antibodies. Open histograms; fluorescence intensities of immature cells and grey histograms: fluorescence intensities of mature cells for, the markers as indicated above.

Implementation of leukemia cell lines in myeloid DC differentiation studies.

Although extensively studied, the delineation of human myeloid DC differentiation is hampered by low frequencies and heterogeneity of DC precursors. For that reason, there is an obvious need for homogenous primary cell systems that allow us to study myeloid DC differentiation, as also mentioned by Platzer and co-workers (80). As described, the transitional DC differentiation requires cytokines that promote monopoiesis or DC differentiation such as macrophage-colony stimulating factor (M-CSF), GM-CSF and TNF- α (81,82). Besides that, the expression CD14, TNF- α R1 and CD86 on primary leukemic blast have also been demonstrated to be beneficial for proper DC differentiation (83-85), as well as the absence of FLT-3 internal tandem duplicates (ITD) (86). As shown in table III, most leukemic cell lines exhibit several characteristics known to be associated with DC differentiation. However, only the MUTZ-3 cell line is dependent on cytokines for its proliferation and survival, indicating that this is an important parameter for the observed DC differentiating capacity and the presence of transitional stages in DC differentiation of leukemic cell lines. The latter is further illustrated by transduction experiments with signal transducer of transcription 5b (STAT5b), which has been described to be involved in cytokine signal transduction of cytokines associated with DC precursor growth and differentiation such as IL-3, GM-CSF, TNF- α , SCF and FLT-3 (87). As described, constitutive expression of STAT5b resulted in cytokine-independent growth of the MUTZ-3 cells, as well as accelerated DC differentiation and instant maturation (reminiscent of other leukemia cell lines with DC differentiating potential), indicating that indeed transitional DC differentiation is cytokine dependent (88). Besides that, the cytokine dependence of MUTZ-3 is further illustrated by the lack of human telomerase reverse transcriptase (hTERT) expression (data not shown), which has been described to be involved in the development of cytokine unresponsiveness of (malignant) hematopoietic progenitor cells (89). Taken together, although the expression of CD14, TNF- α R1 and/or CD86 are indicative for the ability of leukemia cells to differentiate into DC, cytokine-sensitivity of the leukemic cells is the most important parameter for the ability to differentiate along the different precursor stages, indicating that the latter should be taken into account in the search for novel human DC cell lines.

Table III. Differential expression of markers/characteristics associated with DC differentiation.

Cell Line	DC differentiated on capacity †	Cytokine dependence for growth and survival	CD14 expression	TNF- α R1 expression	CD86 expression	References
MUTZ-3	++	yes	10-20%	+	+	(61,85,90)
KG-1	+/-	no	-	+	+/-*	(27,30,37)
THP-1	+/-	no	< 10%	+	-	(30,90,91)
HL-60	-	no	< 5 %	+	+/-*	(26,46,92,93)
U-937	-	no	< 10%	+	no	(90,91)
K562	-	no	-	+	-	(58,93,94)
Monomac-6	-	no	< 10%	unknown	unknown	(90)

† determined by flow cytometry and taken from literature

* conflicting data

Implementation of leukemia cell lines in clinical DC vaccination studies; the case for allogeneic DC vaccines

