Review

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The characterization and role of leukemia cell-derived dendritic cells in immunotherapy for leukemic diseases

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Summary **Usually, an effective anti-leukemia immune response cannot be initiated effectively in patients with leukemia. This is probably related to immunosuppression due to chemotherapy, down-regulation of major histocompatibility complex (MHC) II molecules, and the lack of co-stimulatory molecules on dendritic cells (DC). In light of this problem, some methods had been used to induce leukemia cells to differentiate into mature DCs, causing them to present leukemia-associated antigens and activating naïve T cells. Furthermore, leukemia-derived DCs could be modified with tumor antigens or tumor-associated antigens to provide a new approach to anti-leukemia therapy. Numerous studies have indicated factors related to the induction and functioning of leukemia-derived DCs and the activation of cytotoxic T-lymphocytes (CTLs). These include the amount of purified DCs, cytokine profiles appropriate for inducing leukemia-derived DCs, effective methods of activating CTLs, reasonable approaches to DC vaccines, and the standardization of their clinical use. Determining these factors could lead to more effective leukemia treatment and benefit both mankind and scientific development. What follows in a review of advances in and practices of inducing leukemia-derived DCs and the feasibility of their clinical use.**

Keywords: Dendritic cell, leukemia cell, leukemia-derived DCs

1. Introduction

Dendritic cells (DCs), including myeloid DCs and lymphoid DCs, are derived from pluripotential hematopoietic stem cells. Although widely distributed throughout the body, only a few of DCs can be found in single organs and account for about 1% of the total amount of leukocytes in peripheral blood (*1*). The lack of DCs is partially related to development of some solid tumors or leukemias (*1*). Therefore, DCs have been studied to treat hematologic malignancies (*2*). Since patients with leukemia have small amounts of DCs in the blood and these DCs have poor clinical efficacy, researchers have focused on finding methods to increase the numbers of DCs to induce leukemia-specific CTLs. Several methods have been used to induce leukemia cells to differentiate into mature DCs, and these leukemia-derived DCs are capable of presenting leukemia-associated antigens and are used clinically (*3*).

This review summarizes current understanding of DC functioning in patients with leukemia, the anti-leukemia role of leukemia-derived DCs, and ways to induce DCs from leukemia cells.

2. Deficient immunity and a lack of DCs in patients with leukemia

Although intensive chemotherapy-based approaches, which include stem cell transplantation, have induced complete remission (CR) in 80% of patients with acute myeloid leukemia (AML), many patients still

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relapse due to the persistence of minimal residual disease (MRD), resulting in survival rates of 30-40%. Obviously, the leukemia-bearing host is immunologically tolerant to the remaining leukemia cells and therefore fails to eradicate the disease (*4*). The lack of co-stimulatory molecules on the surface of leukemia cells, as evident in the low expression of CD40 and CD80 and other molecules, can lead to the insufficient recognition and lack of presentation of specific antigens and failure to activate T cells (*5-8*). The expression of CD40 is considered to be crucial for T cell activation and expansion, so the absence of CD40 on blasts might be especially responsible for the insufficient recognition of blasts by the immune system in patients with AML (*5*).

Antigen-presenting cells (APCs) are devoid of co-stimulatory molecules and thus fail to activate T cells to start an effective anti-tumor immune response (*9-11*). At the same time, growing malignant tumor cells can secrete many immunosuppressive factors such as interleukin-10 (IL-10), transforming growth factor-β (TGF-β), and vascular endothelial growth factor (VEGF) that can lead to dysfunction of DCs, resulting in tumor-associated antigens not being effectively presented to lymphocytes (*12,13*). Moreover, immature DCs in patients with leukemia might induce regulatory or suppressive T cells, impairing the quality of the anti-leukemia immune response as would occur with normal immature DCs (*14-16*). Recently, other proteins have been found to correlate with immunity or the prognosis for patients with leukemia. For example, CD56 expression in AML with t(8;21) is associated with a significantly shorter duration of CR and survival (*17*). B7-2 is one of the most crucial factors in the prognosis of adult acute leukemia and has an important role in tumor immunity (*18*), and poliovirus receptor-related (PRR) proteins could play a role in leukemia. Patients with a high level of PRR1 or PRR2 expression exhibit a more favorable prognosis (*19*). Thus far, however, the role of these factors in compromised immunity and the lack of DCs is still being studied. Presumably, the mechanism of immune reaction in patients with leukemia is as shown in Figure 1. Overall, the rapidly growing body of data offers new insights towards understanding AML biology and it provides evidence that DC subsets and dendritopoiesis *in vivo* are affected by leukemogenesis and may contribute to leukemia's evasion of the immune system.

