Generation of potent T_h1 responses from patients with lymphoid malignancies after differentiation of B lymphocytes into dendritic-like cells

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Abstract

Dendritic cells (DC) are a group of potent antigen-presenting cells (APC) specialized for initiating T cell immune responses. They originate from the bone marrow and upon stimulation with bacterial products, cytokines or CD40 ligation they acquire the ability to migrate to the secondary lymphoid organs. In vitro DC can be generated from human CD34^+ bone marrow cells and CD14^+ peripheral blood monocytes after culture with different cytokine combinations. Since most leukemic cells and tumors in general are devoid of APC capacities, various strategies have been used to increase their recognition and confer the capacity of antigen presentation on them. Because of our interest in the design of vaccine immunotherapy protocols for the adjuvant treatment of patients with lymphoid malignancies (LM), we chose to explore the capacity of human acute lymphoblastic leukemia, chronic lymphocytic leukemia and plasma cell leukemia to differentiate into cells with APC and DC features. Our results among a sample of 10 patients demonstrate that such approach is feasible. Leukemic cells could be induced in the presence of IL-4 and CD40L to exhibit a DC morphology with a phenotype of mature DC-like cells. They could also induce a potent proliferative response in naive CD4^+ T cells. In addition, they expressed chemokine receptor CCR7 and CD62L, and could drive T cells towards a T_h1 response with secretion of IFN-γ. Our strategy leading to increased LM cell immunogenicity may have potential clinical applications and LM appear to be attracting candidates for adjuvant vaccination and adoptive immunotherapy.

Introduction

Dendritic cells (DC) are a system of potent antigen-presenting cells (APC) specialized for initiating primary T cell immune responses (1). DC are considered as important elements in the induction of specific antitumor immune responses. DC ultimately derive from hematopoietic precursors, although little is known about their lineage of origin. They can be generated in vitro from CD34 cord blood or bone marrow progenitors in the presence of granulocyte macrophage colony stimulating factor (GM-CSF) and tumor necrosis factor (TNF)-α, as well as from peripheral blood monocytes in the presence of GM-CSF and IL-4 (2,3).

Since most leukemic cells and tumors in general are devoid of APC capacities, various strategies have been used to increase their recognition. Transfection of co-stimulatory molecules can render non-immunogenic malignant cells immunogenic (4). An alternative approach was the fusion of malignant cells with B cells or DC (5–7). All these approaches resulted in induction of cytotoxic T lymphocyte (CTL)-mediated protective antitumor immunity. It has been previously shown that activation of follicular lymphoma cells or pre-B cell leukemias via CD40 could induce or up-regulate both adhesion and B7 co-stimulatory molecules (8,9). Moreover, using...
the CD40-stimulation strategy, autologous anti-leukemia-specific CTL could be generated in some patients with pre-B cell leukemias (10). Another promising approach would be the combination of tumoral cells themselves into DC or at least into cells with APC functions. Such cells are expected to combine both APC function and expression of tumor antigens. Recently, hematopoietic tumors appeared to be candidates for DC differentiation. Several studies, including one from our group, succeeded in in vitro DC differentiation of leukemic blasts, derived from chronic myeloid leukemia or acute myeloid leukemia (11–18). Except for the study of Cignetti et al. (16) who described the generation of DC like cells from two patients with CD34+ acute lymphoblastic leukemia (ALL), without detailed functional characterization, all previous reports concerned leukemic cells belonging to the myeloid lineage.

In the present report, we investigated whether CD19+ B lymphocytes could be induced to differentiate into dendritic-like cells (B-DC). We show that in the presence of IL-4 and CD40L, human CD19+ B lymphocytes can convert after culture in vitro into cells with mature DC features demonstrated by their typical morphology, their phenotype and their powerful capacity to induce naive CD4+ allogeneic T cell proliferation towards a Th1 response profile. Because of our interest in the design of vaccine immunotherapy protocols for the adjuvant treatment of patients with lymphoid malignancies (LM), we then chose to explore the capacity of human ALL, chronic lymphocytic leukemia (CLL) and plasma cell leukemia (PCL) to differentiate into B-DC. Our results among a sample of 10 LM demonstrate that such approach is feasible and make them potential candidates for adjuvant vaccination therapies.

Methods

Blood samples

Peripheral blood mononuclear cells (PBMC) from healthy donors (Regional transfusion center, Marseille, France) and from patients were isolated on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradients prior to cryo-preservation. Blood samples from patients with LM were obtained after informed consent at diagnosis and before any chemotherapy according to institutional guidelines. Relevant clinical and diagnostic laboratory data for all cases are shown in Table 1. All patients were diagnosed and/or treated at the Institut Paoli-Calmettes (Marseille, France) between 1993 and 2000.

Cell lines

Murine L cells transfected with human CD40L were kindly provided by Schering-Plough (Laboratory for Immunological Research, Dardilly, France) (19) and used after a 75 Gy irradiation.