DC have been described as the main orchestrators of the immune system and are increasingly applied as cellular adjuvants in immunotherapy of cancer. Although clinical DC vaccination has been proven to be feasible and safe, clinical results have been demonstrated to be variable (95-97). In the majority of clinical trials, autologous MoDC or CD34+ HPC-derived DC have been used for vaccination (2,98). However, this approach of individual patient-based immunotherapy has several limitations, i.e. the preparation of an individual DC vaccine for each and every patient is laborious, time-consuming, and difficult to standardize, the latter resulting in highly variable results. By making use of allogeneic DC as vaccine vehicles, such problems may be overcome. Although the implementation of an allogeneic DC vaccine might cause HLA compatibility problems, thereby inducing allogeneic responses, the induction of such an allogeneic response has also been hypothesized to be beneficial in anti-tumor immunology (as reviewed by Fabre et al (99)). The use of allogeneic DC vaccines might induce stronger vaccine-specific immune responses than autologous DC vaccines, either via the induction of high-frequency (1-10%) alloreactive T cells that might also display specificity against tumor associated antigens (i.e. cross activation) or via the induction of allogeneic CD4+ T helper cell responses, necessary for optimal activation of TAA-specific CTL. Furthermore, the use of standardized, fully mature allogeneic DC might also be important for the development of type-1 allogeneic responses and subsequent Th1 skewing of the immune response (99). The observation that DC derived from the human CD34+ AML cell line MUTZ-3 exhibit the functional properties that are required for in the generation of CTL-mediated immunity, i.e. proper differentiation and maturation capacity, exquisite T cell stimulatory potential and good lymph node homing potential, indicates that the MUTZ-3 cell line is a good candidate for the development of such an allogeneic DC vaccine. We are therefore currently in the process of developing culture methods to produce large amounts of MUTZ-3 DC according to Good Manufacturing Practice (GMP) guidelines. This entails the use of GMP-manufactured media, the use of low percentage of US Department of Agriculture (USDA)-approved FCS, thereby ensuring the maintenance of the immunostimulatory phenotype and function of the DC. In addition, optimal maturation status and antigen loading methods are also being explored. Of note, to circumvent that repeated vaccination with one particular allo-DC vaccine leads to accelerated clearance of the vaccine, and thus leads to a decrease in the efficacy of vaccination, it would be of great importance to develop a prime-boost regimen consisting of different allogeneic DC vaccines, further illustrating the importance of the development of additional DC cell lines. Alternatively, consecutive immunization could also be performed with antigens only, i.e. boosting with peptide- or DNA-based vaccines or recombinant viruses (100,101).

Concluding remarks

Although monocytic leukemia cell lines such as THP-1, KG-1, K562, monomac-6 and U-937 had originally been predicted to be good candidates for differentiation into DC-like cells, these leukemia cell lines have been demonstrated to be poor models for DC differentiation, most likely because they are already developed too far along the monocyte/macrophage differentiation pathway to allow

redirection towards DC and because of their cytokine refractory phenotype (98,102,103). The relatively undifferentiated myelomonocytic CD34+ acute myeloid leukemia cell line MUTZ-3, in contrast, has been demonstrated to be the most valuable and sustainable model system for myeloid DC differentiation, reflecting all physiologically observed transitional maturation states. In addition, the MUTZ-3 cell line provides us with DC that exhibit the phenotypic and functional properties that are essential for the *in vivo* generation of CTL mediated immunity and thus represent a suitable candidate for both DC differentiation and clinical tumor vaccination studies.

