

Dendritic Cell Vaccine as an Adjuvant Therapy in the Treatment of Chronic Myeloid Leukemia

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ABSTRACT

Dendritic cells (DCs) are bone marrow (BM)-derived antigen-presenting cells (APCs) with a key role in the induction of immunity. This study aimed at generating a DC vaccine expressing leukemia-associated antigen differentiated from myeloid blast cells to boost the immune system and to improve the clinical outcome of chronic myeloid leukemia (CML) patients. Two ml of venous blood were obtained from each patient to generate DCs by suspending them in liquid culture medium containing granulocyte monocyte colony stimulating factor (GM-CSF) and interleukin 4 (IL-4) and activated by adding tumor necrosis factor alpha (TNF α). DCs, identified by CD83 expression using flow cytometry, showed a significant increase after culture. A follow-up of patients by monitoring the immunological response was done by flow cytometric assessment of CD8⁺ T-cells % before and after injection of DCs. This study included 22 patients diagnosed as chronic myeloid leukemia who were divided into 2 groups. Group I was a pathological control group which included 13 age- and sex-matched CML patients that were not given the DC vaccine and were injected with saline. Group II included 9 CML patients that were given the DC vaccine. The WBC count before (141.59 \pm 100.51) and after (94.47 \pm 76.14) vaccination was highly significantly different in group II patients ($P = 0.018$). Although there was no statistically significant difference in the median of the CD8⁺ level and the absolute number of CD8⁺ before and after vaccination, there was an actual increase in the percentages of both medians. We conclude that a DC vaccine may be used as an adjuvant therapy parallel to CML patients' chemotherapeutic regimen.

Keywords: antigen-presenting cells, expression of CD83, generation of immune induction

INTRODUCTION

Chronic myeloid leukemia (CML) is characterized by the increased and unregulated growth of predominantly myeloid cells in the bone marrow (BM) and the accumulation of these cells in the blood. CML is a clonal bone marrow stem cell disorder characterized by proliferation of mature granulocytes and their precursors. It is a type of myeloproliferative disease associated with a characteristic chromosomal translocation called the Philadelphia chromosome (Tefferi 2006; Nowell 2007).

CML presents a unique opportunity to develop therapeutic strategies using vaccination against a truly tumor-specific antigen that is also the oncogenic protein required for neoplasia. CML is characterized by a reciprocal translocation between chromosomes 9 and 22, i.e. t(9;22). As a result, part of the BCR (breakpoint cluster region) gene from chromosome 22 is fused with the ABL (Abelson leukemia virus) gene on chromosome 9 producing the fusion gene *bcr-abl* that generates a protein of p210 KDa (Hehlmann *et al.* 2007). CML was the first cancer to be associated with a defined genetic abnormality, *bcr-abl*, which is necessary and sufficient for initiating chronic phase disease as well as the first cancer to be treated with molecular targeted therapy (Kavalerchik *et al.* 2008).

The concept of tumor "immunosurveillance", whereby the host immune system is thought to protect against the development of primary cancers has been resurrected after being debated for decades. Evidence in support of tumor immunosurveillance includes identification of numerous human tumor-associated or tumor-specific antigens recognized by T cells and from isolation of tumor antigen-specific T cells from metastatic lesions (Finn *et al.* 2003; Xue *et al.* 2010).

Indeed, numerous published reports have shown that vaccination of cancer patients with killed tumor cells, tumor cell lysates or tumor antigen proteins, peptides or DNA administered with cytokines or adjuvants can produce immunologic and clinical responses (Finn *et al.* 2003; Barrett and Rezvani 2007; Rezvani *et al.* 2008; Palmer *et al.* 2009).

Dendritic cells (DCs) are BM-derived antigen presenting cells (APCs) that play a critical role in the induction and regulation of immune responses. They are traditionally divided into two populations: cells with a monocytoid appearance called myeloid DCs (CD11c⁺CD123^{dim}) and cells with morphological features similar to plasma cells and thus called plasmacytoid DCs (PDCs, Cd11c⁺CD123^{bright}). PDCs, found primarily in blood and lymphoid tissues, are the principal interferon α (IFN α)-producing cells in the body and can activate antiviral anti-tumor-antigen responses (O'Neill *et al.* 2004). There is also a third subpopulation called a common DC precursor. The myeloid, lymphoid or common DC precursor differentiates along immature DC precursor states to mature DC (Ardavin *et al.* 2001).