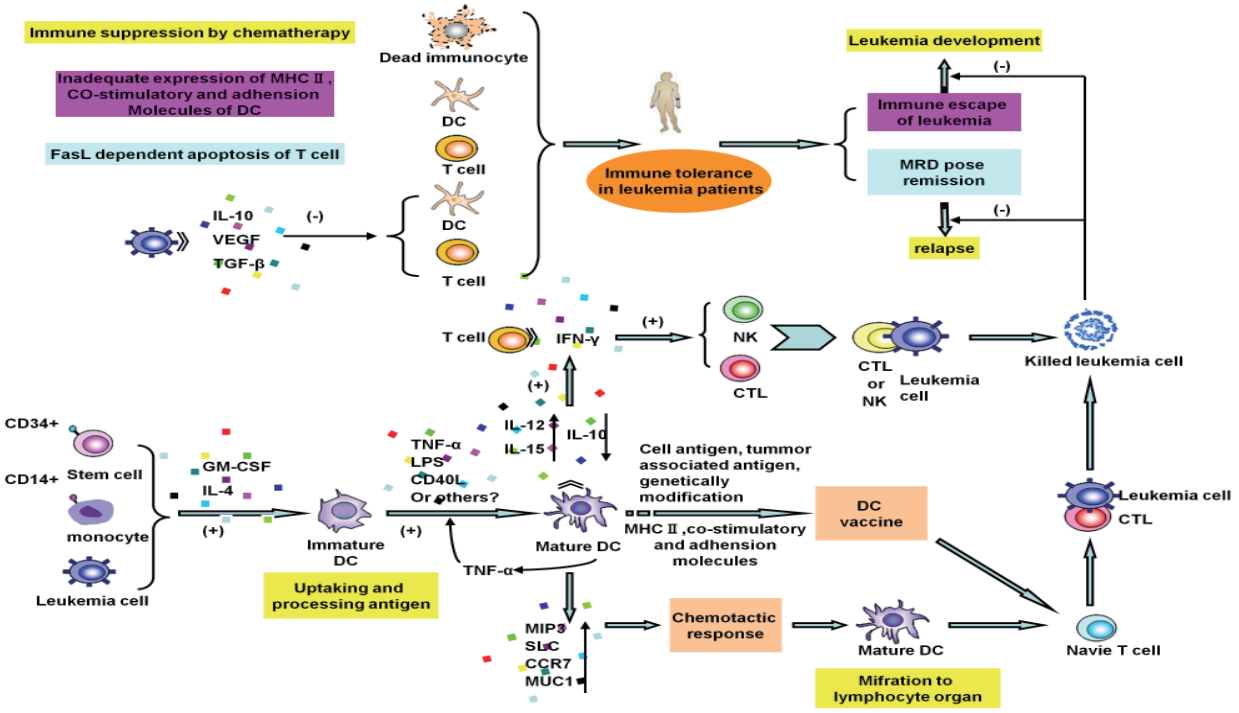

Figure 1. Mechanism of immune reaction in patients with leukemia. Deficient immune responses and MRD in patients with leukemia contribute to immunodepression due to chemotherapy, the down-regulation of MHC-II molecules, the lack of co-stimulatory molecules on DCs, FasL-induced apoptosis of T cells, and inhibition of the functioning of T or DC cells by TGF-β, IL-10, and VEGF. Some cytokine profiles and other agents can induce primary leukemia cells or leukemia cell lines to differentiate into immature or mature DCs. Such leukemia-derived DCs are responsible for presenting leukemia-associated antigens and convert the naïve T cells into CTLs, which can be enhanced by genes, tumor-associated antigen peptides, or cellular tumor antigens. The IFN-γ and chemokines secreted by leukemia-derived DCs can also modulate the functioning of NK and CTL or induce the migration of naïve T cells to lymphocyte organs. The activated CTL will kill leukemia cells and induce remission in patients with leukemia. Although DCs are used to treat patients with leukemia, DC-mediated leukemia immunotherapy is thus far confined to animal and *in vitro* experiments, and many problems remain to be solved.

3. *Ex vivo* generation of DCs

3.1. *DCs produced from primary leukemia cells*

At present, DC amplification mainly focuses on the effects of different cytokine combination profiles. The most conventional cytokines include granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin-4 (IL-4), and tumor necrosis factor-α (TNF-α). The GM-CSF + TNF-α + IL-4 profile can induce CD34+ hemopoietic stem cells and CD14 + monocytes in healthy persons to differentiate into DCs (*20*). T cell immunity against autologous leukemia cells mediated *in vitro* by DCs from patients with AML might prove useful for immunotherapy of AML even in patients with CR (*21,22*). Peripheral blood cells of patients with chronic myelomonocytic leukemia (CMML) can also be induced to acquire DC characteristics upon culturing with GM-CSF plus IL-4 plus TNF-α. CMML-derived DCs are potent stimulators of the allogeneic mixed lymphocyte reaction (MLR) and may serve as a cellular vaccine to induce anti-tumor immunity in patients with CMML (*23*). Some researchers have successfully induced chronic myelogenous leukemia (CML) cells to become CML-DCs using GM-CSF and IL-4, and the CML-DCs produced appear no different from normal DCs in terms of their ability to stimulate the proliferation of T lymphocytes (*3,24,25*). CML-DCs can express high levels of co-stimulatory molecules such as CD1a, CD86, CD40, CD80, and CD83 and stimulate autologous T cells or human leukocyte antigen (HLA)-compatible donor T cells to proliferate substantially and start a CML-specific cytotoxic immune response. DCs can secrete IL-12 in varying degrees and assist lymphocytes to produce IFN-γ. IFN-γ can enhance the activity of CTLs and enhance the ability of NK cells to kill target cells.