References

1. Banchereau J, Briere F, Caux C, et al. Immunobiology of dendritic cells. *Annu Rev Immunol* 2000;18:767-811.
2. Figdor CG, de Vries IJ, Lesterhuis WJ, Melief CJ. Dendritic cell immunotherapy: mapping the way. *Nat Med* 2004;10:475-80.
3. Siegal FP, Kadowaki N, Shodell M, et al. The nature of the principal type 1 interferon-producing cells in human blood. *Science* 1999;284:1835-7.
4. Shortman K, Liu YJ. Mouse and human dendritic cell subtypes. *Nat Rev Immunol* 2002;2:151-61.
5. Romani N, Gruner S, Brang D, et al. Proliferating dendritic cell progenitors in human blood. *J Exp Med* 1994;180:83-93.
6. Caux C, Vanbervliet B, Massacrier C, et al. CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GM-CSF+TNF alpha. *J Exp Med* 1996;184:695-706.
7. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* 1994;179:1109-18.
8. Romani N, Reider D, Heuer M, et al. Generation of mature dendritic cells from human blood. An improved method with special regard to clinical applicability. *J Immunol Methods* 1996;196:137-51.
9. Strobl H, Riedl E, Scheinecker C, et al. TGF-beta 1 promotes in vitro development of dendritic cells from CD34+ hemopoietic progenitors. *J Immunol* 1996;157:1499-507.
10. Zhou LJ, Tedder TF. CD14+ blood monocytes can differentiate into functionally mature CD83+ dendritic cells. *Proc Natl Acad Sci U S A* 1996;93:2588-92.
11. Bender A, Sapp M, Schuler G, Steinman RM, Bhardwaj N. Improved methods for the generation of dendritic cells from nonproliferating progenitors in human blood. *J Immunol Methods* 1996;196:121-35.
12. Paquette RL, Hsu NC, Kiertscher SM, et al. Interferon-alpha and granulocyte-macrophage colony-stimulating factor differentiate peripheral blood monocytes into potent antigen-presenting cells. *J Leukoc Biol* 1998;64:358-67.
13. Luft T, Pang KC, Thomas E, et al. Type I IFNs enhance the terminal differentiation of dendritic cells. *J Immunol* 1998;161:1947-53.
14. Dubsky P, Saito H, Leogier M, et al. IL-15-induced human DC efficiently prime melanoma-specific naive CD8+ T cells to differentiate into CTL. *Eur J Immunol* 2007;37:1678-90.
15. Mohamadzadeh M, Berard F, Essert G, et al. Interleukin 15 skews monocyte differentiation into dendritic cells with features of Langerhans cells. *J Exp Med* 2001;194:1013-20.
16. Choudhury BA, Liang JC, Thomas EK, et al. Dendritic cells derived in vitro from acute myelogenous leukemia cells stimulate autologous, antileukemic T-cell responses. *Blood* 1999;93:780-6.
17. Charbonnier A, Gaugler B, Sainty D, Lafage-Pochitaloff M, Olive D. Human acute myeloblastic leukemia cells differentiate in vitro into mature dendritic cells and induce the differentiation of cytotoxic T cells against autologous leukemias. *Eur J Immunol* 1999;29:2567-78.
18. Cignetti A, Bryant E, Allione B, Vitale A, Foa R, Cheever MA. CD34(+) acute myeloid and lymphoid leukemic blasts can be induced to differentiate into dendritic cells. *Blood* 1999;94:2048-55.
19. Harrison BD, Adams JA, Briggs M, Brereton ML, Yin JA. Stimulation of autologous proliferative and cytotoxic T-cell responses by "leukemic dendritic cells" derived from blast cells in acute myeloid leukemia. *Blood* 2001;97:2764-71.
20. Westers TM, Stam AG, Scheper RJ, et al. Rapid generation of antigen-presenting cells from leukaemic blasts in acute myeloid leukaemia. *Cancer Immunol Immunother* 2003;52:17-27.
21. Houtenbos I, Westers TM, Stam AG, et al. Serum-free generation of antigen presenting cells from acute myeloid leukaemic blasts for active specific immunisation. *Cancer Immunol Immunother* 2003;52:455-62.
22. Santiago-Schwarz F, Coppock DL, Hindenburg AA, Kern J. Identification of a malignant counterpart of the monocyte-dendritic cell progenitor in an acute myeloid leukemia. *Blood* 1994;84:3054-62.
23. Larsson K, Lindstedt M, Borrebaeck CA. Functional and transcriptional profiling of MUTZ-3, a myeloid cell line acting as a model for dendritic cells. *Immunology* 2006;117:156-66.
24. Masterson AJ, Sombroek CC, De Grujil TD, et al. MUTZ-3, a human cell line model for the cytokine-induced differentiation of dendritic cells from CD34+ precursors. *Blood* 2002;100:701-3.