It has been proposed that the manipulation of DCs as a "natural" adjuvant vaccine may prove to be a particularly effective way to stimulate antitumor immunity (Ardavin *et al.* 2004).

DCs can be generated *in vitro* from human CD34⁺ BM or cord blood progenitors and adult monocytes. GM-CSF, stem cell factor (SCF), FLT3 ligand and TNF- α are used in CD34⁺ cultures while GM-CSF and IL-4 are sufficient for monocyte cultures. Intriguingly, cytokines are not essential to induce DC differentiation. Agents such as phorbol esters or calcium ionophores (CIs) can also induce acquisition of DC characteristics by bypassing TNF-receptor-mediated pathways (Davis *et al.* 1998; Tong *et al.* 2008).

DCs originate from the same precursor as acute myeloid

leukemia (AML) and CML. Thus, an alternative approach to generate DCs for vaccination purposes may be the differentiation of leukemic cells in AML and CML towards DCs. Leukemia-derived DCs are thought to conserve the characteristic cytogenetic and phenotypic aberrations of malignant cells (Li *et al.* 2005). Fluorescent *in situ* hybridization (FISH) analysis proved that CML-derived DCs conserve the CML-characteristic chromosomal aberration BCR-ABL. Leukemic DCs allow not only the whole spectrum of known but also unknown leukemia antigens to be presented to the immune system (Harrison *et al.* 2001).

Leukemia-derived DCs can induce the generation of autologous cytotoxic cells capable of lysing leukemic cells *in vitro* (Cignetti *et al.* 2004; Wang *et al.* 2009). Moreover, intradermal (ID) vaccination of *in vitro* generated autologous CML-DCs in patients in late chronic phase CML may result in an anti-leukemia immune response as demonstrated by a strong delayed-type of hypersensitivity (DTH) (Ossenkoppele *et al.* 2003). This underscores that leukemia-derived DC-based immunotherapy might provide a tool to control or eradicate minimal residual disease (MRD) in AML and CML (Westers *et al.* 2006; Fujii *et al.* 2009). Our work aimed at generating a DC vaccine expressing leukemia-associated Ag differentiated from myeloid blasts to boost the immune system and improve the clinical outcome of CML patients so it can be used as an adjuvant therapy alongside their chemotherapeutic regimen.

MATERIALS AND METHODS

Subjects

This study included 22 patients recently diagnosed as having chronic CML with a t(9;22) selected from the Kasr El-Aini Teaching Hospital. They were divided into 2 groups. Group I was a pathological control group which included 13 age- and sex-matched CML patients that were not given the DC vaccine and were injected with saline. There were 5 males (38.5%) and 8 females (61.5%) whose ages ranged from 20-75 years with a mean value of 45.85 ± 14.57 years. Group II included 9 CML patients that were given the DC vaccine. There were 3 males (33.3%) and 6 females (66.7%). Their ages ranged from 32-75 years with a mean value of 47.44 ± 13.73 years. Informed and written consent was obtained from all patients, who received hydroxyl-urea as a chemotherapeutic agent.

All patients were subjected to full history taking, clinical examination, and laboratory analysis before and after vaccination. The laboratory analysis included complete blood picture (CBC), liver functions, kidney functions, lactate dehydrogenase (LDH), uric acid (UA), CD83 and CD8 assay.

Methods

Sampling

Twenty ml of venous blood were obtained from each patient by a sterile venipuncture and divided; 4 ml were placed into sterile vacutainers (Greiner Bio-One, Frickenhausen, Germany) containing preservative-free heparin (Sigma-Aldrich, St. Louis, USA) for mononuclear cell separations and cell culture and 2 ml into a sterile EDTA vacutainer for performing a complete blood picture and flow cytometric detection of CD8⁺ T cells.