The leukemic blasts of AML collected from the peripheral blood or the bone marrow of patients with different types of leukemias may be induced to differentiate *ex vivo* into fully functional DCs. These leukemia-derived DCs can be obtained after short-term culturing in the presence of GM-CSF, IL-4, CD40L, or other cytokines. The derived DCs exhibit a typical DC morphology, have a phenotype of mature DCs particularly with regard to the expression of co-stimulatory molecules, and can induce a potent proliferative response in naïve CD4+ T cells (*9, 26-29,44-46*).

Usually, cytokines including GM-CSF can induce AML leukemic cells to become immature DCs and further develop into mature DCs after induction by IFN-α, IL-1β, IL-6, or prostaglandin E2 (PGE2) (*30,31*). The ability of leukemia-derived DCs to induce proliferation of allogeneic T cells increases significantly and offers a useful path to active immunotherapy for patients with AML (*32-34*). Other factors also affect AML-DC formation and functioning. For example, only AML primary cells expressing CD14 and TNF-α-RI can be successfully induced to differentiate into DCs (AML-DCs) by cytokines (*35,36*). The FLT3-ITD (FLT3 internal tandem duplication) can inhibit AML-DC formation and down-regulate VEGF expression and induce the production of functional AML-DCs (*34*). Ganoderma lucidum polysaccharides (GL-PS) can stimulate the maturation of monocyte-derived DCs in patients with monocytic leukemia (AML-M4 and M5) (*37*). DCs are harder to cultivate from AML samples than from CML samples, and DCs cannot be obtained from some patients with AML by conventional methods and with low levels of expression of several key molecules.

Furthermore, myeloid-leukemic cells in myelodysplastic syndromes (MDS) can be induced to differentiate into leukemia-derived DCs. In all, 31% to 52% of leukemic blasts can be converted to leukemia-derived DCs and 39% to 64% of these DCs are mature (*38,39*). Lymphocytic leukemia cells can be also induced to differentiate into leukemic DCs with phenotypical and functional characterization of DCs (*40*). At the current point in time, 2 to 5 cytokine combination profiles, consisting of GM-CSF, TNF, IL-4, CD40L, SCF, FL, and TGF-α, are mainly used to induce leukemic cells to differentiate (*39,56*) (Table 1). Methods of inducing leukemic cells to differentiate into DCs still need further improvement. There are positive

Table 1. Induced differentiation of DCs from primary leukemia cells

Leukemia	Methods of producing DCs*							DC differentiation capacity	Antigen-presenting ability	Source
	GM-CSF, IL-4, TNF-a, FL, CD40L	GM-CSF, IL-4, TNF-a, FL, IL-1b, IL-6, PGE2	IL-4, CI (A23187)	GM-CSF, IL-4, OK-432, PGE2	GM-CSF, TNF-a, IL-4, IL-3, SCF, FL	GM-CSF, IL-4, Poly (I:C)	IL-4 CD40L			
AML	+[a,b]	+	+	+	+	+	–	+	+	*28-40*
ALL	+[b]	–	–	–	–	–	–	+	+	*22*
CML	+[a]	–	–	–	–	–	–	++	++	*15-17*
CLL	–	–	–	–	–	–	+	+	+	*41*
MDS	+[a]	+	+	+	+	+	–	+	+	*28, 44-46*
CMML	+[c]	–	–	–	–	–	–	+	+	*42, 43*

[a] GM-CSF + IL-4 + TNF-α + FL; [b] GM-CSF + IL-4 + TNF-α + CD40L; [c] GM-CSF + IL-4 + TNF-α.
* In some other studies, 4-1BBL, LPS, or HDAC inhibitors were used to promote the maturation of leukemia-derived DCs.

signs regarding the role of effective adjuvants in the induction or functioning of leukemia-derived DC cells. In the presence of GM-CSF, TNF-α, and/or IL-4, the leukemia-derived DCs that are usually obtained display features of immature DCs, so immature DCs must be induced to differentiate into mature DCs. With CD40L as a maturating agent, leukemic immature DCs can differentiate into cells that can fulfill the phenotypic criteria of mature DCs (*40*). Ionomycin calcium (CI) A23187 can induce human granulocytes to take on DC-like characteristics (*41*). Histone deacetylase inhibitors (HdI) could potentially improve the differentiation of leukemia-derived DCs induced from bone marrow samples of patients with ALL, as indicated by the up-regulation of CD86 (+) CD80 (–) cells (*42*). The role of the 4-1BB ligand (4-1BBL) in the T cell response induced by AML-DCs was examined and addition of 4-1BBL to cocultures of AML-DC and T cells was found to induce a preferential increase in the proliferation of CD8+ T cells. 4-1BBL was found to be an effective adjuvant to enhance the T cell response elicited by AML-DC (*43*).