25. Czerniecki BJ, Carter C, Rivoltini L, et al. Calcium ionophore-treated peripheral blood monocytes and dendritic cells rapidly display characteristics of activated dendritic cells. *J Immunol* 1997;159:3823-37.
26. Koski GK, Schwartz GN, Weng DE, et al. Calcium mobilization in human myeloid cells results in acquisition of individual dendritic cell-like characteristics through discrete signaling pathways. *J Immunol* 1999;163:82-92.
27. St Louis DC, Woodcock JB, Franzoso G, et al. Evidence for distinct intracellular signaling pathways in CD34+ progenitor to dendritic cell differentiation from a human cell line model. *J Immunol* 1999;162:3237-48.
28. Davis TA, Saini AA, Blair PJ, et al. Phorbol esters induce differentiation of human CD34+ hemopoietic progenitors to dendritic cells: evidence for protein kinase C-mediated signaling. *J Immunol* 1998;160:3689-97.
29. Tsuchiya S, Yamabe M, Yamaguchi Y, Kobayashi Y, Konno T, Tada K. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). *Int J Cancer* 1980;26:171-6.
30. Berges C, Naujokat C, Tinapp S, et al. A cell line model for the differentiation of human dendritic cells. *Biochem Biophys Res Commun* 2005;333:896-907.
31. Tsuchiya S, Kobayashi Y, Goto Y, et al. Induction of maturation in cultured human monocytic leukemia cells by a phorbol diester. *Cancer Res* 1982;42:1530-6.
32. Hara H, Tanishita H, Yokoyama S, Tajima S, Yamamoto A. Induction of acetylated low density lipoprotein receptor and suppression of low density lipoprotein receptor on the cells of human monocytic leukemia cell line (THP-1 cell). *Biochem Biophys Res Commun* 1987;146:802-8.
33. Tajima S, Hayashi R, Tsuchiya S, Miyake Y, Yamamoto A. Cells of a human monocytic leukemia cell line (THP-1) synthesize and secrete apolipoprotein E and lipoprotein lipase. *Biochem Biophys Res Commun* 1985;126:526-31.
34. Koeffler HP, Golde DW. Acute myelogenous leukemia: a human cell line responsive to colony-stimulating activity. *Science* 1978;200:1153-4.
35. Koeffler HP, Bar-Eli M, Territo MC. Phorbol ester effect on differentiation of human myeloid leukemia cell lines blocked at different stages of maturation. *Cancer Res* 1981;41:919-26.
36. Kiss Z, Deli E, Shoji M, et al. Differential effects of various protein kinase C activators on protein phosphorylation in human acute myeloblastic leukemia cell line KG-1 and its phorbol ester-resistant subline KG-1a. *Cancer Res* 1987;47:1302-7.
37. Hulette BC, Rowden G, Ryan CA, et al. Cytokine induction of a human acute myelogenous leukemia cell line (KG-1) to a CD1a+ dendritic cell phenotype. *Arch Dermatol Res* 2001;293:147-58.
38. Hajas G, Zsiros E, Laszlo T, et al. New phenotypic, functional and electrophysiological characteristics of KG-1 cells. *Immunol Lett* 2004;92:97-106.
39. Collins SJ, Ruscetti FW, Gallagher RE, Gallo RC. Normal functional characteristics of cultured human promyelocytic leukemia cells (HL-60) after induction of differentiation by dimethylsulfoxide. *J Exp Med* 1979;149:969-74.
40. Breitman TR, Selonick SE, Collins SJ. Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. *Proc Natl Acad Sci U S A* 1980;77:2936-40.
41. Miyaura C, Abe E, Kuribayashi T, et al. 1 alpha,25-Dihydroxyvitamin D3 induces differentiation of human myeloid leukemia cells. *Biochem Biophys Res Commun* 1981;102:937-43.
42. Ball ED, Guyre PM, Shen L, et al. Gamma interferon induces monocytoid differentiation in the HL-60 cell line. *J Clin Invest* 1984;73:1072-7.
43. Huberman E, Callahan MF. Induction of terminal differentiation in human promyelocytic leukemia cells by tumor-promoting agents. *Proc Natl Acad Sci U S A* 1979;76:1293-7.
44. Rovera G, Santoli D, Damsky C. Human promyelocytic leukemia cells in culture differentiate into macrophage-like cells when treated with a phorbol diester. *Proc Natl Acad Sci U S A* 1979;76:2779-83.
45. Fischkoff SA, Pollak A, Gleich GJ, Testa JR, Misawa S, Reber TJ. Eosinophilic differentiation of the human promyelocytic leukemia cell line, HL-60. *J Exp Med* 1984;160:179-96.
46. Koski GK, Schwartz GN, Weng DE, et al. Calcium ionophore-treated myeloid cells acquire many dendritic cell characteristics independent of prior differentiation state, transformation status, or sensitivity to biologic agents. *Blood* 1999;94:1359-71.
47. Ziegler-Heitbrock HW, Thiel E, Futterer A, Herzog V, Wirtz A, Riethmuller G. Establishment of a human cell line (Mono Mac 6) with characteristics of mature monocytes. *Int J Cancer* 1988;41:456-61.
48. Cross AK, Richardson V, Ali SA, Palmer I, Taub DD, Rees RC. Migration responses of human monocytic cell lines to alpha- and beta-chemokines. *Cytokine* 1997;9:521-8.