Generation of dendritic cells

MNCs were separated under aseptic conditions using a Ficoll Hypaque density gradient (density 1.077, GibcoBRL, Grand Island, NY, USA) by centrifugation at 1800 rpm for 20 min and adjusted at 1×10^6 cells/ml by counting the cells using a hemocytometer (Newbauer, Germany) according to Inaba *et al.* (1992) with some modifications (including no addition of recombinant flt3-ligand to culture and no pulsing of DCs with tumor lysate). MNCs were differentiated into DCs by suspending them in liquid culture medium containing minimum essential Eagle medium with L-glutamine (EMEM/ L-G), 10% heat-inactivated fetal calf serum (FCS), penicillin (100 U/ml), streptomycin (10 mg/ml), amphoteri-

cin B and gentamycin (all from GibcoBRL) and adding the growth factors recombinant human IL4 (rhIL-4) 20 ng/ml and recombinant human GM-CSF (rhGM-CSF) 100 U/ml (R&D System, Minneapolis, MN) to the suspension in sterile tissue culture tubes (Nunc A/S, Kamstrup, Denmark) that were incubated at 37°C in 5% CO₂ for 14 days. The medium was changed every 5-7 days.

On day 13, the cultured MNCs were evaluated for morphological changes using cytopsin preparation stained with Giemsa. Cells having a large size, copious gray cytoplasm, and long cytoplasmic processes were identified as leukemic DCs. Viability of generated DCs was assessed by mixing an equal volume of MNCs and trypan blue (Merck, Darmstadt, Germany). Cells were examined using inverted phase microscopy (Leitz, Wilovert, Germany) and viability was assessed by trypan blue dye exclusion.

Activation of DCs was done on day 13 by adding 10 ng/ml TNF α (R&D System).

DCs were detected by flow cytometric analysis using monoclonal antibody (mAb) against CD83, which is a maturation marker for DCs. DCs (1×10^6 cells/100 μ l) were suspended in phosphate buffer saline (PBS, GibcoBRL) containing 1% BSA and were stained with fluorochrome-conjugated mAb (anti-mouse mAnti-CD83) (BD Biosciences, San Diego, CA) for 20 min on ice. Flow cytometric analysis was performed using FACSCaliber (BD Biosciences) equipped with CellQuest Software (BD Biosciences). 10,000 cells were passed in front of the laser for each sample. Each sample was analyzed in duplicate. A cut off value at 20% was set to categorize samples as positive (Fig. 1).

On day 14 DCs were separated by incubation with trypsin/EDTA (GibcoBRL) and then suspended in 1 ml of sterile saline and the patient was given five I.D injections on each forearm.

Follow-up by monitoring immunological response

The patients were monitored before and after injection of DCs by flow cytometric assessment of the percentage of CD8⁺ T-cells (Table 2). The DC suspension (1×10^6 cells/100 μ l) was mixed with 10 μ l fluorochrome-conjugated anti-CD8 (anti-mouse mAnti-CD8) (BD Biosciences). The mixture was incubated in the dark at 4°C for 30 min followed by washing with PBS containing 2% BSA. A non-reactive mAb of the same isotype, and conjugated with the same fluorochrome was used as a negative control. Flow cytometric analysis was performed using FACSCaliber (BD Biosciences) equipped with CellQuest Software (Fig. 2).

Statistical analysis of data

Quantitative values are expressed as the mean \pm S.D, and were compared using a Student's *t*-test. Qualitative data were compared using a χ^2 test. *P* < 0.05 was considered to be significant while *P* < 0.01 was considered to be highly significant. Pearson's correlation coefficients for the different variables were calculated. SPSS v. 12 was used.

Declaration of ethics

This study was approved by the review board of our hospital, and written informed consent was obtained from all patients according to Helsinki guidelines of research ethics.

Disclosure statement

The authors declare no conflicts of interest.