3.2. *DCs produced from leukemia cell lines*

K562 cells, a CML cell line that includes multidrug-resistant leukemia K562/A02 cells, has been used in attempts to induce leukemia-derived DCs with different cytokine profiles, but the cells differentiated into K562-DCs only after exposure to GM-CSF + TNF-α + IL-4, which activated lymphocytes to produce CTLs (*44, 45*). IFN-γ was also detected in the supernatant, which enhanced the activity of CTLs and enhanced the ability of NK cells to kill target cells. There are contradictory findings regarding the DC differentiation capacity of THP-1 cells. Although THP-1 cells have been noted to acquire DC-like properties upon stimulation with cytokines, THP-1 cells have a relatively low DC differentiation capacity since less than 5% of THP-1 cells express the classic myeloid DC marker CD1a (*46, 47*). Moreover, THP-1 DCs fail to function like DCs, as indicated by the absence of allogeneic T-cell stimulatory capacity. Other reports, however, indicated that THP-1 leukemia cell lines can be induced to differentiate rapidly into mature DCs when cultured in serum-free medium containing GM-CSF, TNF-α, and ionomycin. Cell line-derived mature DCs are capable of stimulating allogeneic CD4+ and CD8+ T cells, ultimately defining them as potent APCs (*48*). Mature DCs were induced from human monocytic cell THP-1 by treatment with IL-4, GM-CSF, TNF-α, and ionomycin, and some cells were pretreated with PPAR-gamma agonists. Results indicated that induction of junctional adhesion molecule (JAM)-A occurred during differentiation of human THP-1 DCs and was independent of PPAR-gamma and the p38 MAPK pathway (*49*).

KG-1 is a cytokine-responsive, CD34+ myelo-monocytic cell line derived from a patient with erythroleukemia. Recently, KG-1 cells have also been described as acquiring DC-like properties upon stimulation with cytokines or PMA ± CI. Although KG-1 cells respond to stimulation with a number of factors known to induce differentiation and/or maturation of DCs *in vitro*, they usually do not differentiate in response to LPS, CpG oligodeoxynucleotide, or CD40L. Only treatment with PMA and ionomycin (with or without prior culturing in GM-CSF and IL-4) induced morphological and phenotypic changes consistent with DC-like maturation, and even these maximally differentiated KG-1 cells showed lower levels of surface marker expression and ability to stimulate an allogeneic MLR compared to monocyte-derived DCs *in vitro*. Although KG-1 cells differentiate into cells with DC-like functional characteristics *in vitro*, they lack the potential of mature DCs in terms of key aspects of specific antigen-presenting cells (*46,50,51*). Cyclophilin A (CypA) is a ubiquitously distributed intracellular protein that can enhance DC differentiation and maturation by up-regulating CD11b and CD11c expression and also augment DC antigen uptake and antigen presentation (*52*). The HL-60 acute promyelocytic leukemia cell line is a multipotent cell line capable of differentiating into granulocytes (*53,54*), monocyte-macrophage-like cells (*55,56*), or eosinophilic granulocytes (*57*). HL-60 cells have also been found to be insensitive to cytokine-induced DC differentiation (*58*). Upon CI treatment, HL-60 cells rapidly up-regulated CD86 and demonstrated de novo expression of CD83, CD80, and CD54. Expression of CD40 and CD1a only became apparent after 72-96 h. In addition, CI treatment also resulted in a marked increase in APC function, as determined by enhanced allogeneic T cell stimulation capacity. However, the fact that HL-60 cells failed to express MHC class II molecules and down-regulated MHC class I molecules upon CI treatment suggests that they may have limited antigen-specific T-cell stimulatory capacity (*58,59*). A large number of cells with a typical dendritic appearance were observed after culturing with CI A23187 for 72 hours, and the expression of CD80 and CD86 was continuously up-regulated. Allo-MLR revealed that DCs derived from HL-60 cells treated with A23187 or plus rhIFN-γ stimulated the proliferation of allogeneic human T cells (*60*).The Monomac-6 human acute monocytic leukemia cell line exhibits a well-differentiated monocyte phenotype with phagocytotic activity and the expression of the mature monocyte marker CD14 (*61*), though they are unable to differentiate into DCs.