49. Neustock P, Brand JM, Kruse A, Kirchner H. Cytokine production of the human monocytic cell line Mono Mac 6 in comparison to mature monocytes in peripheral blood mononuclear cells. *Immunobiology* 1993;188:293-302.
50. Ziegler-Heitbrock HW, Strobel M, Kieper D, et al. Differential expression of cytokines in human blood monocyte subpopulations. *Blood* 1992;79:503-11.
51. Ziegler-Heitbrock HW, Blumenstein M, Kafferlein E, et al. In vitro desensitization to lipopolysaccharide suppresses tumour necrosis factor, interleukin-1 and interleukin-6 gene expression in a similar fashion. *Immunology* 1992;75:264-8.
52. Sundstrom C, Nilsson K. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). *Int J Cancer* 1976;17:565-77.
53. Nilsson M, Nilsson K, Forsbeck K. Increased endocytosis and formation of multivesicular bodies in phorbol ester-stimulated human monoblastic U-937 cells. *Exp Cell Res* 1989;181:551-65.
54. Minta JO, Pambrun L. In vitro induction of cytologic and functional differentiation of the immature human monocytelike cell line U-937 with phorbol myristate acetate. *Am J Pathol* 1985;119:111-26.
55. Tetteroo PA, Massaro F, Mulder A, Schreuder-van Gelder R, dem Borne AE. Megakaryoblastic differentiation of proerythroblastic K562 cell-line cells. *Leuk Res* 1984;8:197-206.
56. Lozzio BB, Lozzio CB. Properties of the K562 cell line derived from a patient with chronic myeloid leukemia. *Int J Cancer* 1977;19:136
57. Sutherland JA, Turner AR, Mannoni P, McGann LE, Turc JM. Differentiation of K562 leukemia cells along erythroid, macrophage, and megakaryocyte lineages. *J Biol Response Mod* 1986;5:250-62.
58. Lindner I, Kharfan-Dabaja MA, Ayala E, et al. Induced dendritic cell differentiation of chronic myeloid leukemia blasts is associated with down-regulation of BCR-ABL. *J Immunol* 2003;171:1780-91.
59. Hu ZB, Ma W, Zaborski M, MacLeod R, Quentmeier H, Drexler HG. Establishment and characterization of two novel cytokine-responsive acute myeloid and monocytic leukemia cell lines, MUTZ-2 and MUTZ-3. *Leukemia* 1996;10:1025-40.
60. Quentmeier H, Duschl A, Hu ZB, Schnarr B, Zaborski M, Drexler HG. MUTZ-3, a monocytic model cell line for interleukin-4 and lipopolysaccharide studies. *Immunology* 1996;89:606-12.
61. Santegoets SJ, Masterson AJ, van der Sluis PC, et al. A CD34+ human cell line model of myeloid dendritic cell differentiation: evidence for a CD14+CD11b+ Langerhans cell precursor. *J Leukoc Biol* 2006;80:1337-44.
62. Wollenberg A, Mommaas M, Oppel T, Schottdorf EM, Gunther S, Moderer M. Expression and function of the mannose receptor CD206 on epidermal dendritic cells in inflammatory skin diseases. *J Invest Dermatol* 2002;118:327-34.
63. Headington JT. The dermal dendrocyte. *Adv Dermatol* 1986;1:159-71.
64. Cerio R, Griffiths CE, Cooper KD, Nickoloff BJ, Headington JT. Characterization of factor XIIIa positive dermal dendritic cells in normal and inflamed skin. *Br J Dermatol* 1989;121:421-31.
65. Lenz A, Heine M, Schuler G, Romani N. Human and murine dermis contain dendritic cells. Isolation by means of a novel method and phenotypical and functional characterization. *J Clin Invest* 1993;92:2587-96.
66. Turville SG, Cameron PU, Handley A, et al. Diversity of receptors binding HIV on dendritic cell subsets. *Nat Immunol* 2002;3:975-83.
67. Birbeck M, Breathnach A, Everall J. An electron microscopic study of basal melanocytes and high level clear cells (Langerhans cells) in vitiligo. *J Invest Dermatol* 1961;37:51-64.
68. Charbonnier AS, Kohrgruber N, Kriehuber E, Stingl G, Rot A, Maurer D. Macrophage inflammatory protein 3alpha is involved in the constitutive trafficking of epidermal langerhans cells. *J Exp Med* 1999;190:1755-68.
69. Tang A, Amagai M, Granger LG, Stanley JR, Udey MC. Adhesion of epidermal Langerhans cells to keratinocytes mediated by E-cadherin. *Nature* 1993;361:82-5.
70. Valladeau J, Ravel O, Dezutter-Dambuyant C, et al. Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. *Immunity* 2000;12:71-81.
71. Kim KD, Choi SC, Noh YW, et al. Impaired responses of leukemic dendritic cells derived from a human myeloid cell line to LPS stimulation. *Exp Mol Med* 2006;38:72-84.
72. Nestle FO, Banachereau J, Hart D. Dendritic cells: On the move from bench to bedside. *Nat Med* 2001;7:761-5.