RESULTS AND DISCUSSION

Dendritic cells and their immunotherapeutic role

Philadelphia chromosome (Ph) encodes a 210-kDa (p210^{BCR-ABL}) fusion protein which is a constitutively active tyrosine kinase and probably initiates the neoplastic process (Mumprecht *et al.* 2009).

DCs are responsible for the initiation of immune responses (Dong *et al.* 2003), and are involved in mediating anti-cancer immunity, they have been the APC of choice for most cell therapy studies against cancer (Banchereau *et al.*

Table 1 Statistical comparison between group I and group II for age and sex.

Items	Group I (No= 13)	Group II (n= 9)	P- value	Significance
Age (years)				
Range	(20 – 75)	(32 – 75)		
Mean ± SD	45.85 ± 14.57	47.44 ± 13.73	0.798	NS
Sex				
Male (No; %)	(5; 38.5 %)	(3; 33.3 %)		NS
Female (No; %)	(8; 61.5 %)	(6; 66.67 %)	1.00	

Table 2 Clinical data of group I (pathological control) before and after two weeks of monitoring.

Items	Group I (n= 13)			
	Before		After	
	No.	%	No.	%
Fever				
Present	9	69.2%	8	61.5%
Absent	4	30.8%	5	38.5%
Bony aches				
Present	8	61.5%	8	61.5%
Absent	5	38.5%	5	38.5%
Bleeding				
Present	0	0%	0	0%
Absent	13	100%	13	100%
Weight loss				
Present	10	76.9%	11	84.6%
Absent	3	23.1%	2	15.4%
Anemic manifestations				
Present	13	100%	11	84.6%
Absent	0	0%	2	15.4%
Splenomegaly				
Present	13	100%	13	100%
Absent	0	0%	0	0%
Hepatomegaly				
Present	6	46.2%	7	53.8%
Absent	7	53.8%	6	46.2%
Lymphadenopathy				
Present	2	15.4%	3	23.1%
Absent	11	84.6%	10	76.9%

2001; Ilett *et al.* 2010).

They are heterogenous and can be divided into myeloid and PDCs based on their origin, expression of surface markers, and function. They are defined as leucocytes with high expression of major MHC antigens and a lack of other leucocyte lineage markers CD3 (T-cells), CD14 (monocytes), CD 19/20 (B-cells), CD56 (NK cells) and CD34 (hematopoietic progenitors) (O’Neill 2004).

Since DCs have the broadest range of antigen presentations, and CML presents a unique opportunity to develop therapeutic strategies using vaccination against a truly tumor-specific Ag that is also the oncogenic protein required for neoplasia (Pinilla-Ibarz *et al.* 2000). CML may be a good target for immunological approach, because the data *in vitro* provide the rationale for developing *bcr-abl* peptide-based vaccines (Takahashi *et al.* 2003). Moreover, many DCs in CML patients may originate from leukemic cells, and thus DCs may also express leukemia-specific antigens (Muller *et al.* 2001; Greiner and Schmitt 2008).

The purpose of immunotherapy is to overcome tolerance and boost immunity to elicit an efficient immune response against leukemia (Robin *et al.* 2005). Because DCs are clinically well tolerated, it makes them interesting candidates for anti-tumor strategies (Nestle *et al.* 2001; Janikashvili *et al.* 2010).

This study aimed at generating a DC vaccine expressing leukemia-associated Ag differentiated from myeloid blasts to boost the immune system and improve the clinical outcome of CML patients so it can be used as an adjuvant therapy parallel to chemotherapeutic regimen.

Cytokines induced differentiation of DCs

MNCs were separated under aseptic conditions using a Ficoll Hypaque density gradient, then MNCs were differentiated into DCs by suspending them in liquid culture medium containing EMEM, 10% FCS, penicillin (100 U/ml), streptomycin (10 mg/ml), amphotericin B and gentamycin and adding the growth factors rhIL-4 (20 ng/ml) and rhGM-CSF (100 U/ml) to the suspension in sterile tissue culture tubes that were incubated at 37°C in 5% CO₂ for 14 days. The medium was changed every 5-7 days. Activation of DCs was done on day 13 by adding 10 ng/ml TNF α . The cultured MNCs were evaluated for morphological changes using cytospin preparation stained with Giemsa. Cells having a large size, copious gray cytoplasm, and long cytoplasmic processes were identified as leukemic DCs (**Fig. 1**). To ensure that the DCs were mature, they were immuno-