The U-937 histiocytic lymphoma cell line also exhibits monocytic characteristics, displaying a monoblast morphology (*62*). In contrast to Monomac-6 cells, U-937 cells are able to acquire mature, monocyte-like morphologic and phenotypic characteristics

upon stimulation with PMA. Despite its monocyte-like characteristics, the U-937 cell line is unable to differentiate into DCs. The MUTZ-3 cell line is derived from the peripheral blood of a patient with AML (*63*) and was used to generate immature dendritic-like cells (MUTZ-3 DC) (*64,65*). Compared to monocyte-derived DCs, the MUTZ-3 cell line has been shown to downregulate CD14 in response to GM-CSF and IL-4 and to exhibit characteristics of CD34-derived DC precursors (*46*). 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 differentiation such as GM-CSF-R and TNF-α-RI and -RII are up-regulated. In contrast to other AML-derived cell lines, MUTZ-3 cells have a marked DC differentiation capacity since CD1a expression after DC differentiation ranges between 60% and 90% for MUTZ-3 IDC and LC, respectively (*66,67*). Moreover, 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 a true DC phenotype (*68*). Importantly, MUTZ-3-derived IDC and LC may also mature further in the presence of cytokines or CD40L, resulting in up-regulation of costimulatory and adhesion molecules CD80, CD86, CD40, CD54, and HLA-DR and de novo expression of CD83 (*69*). All-trans retinoic acid (ATRA) can also induce the NB4 retinoic acid (RA)-sensitive promyelocytic leukemic cell line to differentiate into DC-like cells and these differentiated cells can activate T cells. Results suggest that the differentiation of NB4 cells by ATRA causes the cells to express DC markers and that ATRA-differentiated NB4 cells are able to present antigens to T cells (*70*).

Jin *et al.* investigated whether phosphatidic acid (PA) can induce NB4 cells to differentiate into DC-like cells and they found dioctanoyl-PA alone upregulated the expression of DC markers. The expression of DC markers on NB4 cells was facilitated by the overexpression of phospholipase D and upregulation was blocked by the addition of n-butanol, an inhibitor of PA production. The expression of CD11c, CD83, and CCR7 in PA-treated NB4 cells was further increased by TNF-α treatment. These results suggest that PA induces differentiation of NB4 cells into DC-like cells and that the upregulation of antigen-presenting cell markers is mediated by the activation of ERK and the downregulation of PML-RAR alpha levels (*71*).

To summarize, attempts at inducing K562, THP-1, KG-1, Monomac-6, U-937, MUTZ-3, and NB4 cells to differentiate into DC-like cells have been tried with a similar cytokine profile or special drugs. Although they were all induced to differentiate into DC-like cells with morphologic and phenotypic characteristics of DCs, K562, THP-1, KG-1, MUTZ-3, and NB4 cells have obvious potential as mature DCs in terms of key aspects of specific antigen-presenting capacity (Table 2).

4. Modified DCs for clinical use

4.1. *Genetically modified DCs*

Specific mRNA fragments encoding the tumor antigens can be transduced into DCs to generate DC vaccines. Methodologically, several recombinant DNA delivery techniques have been used. In one study, nucleofection and adenoviral transduction were compared in terms of their efficiency at transducing human MoDCs *in vitro*. The use of a fiber-modified adenoviral vector may therefore be preferable to non-viral gene delivery systems for DCs that will be used in cancer immunotherapy (*72*). For example, Muller *et al.* successfully transduced all of the mRNA isolated from type-B leukemic cells into DCs, further activating CTLs to kill leukemic cells (*73*). Nikitina *et al.* utilized the adenovirus vector Ad2p53 to transduce the wild type p53 gene into DCs, further stimulating T cells to generate p53-specific CTLs capable of killing the corresponding K562 leukemic cell strain (*74*). Therefore, electroporation of mRNA-encoding tumor antigens is a powerful technique to charge human DCs with tumor antigens and could provide tumor vaccines (*75*). The genes encoding some cytokines, co-stimulators, and chemokines can also be transduced into DCs to generate DC vaccines, enhancing the immunogenicity of the cells and starting a specific anti-tumor immune response. The expression of CD40 is considered to be crucial for T cell activation and expansion (*5*). Moreover, its expression on blasts was

Table 2. Induced differentiation of DCs from leukemia cell lines

Cell line	DC differentiation capacity	Cytokine profile	DC markers	Induced CTL activity	Source
K562	+	GM-CSF + IL-4 + TNF-α	+	++	*33, 61-65*
THP-1	+/–	GM-CSF + IL-4 + TNF-α, or + CI	+	+/–	*68-79*
KG-1	+/–	GM-CSF + IL-4 + TNF-α, or PMA + CI	+	+/–	*66-67, 71-74*
HL-60	+/–	CI or CI + IFN-γ; Insensitive to cytokines	+	+	*80-82*
U-937	–	GM-CSF + IL-4 + TNF-α	–	–	*84*
Monomac-6	–	GM-CSF + IL-4 + TNF-α	–	–	*67-68, 83*
MUTZ-3	++	GM-CSF + IL-4 + TNF-α	+	++	*66,68, 85-93*
NB4	+	ATRA; ATRA + GM-CSF, CD40L; PA + TNF-α	+	+	*94, 95*

almost absent. Thus, the absence of CD40 or CD80 on blasts in particular might be responsible for the insufficient recognition of blasts by the immune system of patients with AML. Thus, enhancing the expression of CD80 on leukemia blasts could increase their costimulatory activity on autologous T cells (*76*).