73. Mailliard RB, Wankowicz-Kalinska A, Cai Q, et al. alpha-type-1 polarized dendritic cells: a novel immunization tool with optimized CTL-inducing activity. *Cancer Res* 2004;64:5934-7.
74. Santegoets SJ, Schreurs MW, Masterson AJ, et al. In vitro priming of tumor-specific cytotoxic T lymphocytes using allogeneic dendritic cells derived from the human MUTZ-3 cell line. *Cancer Immunol Immunother* 2006;55:1480-90.
75. Curti A, Pandolfi S, Aluigi M, et al. Interleukin-12 production by leukemia-derived dendritic cells counteracts the inhibitory effect of leukemic microenvironment on T cells. *Exp Hematol* 2005;33:1521-30.
76. Luft T, Jefford M, Luetjens P, et al. Functionally distinct dendritic cell (DC) populations induced by physiologic stimuli: prostaglandin E(2) regulates the migratory capacity of specific DC subsets. *Blood* 2002;100:1362-72.
77. Scandella E, Men Y, Legler DF, et al. CCL19/CCL21-triggered signal transduction and migration of dendritic cells requires prostaglandin E2. *Blood* 2004;103:1595-601.
78. Kalinski P, Hilkens CM, Snijders A, Snijdwint FG, Kapsenberg ML. IL-12-deficient dendritic cells, generated in the presence of prostaglandin E2, promote type 2 cytokine production in maturing human naive T helper cells. *J Immunol* 1997;159:28-35.
79. Kalinski P, Schuitemaker JH, Hilkens CM, Wierenga EA, Kapsenberg ML. Final maturation of dendritic cells is associated with impaired responsiveness to IFN-gamma and to bacterial IL-12 inducers: decreased ability of mature dendritic cells to produce IL-12 during the interaction with Th cells. *J Immunol* 1999;162:3231-6.
80. Platzer B, Jorgl A, Taschner S, Hocher B, Strobl H. RelB regulates human dendritic cell subset development by promoting monocyte intermediates. *Blood* 2004;104:3655-63.
81. Jorgl A, Platzer B, Taschner S, et al. Human Langerhans-cell activation triggered in vitro by conditionally expressed MKK6 is counterregulated by the downstream effector RelB. *Blood* 2007;109:185-93.
82. Jaksits S, Kriehuber E, Charbonnier AS, Rappersberger K, Stingl G, Maurer D. CD34+ cell-derived CD14+ precursor cells develop into Langerhans cells in a TGF-beta 1-dependent manner. *J Immunol* 1999;163:4869-77.
83. Mohty M, Isnardon D, Blaise D, et al. Identification of precursors of leukemic dendritic cells differentiated from patients with acute myeloid leukemia. *Leukemia* 2002;16:2267-74.
84. Houtenbos I, Westers TM, Ossenkoppele GJ, van de Loosdrecht AA. Identification of CD14 as a predictor for leukemic dendritic cell differentiation in acute myeloid leukemia. *Leukemia* 2003;17:1683-4.
85. Houtenbos I, Westers TM, de Gruijl TD, Scheper RJ, Ossenkoppele GJ, van de Loosdrecht AA. TNF-alpha receptor 1 expression on acute myeloid leukemic blasts predicts differentiation into leukemic dendritic cells. *Leukemia* 2004;18:1149-53.
86. Houtenbos I, Westers TM, Hess CJ, Waisfisz Q, Ossenkoppele GJ, van de Loosdrecht AA. Flt-3 internal tandem duplication hampers differentiation of AML blasts towards leukemic dendritic cells. *Leukemia* 2006;20:1892-5.
87. Anderson P, Sundstedt A, Li L, et al. Differential activation of signal transducer and activator of transcription (STAT)3 and STAT5 and induction of suppressors of cytokine signalling in T(h)1 and T(h)2 cells. *Int Immunol* 2003;15:1309-17.
88. Bontkes HJ, Ruizendaal JJ, Kramer D, et al. Constitutively active STAT5b induces cytokine-independent growth of the acute myeloid leukemia-derived MUTZ-3 cell line and accelerates its differentiation into mature dendritic cells. *J Immunother* 2006;29:188-200.
89. Li S, Ferguson MJ, Hawkins CJ, Smith C, Elwood NJ. Human telomerase reverse transcriptase protects hematopoietic progenitor TF-1 cells from death and quiescence induced by cytokine withdrawal. *Leukemia* 2006;20:1270-8.
90. Drexler HG. Monocytic Cell Lines. *Human Cell Culture* 2000;3:237-57.
91. Goppelt-Strube M, Reiser CO, Schneider N, Grell M. Modulation of tumor necrosis factor (TNF) receptor expression during monocytic differentiation by glucocorticoids. *Inflamm Res* 1996;45:503-7.
92. Reiterer G, Yen A. Platelet-Derived Growth Factor Receptor Regulates Myeloid and Monocytic Differentiation of HL-60 Cells. *Cancer Res* 2007;67:7765-72.
93. Suthesophon K, Nishimura N, Kobayashi Y, et al. Involvement of the tumor necrosis factor (TNF)/TNF receptor system in leukemic cell apoptosis induced by histone deacetylase inhibitor depsipeptide (FK228). *J Cell Physiol* 2005;203:387-97.
94. Cheng T, Wang Y, Dai W. Transcription factor egr-1 is involved in phorbol 12-myristate 13-acetate-induced megakaryocytic differentiation of K562 cells. *J Biol Chem* 1994;269:30848-53.
95. Nestle FO, Aljagic S, Gilliet M, et al. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nat Med* 1998;4:328-32.