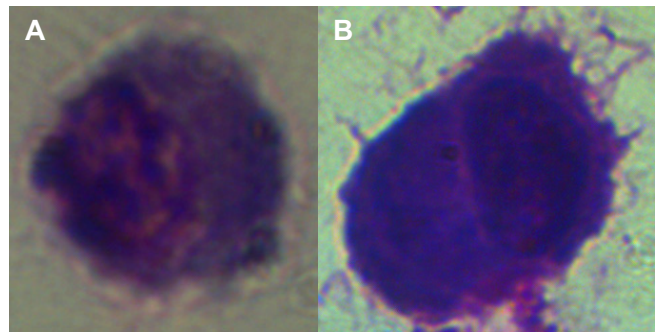


Fig. 1 Light microscopy of DCs. (A) Generation of inactivated DCs after MNCs were separated under aseptic conditions using a Ficoll Hypaque density gradient, then MNCs were differentiated into DCs by suspending them in liquid culture medium containing EMEM, 10% FCS, penicillin (100 U/ml), streptomycin (10 mg/ml), amphotericin B and gentamycin and adding the growth factors rhIL-4 (20 ng/ml) and rhGM-CSF (100 U/ml) to the suspension in sterile tissue culture tubes that were incubated at 37°C in 5% CO₂ for 14 days. The medium was changed every 5-7 days. (B) Generation of activated DCs Activation of DCs was done on day 13 by adding 10 ng/ml TNF α , the cultured MNCs were evaluated for morphological changes using cytospin preparation stained with Giemsa. Cells having a large size, copious gray cytoplasm, and long cytoplasmic processes were identified as leukemic DCs.

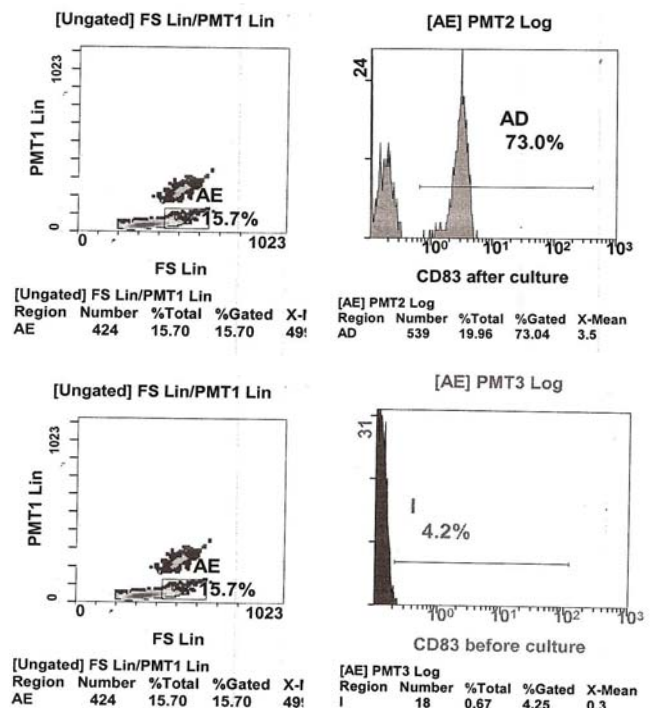


Fig. 2 CD83 expression before and after culture by flow cytometry, revealing positive expression after culture.

Table 3 Statistical comparison of laboratory data of group I (pathological control) before and after two weeks of monitoring. Values = mean ± SD.