4.2. *DC vaccines modified with tumor antigens*

Previous studies (*77-79*) found that there was extensive expression of leukemia-associated antigens (LAAs) such as the preferentially expressed antigen of melanoma, the receptor for hyaluronic acid-mediated motility, and Wilms' tumor gene (WT-1) on AML blasts in contrast to PBMCs from healthy volunteers. Greater expression of LAAs by AML-DCs was observed in comparison to AML blasts. The amount of tumor antigens expressed in leukemia-derived DCs will affect their ability to initiate an immune response (*8*).

Recently, other leukemia-associated antigens that are recognized by CTL in the context of HLA class I molecules have been identified. These include fusion gene products such as BCR-ABL and ETV6-AML1, proteinase 3, human telomerase reverse transcriptase, and cyclophilin B. These findings have led to various clinical trials involving peptide modification and DC therapy (*80*). Tumor lysates can be utilized to load DCs to generate DC vaccines. DCs with strong phagocytosis can take up and present effective constituents such as tumor-associated antigens (TAA) in lysates, the MHC-II molecules in oncocyte extracts and the minor histocompatibility antigens to activate CTLs and induce an immune reaction. Apoptotic tumor cells can also be utilized in DC loading. DCs can take up apoptotic bodies and further process and present tumor antigens to induce tumor-specific CTL action (*81*). Klammer *et al.* (*82*) used leukemic cells and DCs collected from the peripheral blood to produce fusion vaccines, and their results indicated that the fusion cells not only expressed tumor antigens but also expressed the surface markers and co-stimulatory factors of DCs. Fusion vaccines made from tumor cells and allogenic DCs mainly secrete IFN-γ, which can induce Th1 differentiation, while fusion vaccines made from syngeneic DCs can induce the differentiation of both Th1 and Th2. Since the anti-tumor immune response is mainly a Th1-induced cellular immune response, allogenic DCs are more suitable for fusion vaccine production. DC modification with cellular tumor antigens is relatively simple and easy, but it still has some disadvantages. For example, a large amount of tumor cells is required and there are large amounts and various types of non-associated antigens that can induce auto-immune diseases; the utilized antigen peptides cannot always induce optimal anti-tumor immune responses because of antigenic modulation, antigenic deletion, low antigen immunogenicity, and the difficulty of determining the stimulating dose. AML-DC vaccines might be provided by the addition of adjuvants such as LPS or CPG-rich oligodeoxyribonucleotide binding to TLR and induction of a greater Type 1 T cell response. AML-DCs strongly expressed TLR-2 and TLR-4, while TLR-9 was expressed at a lower level. In accordance with the TLR expression levels, DCs produced from patients with AML responded to the known microbial ligands peptidoglycan (PGN) and lipoteichoic acid for TLR-2 and LPS as a ligand for TLR-4 by producing TNF-α and IL-6. A response to the ODNs 2006 and 2216 binding to TLR-9 was only detected in AML-DCs (*83*).

5. Quantity and quality control of leukemia-derived DCs

The leukemic derivation of AML-DC may be indicated by the persistence of clonal cytogenetic aberrations in DCs or by coexpression of leukemic antigens on DCs. For example, proof of the clonal derivation of DC was obtained in five AML and four MDS cases (*38*) with a combined FISH/immunophenotype analysis (FISH-IPA). The clonal numerical chromosomal aberrations of the diseases were regularly detected along with DC markers, but not with all clonal cells being converted to leukemia-derived DCs (on average, 53% of blasts in AML or MDS). Instead, not all DCs had clonal aberration (on average, 51% of DCs). On average, only 57% of blasts in AML and 64% of blasts in MDS were converted to leukemia-derived DCs (DC (leu)).

In order to establish the value of DC vaccination in patients with leukemia, some consensus on quality criteria and immune monitoring is essential. Although leukemic DCs meet most quality criteria, the optimal level of maturation needed to elicit an immune response should be determined. The quality of DCs can be defined by morphological, immunophenotypic, and functional criteria (*84*).

Cut-off proportions of mature DCs, DC (leu), proliferating, CD4 (+), CD8 (+), and non-naïve T cells after DC-stimulation were predictive for the anti-leukemic-activity of stimulated T cells as well as a response to immunotherapy. Interestingly, ratios > 1 of CD4:CD8 or CD45RO:CD45RA T cells were predictive for anti-leukemic function after DC stimulation. In an attempt to further characterize the DC/DC leu-induced T cell response pattern, immunoscope spectratyping was used to detect T cell receptor (TCR) rearrangements in combination with functional flow cytometry and a non-radioactive fluorolysis assay. Results indicated that a combined strategy using spectratyping with functional tests might not only provide useful information about the specificity and efficacy of the induced T cell response but also pave the way to effective T cell clones for therapy (*85*).