96. Thurner B, Haendle I, Roder C, et al. Vaccination with mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. *J Exp Med* 1999;190:1669-78.
97. Banchereau J, Palucka AK, Dhodapkar M, et al. Immune and clinical responses in patients with metastatic melanoma to CD34(+) progenitor-derived dendritic cell vaccine. *Cancer Res* 2001;61:6451-8.
98. Davis ID, Jefford M, Parente P, Cebon J. Rational approaches to human cancer immunotherapy. *J Leukoc Biol* 2003;73:3-29.
99. Fabre JW. The allogeneic response and tumor immunity. *Nat Med* 2001;7:649-52.
100. Harrop R, John J, Carroll MW. Recombinant viral vectors: cancer vaccines. *Adv Drug Deliv Rev* 2006;58:931-47.
101. Doria-Rose NA, Haigwood NL. DNA vaccine strategies: candidates for immune modulation and immunization regimens. *Methods* 2003;31:207-16.
102. Brouwer RE, van der HM, Kluin-Nelemans HC, van Zelderen-Bhola S, Willemze R, Falkenburg JH. The generation of dendritic-like cells with increased allostimulatory function from acute myeloid leukemia cells of various FAB subclasses. *Hum Immunol* 2000;61:565-74.
103. Rasaiyaah J, Yong K, Katz DR, Kellam P, Chain BM. Dendritic cells and myeloid leukaemias: plasticity and commitment in cell differentiation. *Br J Haematol* 2007;138:281-90.