	Group I (n= 13)		P-value	Significance
	Before	After		
Hb (g/dl)	9.38 ± 2.01	9.38 ± 2.69	0.990	NS
WBCs (mm) ³	83.02 ± 63.68	94.51 ± 88.46	0.611	NS
Plt (mm) ³	376.15 ± 272.44	360.08 ± 260.47	0.716	NS
SGOT (u/L)	38.54 ± 23.14	43.77 ± 29.36	0.397	NS
SGPT (u/L)	39.62 ± 23.36	40.46 ± 25.02	0.715	NS
Urea (mg/dL)	19.85 ± 7.30	28.74 ± 13.59	0.066	NS
Creatinine (mg/dL)	0.78 ± 0.26	0.79 ± 0.28	0.759	NS
LDH (u/L)	674.31 ± 407.46	684.46 ± 544.33	0.947	NS
Uric acid (mg/dl)	6.15 ± 2.41	6.22 ± 2.47	0.708	NS

Table 4 Clinical data of group II (vaccinated CML patients) before and after vaccination.

Patients	Group II (n= 9)			
	Before		After	
	No.	%	No.	%
Fever				
Present	6	66.7%	4	44.4%
Absent	3	33.3%	5	55.6%
Bony aches				
Present	5	55.6%	3	33.3%
Absent	4	44.4%	6	66.7%
Bleeding				
Present	0	0%	1	11.1%
Absent	9	100%	8	88.9%
Weight Loss				
Present	6	66.7%	4	44.4%
Absent	3	33.3%	5	55.6%
Anemic manifestations				
Present	9	100%	8	88.9%
Absent	0	0%	1	11.1%
Splenomegaly				
Present	9	100%	9	100%
Absent	0	0%	0	0%
Hepatomegaly				
Present	3	33.3%	3	33.3%
Absent	6	66.7%	6	66.7%
Lymphadenopathy				
Present	2	22.2%	4	44.4%
Absent	7	77.8%	5	55.6%

phenotyped by flow cytometry using monoclonal antibodies against CD 83 that was significantly increased after culture.

In our study, we used both GM-CSF and IL-4. Both GM-CSF and IL-4 are the key cytokines for DC generation (Sallusto and Lanzavecchia 1994). The majority of protocols rely on the combination of GM-CSF and IL-4 for the generation of mature DC *in vitro* (Thurner *et al.* 1999). However Chitta *et al.* (2008) preferred exclusive use of GM-CSF for the differentiation of functional DCs from its precursors. In human CML, malignant cells incubated with cytokines such as GM-CSF, IL-4 or TNF- α undergo differentiation to DC

capable of inducing tumor-specific cytotoxic T lymphocytes (CTL) (Brossart 2002; Zhang *et al.* 2006).

The mature DCs were injected intradermally in the forearms of each patient. ID injection proved to be effective and is supported by Ossenkoppele *et al.* (2003) who said that ID vaccination of *in vitro* generated autologous CML-DCs in patients in the late chronic phase CML resulted in an anti-leukemia immune response as demonstrated by a strong delayed type of hypersensitivity (DTH).

Statistical significance of clinical, laboratory and immunological data of CML patients

There were no statistically significant differences between groups I (pathological control) and II regarding age and sex prevalence ($P = 0.798$ and 1.00 respectively) (Table 1).

When comparing group I before and after monitoring for 2 weeks, there was no significant difference regarding all clinical and laboratory parameters (Table 3).

In this study, there was an improvement in fever, bone aches, weight loss, and anemic manifestations in group II (Table 4). This improvement agrees with Shabowsky *et al.* (2009) who said that DCs pulsed with tumor-associated antigens induce durable anti-tumor responses with demonstrable clinical efficacy. This also agrees with Ballestrero *et al.* (2007) who said that in terms of clinical activity, objective clinical responses have been detected in a minority of the patients. On the other hand, this improvement disagrees with Takashi *et al.* (2003) who said that no clinical response was observed after using mature monocyte-derived DCs injected intradermally. However in our study, there was no improvement in bleeding tendency and there was no change in the number of patients presenting splenomegaly and hepatomegaly before and after vaccination, which agrees with Takashi *et al.* (2003) (Table 4).