The composition and quality of DCs after a mixed lymphocyte culture-priming phase is predictive for a successful *ex vivo* antileukemic response, especially with respect to proportions of mature and leukemia-derived DC. These findings may help to predict DC-mediated functions or the clinical course of the disease and they may also help with the development and refining of DC vaccination strategies that may pave the way to development and modification of adoptive immunotherapy particularly for patients relapsing after allogeneic stem-cell transplantation (*86*).

6. Leukemia-derived DCs for clinical use and results of clinical trials

Regarding administration routes, intracutaneous or subcutaneous injection is preferable to intravenous injection for T cell induction, but injection dependence will affect the ability of the DCs to migrate to the lymph nodes. Intra-lymph nodal injection can avoid this problem and it facilitates the delivery of a known quantity of DCs to the required location, potentially enhancing the immune function of T cells. Nodal injection also requires specialized techniques and carries the risk of damaging lymphatic tissues. An optimal route of administration providing a better T cell response has yet to be identified. Intranodal administration circumvents this problem and allows delivery of a known amount of DCs to the desired anatomic region, potentially leading to increased T cell immunity (*87,88*).

Furthermore, the route of administration may determine the location of the primary immune response, the distribution of memory cells, and the ability to control the tumors at different sites in the body (*89*).

DCs were cultivated from the peripheral leukemic cells of patients with lymphoma who had undergone chemotherapy mobilization and then retransfused into the body after they were loaded with autologous lymphoma antigens. This approach was clinically efficacious and facilitated anti-tumor immunotherapy with DCs. In a study of 19 patients with AML, Choudhury *et al*. successfully cultivated DCs *in vitro* from the leukemic cells of 18 patients, and after incubation with autologous lymphocytes these DCs induced obvious cytolysis of autologous AML cells (*28*). Reichardt *et al.* used the idiotype peptide of the multiple myeloma to affect mature DCs to produce vaccines to treat multiple myeloma. Among the 12 treated patients, two patients achieved partial remission (PR) and remission lasted for 25 months and 29 months, respectively (*90*). Fujii *et al.* used cytokines to induce the peripheral blood monocytes of a patient with CML and they retransfused the induced DCs back into the body of the patient. They later observed the amplification of T cells expressing receptors like CTLs and DC-mediated reduction of Ph+ leukemia cells in both peripheral blood and bone marrow (*91*). In another clinical experiment, two groups of patients with CML in the chronic phase and the acute phase were inoculated 4 times with 3 × 106 and 15 × 106 CML-DC cells, respectively. Results revealed no obvious clinical therapeutic response. This was probably because all of the selected patients were in the advanced stage and the amount of the inoculated DCs was rather small (*92*). Thus, at least 10 × 106 DCs are required to elicit an immune response in patients with CML, and an autologous CML-specific T cell response has been detected (*93-95*). Additionally, infused CML-DCs induced the appearance of T cell clones expressing the same T cell receptor, suggesting that the immune repertoire included tumor-reactive T cells. Other groups treated patients with chronic-phase CML (CP-CML) with various leukemic antigen peptides, resulting in an apparent immune response and clinical response. Imatinib mesylate is currently used as the first line therapy for CP-CML. Although it selectively targets the ABL portion of BCR-ABL protein as a reversible tyrosine kinase inhibitor, it cannot kill the leukemic stem cells of CML. Immunity could be enhanced in patients with CML treated with imatinib by combining it with immunotherapy, so the immune response of innate and adaptive immunity in CML has been summarized. Development of such immunotherapeutic strategies would be a promising approach to treat patients with CML-CP treated with imatinib (*96*).

To summarize, the aforementioned groups were not inoculated with the same amounts of DCs; most were between 106 to 107. Inoculation timing was nearly the same and most patients were in remission after chemotherapy (*97*). Roddie *et al*. reported the results of their Phase I/II clinical studies to treat acute leukemia by inoculating patients with autologous AML-DCs. They stimulated the leukemic cells of 5 patients with AML with GM-CSF + IL-4 for 4 days, GM-CSF + TNF-α for 3 days, and finally IFN-γ + poly (I : C) for 24 hours, and then they inoculated the 5 patients in the remission stage after chemotherapy with these cells. Their results showed that two patients still had CR 12 months after inoculation (*32*). Li *et al.* inoculated 5 elderly patients with AML with AML-DCs as a second or third-line treatment, and 3 patients lived stably for 5.5 months to 13 months while 2 patients died from rapid progression and deterioration (*98*). Five end-stage patients with AML were subcutaneously injected with AML-DCs up to four times at a biweekly interval, and sufficient amounts of MNCs were collected in leukopenic patients. Large-scale production of AML-DCs in cell factories under GMP conditions yielded an adequate quantity of viable and mature AML-DCs (*99*). In another study, monocyte-derived DCs induced a peptide-specific cellular response (*100*). An effective AML-DC vaccination regimen for AML requires four administrations of 10×10^6 cells. The possibility of DCs

vaccinations has also been tested in animal models. Pawlowska *et al.* reported that tumor-lysate pulsed DCs effectively prevented mice from developing leukemia, but mice with established disease could not be cured (*101*).

Leukemic-specific T cell responses were detected in some patients with AML with administration of AML lysate-pulsed monocyte-derived DCs (*102,103*). Immunotherapy is thought to be most effective at eradicating MRD, which is not done by immune cells themselves because they have been compromised by high-dose chemotherapy or radiation therapy (*104,105*).

During early remission, immune responses seem to be largely MHC-restricted whereas later on the immune response shifts towards being non-MHC restricted (*106*). Patients with high levels of MRD after chemotherapy are particularly likely to benefit from an early start of a vaccination or with the adoptive transfer of leukemia-specific T cells enhanced *ex vivo* (*89,107,108*). Important evidence suggesting that only mature DCs are suitable for use is because loading immature DCs with antigens will lead to the functional quiescence of T cells resulting from T cell removal or amplification of only regulated T cells (*15*). An important argument for the use of only mature DC is that antigen-loaded immature DCs silence T cells either by eliminating them or by enhancing regulatory T cells (*109*) or prolonging periods of maturation, hampering their capacity to stimulate a Th1 response (*110*). These results demonstrate that AML-DCs have certain therapeutic effects in patients whether they are in remission or not. All of these findings confirm that retransfusion of autologous DC vaccines is safe and practical and can stimulate an immune response in patients and enhance their immunity. Claxton *et al.* co-cultured CML-DCs with autologous T cells at a rate of 1:10 for 3 days and then retransfused all of these cells back into patients with CML; this resulted in control of leukocytosis in one patient for several months afterwards. The second patient took hydroxyurea (HU) orally before DC retransfusion and the Ph+ cells showed no obvious decrease after 3 DC transfusions (*111*).

Although DC therapy has been successful in inducing specific anti-tumor immune responses, data on clinical responses are not yet conclusive and most studies only report minimal antitumor effects (*89, 112*). New approaches are needed to warrant use of DC vaccines in treating leukemia (*113*), and more strategies are required to sensitize residual leukemic cells. This aspect warrants further investigation in order to increase the immune stimulatory effect of leukemic DCs (*114*). Areas that are ripe for study are the components needed to produce DCs for therapy, including their culture and cytokine profile, antigen loading and delivery, and the potential for combination of DC-based immunomodulatory strategies (*115*). New methods of LAA antigen loading and maturation of leukemia-derived DCs should be explored their efficacy. Evolving DC-based therapeutic strategies addressing multiple components of tumor-immune system interactions may yield substantial benefits for patients (*116,117*).

7. Conclusion and perspectives

As specific APCs with important immunomodulatory action, DCs are a new concept of anti-tumor vaccine with promising prospects. Specific CTLs induced by DCs can initiate specific immunity and provide a new approach to anti-tumor immunotherapy. That said, the exact mechanisms for differentiation of DCs and the molecular mechanisms of DC functions are still unclear, so ways to regulate DCs to function selectively and thoroughly must be investigated further. Furthermore, studies on DC-mediated leukemia immunotherapies are still confined to animal and *in vitro* experiments, and there are many problems remaining. These include ways to obtain a large amount of purified DCs, the appropriate cytokine profiles for inducing DCs from leukemic cells, efficient methods of activating CTLs, reasonable approaches to DC vaccines, and the standardization of their clinical use. Moreover, whether CTLs induced by DCs cause adverse effects must be answered and ways to evaluate the characteristics of mature or immature DCs must be ascertained. Although studies of leukemic DCs provide new insights towards understanding both leukemogenesis and the physiology of DCs and studies on DCs used in leukemia treatment have made considerable progress, there is a fine line between immune tolerance and activation. The availability of leukemia-derived DCs and their capacity to enhance tumor recognition is a promising approach to immunotherapy for AML and other kinds of leukemias. The design of a clinical DC-based vaccine immunotherapy protocol requires a concise functional characterization of DCs as well as reflection on the crucial role of routes and timing of vaccine delivery to ensure delivery of specific cytotoxic effectors and helper T lymphocytes. If DC-based therapy is to benefit patients, it will probably do so for patients with minimal residual disease following or accompany other established therapies. The optimization of DC-based vaccines is on par with the development of sensitive techniques to monitor minimal residual disease and reliable methods of measuring patient responses to DC vaccines. Effective provision of leukemia-derived DCs in anti-tumor biotherapies will benefit both mankind and scientific development.

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