The number of patients with lymphadenopathy increased after vaccination in group II (Table 4). This may be due to migration of the DCs to the lymph nodes which caused proliferation of lymphocytes leading to their enlargement. This finding is supported by Mullins *et al.* (2003) who said that subcutaneous (S.C) or ID vaccination lead to improved DC migration to lymph nodes. Morse *et al.* (1999) further support this by stating that Indium-labeled DCs, generated from monocytes of cancer patients and injected ID, were partly cleared from the injection site and,

Table 5 Statistical comparison of laboratory data of group II (vaccinated CML patients) before and after vaccination. Values = mean ± SD.

	Group II (n= 9)		P value	significance
	Before	After		
Hb (g/dl)	10.06 ± 1.64	9.41 ± 1.73	0.045	S
WBCs (mm) ³	141.59 ± 100.51	94.47 ± 76.14	0.018	HS
Plt (mm) ³	364.33 ± 284.22	507.67 ± 342.85	0.026	S
SGOT (u/L)	25.44 ± 8.65	24.78 ± 6.63	0.766	NS
SGPT (u/L)	23.89 ± 12.32	24.11 ± 13.57	0.899	NS
Urea (mg/dL)	29.44 ± 8.06	28.67 ± 7.38	0.586	NS
Creatinine (mg/dL)	0.93 ± 0.32	0.81 ± 0.23	0.092	NS
LDH (u/L)	1666.56 ± 613.64	1124.33 ± 583.57	0.038	S
Uric acid (mg/dl)	6.79 ± 1.88	6.97 ± 2.08	0.708	NS

HS highly significant, S significant, N.S not significant

Table 6 Statistical comparison of CD83% analyzed by flow cytometry before and after culture in group II (vaccinated CML patients).

	Group II (n= 9)		P value	Significance
	Before	After		
CD 83 Median (25 th -75 th percentile)	1.00 (0.50 – 1.10)	75.00 (44.50 – 81.50)	0.008	HS

HS highly significant

Table 7 Statistical comparison of CD8% analyzed by flow cytometry before and after vaccination in group II (vaccinated CML patients).

	Group II (n= 9)		P-value	Significance
	Before	After		
CD 8 Median (25 th -75 th percentile)	12.10 (9.70-20.50)	17.00 (8.00-27.40)	0.139	NS
CD 8 Absolute No. Median (25 th -75 th percentile)	620.00 (414.80-2268.20)	681.60 (221.50-1487.40)	0.953	NS

N.S not significant

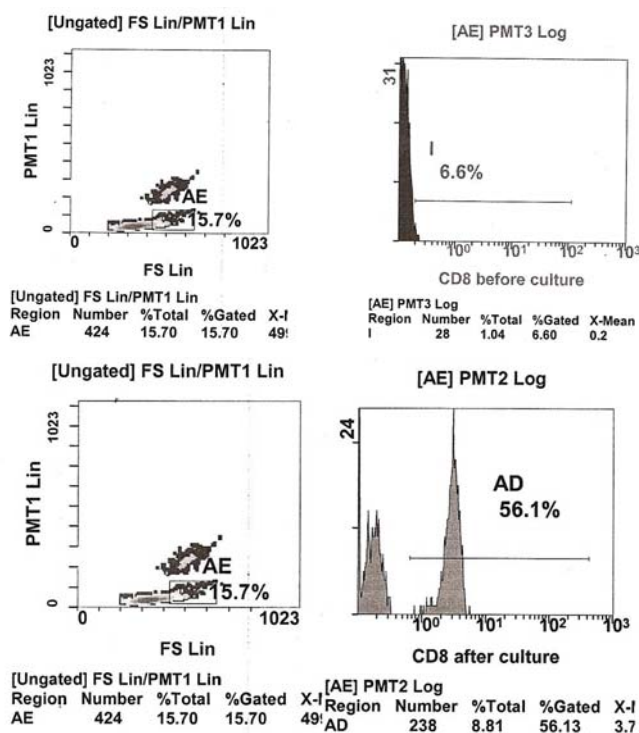
although low in number (0.1-0.4%), migrated to regional lymph nodes. De Vries *et al.* (2003) also said that most human studies have proven that subcutaneous or ID administered mature DCs are found indeed in draining lymph nodes. Morse *et al.* (1999) reported that DCs injected IV initially localized in the lungs and then redistributed to the liver, spleen, bone marrow, but apparently not to the lymph nodes, while DCs injected ID migrated to regional lymph nodes.

In our current study there was improvement in WBC and platelets count, SGOT, urea, creatinine and LDH levels in group II (Table 5). Pinalla-Ibarz *et al.* (2000) sowed a different finding in which there was no evidence of significant changes in blood cell counts, hemoglobin levels or blood chemistry caused by vaccinations in their trial.

Our study showed generation of DCs in all cases of group II (100%). DCs were detected by flow cytometric analysis before and after culture using monoclonal antibody (mAb) against CD83, which is a maturation marker for DCs (Fig. 2). CD83 expression showed a highly statistical significant difference before and after culture with an increase in its level at the end of culture ($P = 0.008$) (Table 6). This is consistent with a study done by Lanzavecchia and Sallusto (2001) in which up-regulation of co-stimulatory molecules such as CD80, CD86, and CD40 and expression of CD83 occurred upon DC maturation. Tscheop *et al.* (2003) also obtained 61% positivity of CD83 after differentiation of immature into mature DCs. Any differences in results may arise from differences in culture conditions or intrinsic variations in ability and default pathways of differentiation of leukemic cells obtained from various patients, since the ability of a given leukemia to differentiate towards DCs may be tied to the level of maturation block in the monocytoid lineage (Chaudary *et al.* 1999).

In our study, we measured CD8⁺ T cells as an indicator of an immune response to vaccination by flow cytometry. The median of CD8⁺ increased from 12.1 to 17.0% and the median of the absolute number of CD8⁺ increased from 620 to 681 lymphocytes/ μ l before and after vaccination (Table 7, Fig. 3). Although there was no significant statistical difference in the median of CD8 ($P = 0.139$) and the median of the absolute number of CD8 ($P = 0.953$), there was an increase in the actual percentages of both medians. This may indicate that DC vaccination helped in boosting the immunity of the CML patients. This agrees with Westerman *et al.* (2007) who said that *in vitro*-generated and matured CML DCs from human PBMCs were able to induce CML-specific CTL responses *in vitro* and *in vivo*. This also agrees with Mumprecht *et al.* (2009), who said that BCR-ABL-expressing DCs are able to effectively stimulate the proliferation of allogeneic and autologous T cells.

All these phase I/II clinical trials have been performed in heavily pretreated patients who were most often immunocompromised by their disease or by multiple courses of chemo- or radiotherapy. It is therefore not surprising that the immunological and even more clinical results obtained so far are not as good as expected early on. It can be speculated that we will need to optimize the source of DC, the loading/pulsing of DC and even the dose and route of vaccination first, before we can expect this immunological

**Fig. 3** CD8 expression before and after injection by flow cytometry, revealing positive expression after injection.

maneuver to become an adjuvant therapy with chemotherapeutic agents. Moreover, the choice of patients is obviously crucial in the light of different rates of immune and clinical responses reported when treating patients with comparable DC preparations in different centers (Reichardt *et al.* 2004).

CONCLUDING REMARKS

From the results obtained in our work, it can be concluded that CML is characterized by its BCR-ABL gene, which is a specific gene that one can study and develop peptide-based vaccines. GM-CSF and IL-4 provide a good medium for the differentiation of myeloid mononuclear cells into DCs and activation by TNF α and they proved to be mature by expression of CD83. ID injection of DCs proved to be an effective route of administration because it allowed DCs to migrate successfully to neighboring lymph nodes to stimulate an immune response. In our results patients injected with mature DCs showed slight clinical improvement and slight positive immune response as shown by the slight increase in both percentage and absolute number of CD8.

ACKNOWLEDGEMENTS

This study was supported in part by grants from Kasr El-Aini Teaching Hospital. We also thank El-Ansary private laboratories, our patients for their willing participation in our research, chemist

Bassant Mohamed for technical assistance and the receipt of our manuscript DBPBMB dedicated to ICAB2009 Proceedings.

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