Cell separation and DC generation

CD14+ monocytes were immunomagnetically purified with CD14 mAb-conjugated microbeads. (Miltenyi Biotec). Purity of the CD14+ and CD19+ cells by flow cytometry analysis was always >98%. Culture experiments were performed in RPMI 1640 medium containing 10% FCS (Biowhittaker, Verviers, Belgium) in the presence of 100 ng/ml GM-CSF (kind gift of Novartis, Bern, Switzerland) and 20 ng/ml IL-4 (kind gift of Schering-Plough Research Institute, Kenilworth, NJ) for Mo-DC generation. On day 6, final maturation of Mo-DC was induced by adding 75-Gy-irradiated CD40L-transfected L cells (2 x 10^5/well). For B-DC generation, CD19+ cells or leukemic cells which had no proliferative capacity were cultured in the presence of 20 ng/ml IL-4 and 75-Gy-irradiated CD40L-transfected cells (2 x 10^5/well). The medium was replenished with cytokines every 3 days.

T cell separation

CD4+CD45RA+ naive T cells were purified by negative selection of adult blood PBMC using goat anti-mouse Ig-coated magnetic beads (Beckman-Coulter, Marseille, France) incubated with mAb against CD8, CD14, CD56 (D. Olive, INSERM U119), CD19 (Diaclone) and CD45RO (Beckman-Coulter). Purity was >99% as controlled by FACS analysis.

Flow cytometry analysis

The following mAb (clone names) were used in this study for flow cytometry: CD1a (BL6), CD4 (13B8.2), CD5 (BL1a), CD13 (Immu103.44), CD14 (RM052), CD19 (J4.119), CD33 (D3HL60.251), CD40 (MAB89), CD54 (84H10), CD58 (AICD58), CD62L (DREG56), CD80 (MAB104), CD83 (HB15a), CD116 (S06), anti-HLA-DR (Immu-357), anti-mouse IgG1(679.1Mc7), anti-mouse IgG2a (U7.27), anti-mouse IgG2b (MOPC-195) and anti-mouse IgM (GC323) from Coulter-Immunotech (Marseille, France). CD86 (IT2.2), CD123 (9F5), CCR5 (2D7) and CCR7 (2H4) were purchased from PharMingen (San Diego, CA). All mAb were used as FITC-, phycoerythrin-, Cy5- or allophycocyanin-conjugated mAb, or with phycoerythrin-conjugated F(ab')2-fragments of goat anti-mouse IgM antibody when using CCR7 mAb. Samples were analyzed using a FACSCalibur (BD Biosciences, Le Pont de Claix, France). Data for at least 10 x 10^3 cells/sample were acquired and analyzed using CellQuest software (BD Biosciences).

FITC–dextran capture analysis

To assess endocytosis of Mo-DC and B-DC, FITC–dextran (Sigma, St Quentin Fallavier, France) was used according to the method described previously (2). Briefly, the cells were incubated with 0.1 mg/ml FITC–dextran at 37°C for 1 h. The results were analyzed as mean fluorescence intensity after subtracting the background in which cells were incubated with FITC–dextran at 4°C.

Confocal microscopy

Cells were adhered to polylysine-coated glass slides for 30 min at room temperature, fixed in 4% paraformaldehyde and permeabilized in 0.1% Triton in PBS. Cells were then labeled with different primary mAb and revealed by species-specific Alexa 488-, TRITC- (Molecular Probes, Eugene, OR) or Cy5-labeled secondary antibodies (Jackson ImmunoResearch, West Baltimore Pike, PA). Slides were then mounted using fluorescent mounting medium (Dako, Trappes, France).
Cytokine production assay

Allogeneic naive CD4+CD45RA+ T cells were co-cultured with freshly isolated monocytes, B lymphocytes, leukemic blasts, immature Mo-DC, matured Mo-DC and B-DC. Cells were harvested after 6 days, and replated in 48-well culture plates at 5 × 10^5 cells/well in the presence of phorbol myristate acetate (Sigma; 25 ng/ml) and ionomycin (Sigma; 1 µg/ml). After 48 h supernatants were harvested and frozen until analysis. Cytokines were analyzed by ELISA using the OptEIA set for IFN-γ and IL-10 (BD Biosciences).

Intracellular analysis of cytokine production

Anti-IL-4–FITC, anti-IL-10–phycocerythrin, anti-IFN-γ–allophycocyanin and FITC/phycocerythrin/allophycocyanin-conjugated isotopic mAb (PharMingen) were used according to the manufacturer’s instruction. In brief, 6 days after stimulation, T cells were activated with phorbol myristate acetate and ionomycin for 6 h in the presence of 10 µg/ml of Brefeldin A (Sigma). Cells were collected, washed, fixed and permeabilized using the CytoStain Kit (PharMingen), and stained with 0.5 µg/test of cytokine-specific mAb.

Results

In vitro generation of B-DC from B lymphocytes

Normal CD14+ peripheral blood monocytes can be induced to differentiate into immature Mo-DC by GM-CSF and IL-4. Immature Mo-DC can acquire a mature phenotype following exposure to TNF-α, CD40L or other maturation agents like lipopolysaccharides. We assessed whether CD19+ B lymphocytes can be similarly induced to differentiate into cells with DC features. CD19+ B cells were sorted by immunomagnetic selection from PBMC from different healthy donors and cultured in the presence of various concentrations of GM-CSF, IL-3, IL-4 or in combination with CD40L. A combination of IL-4 and CD40L was found to be optimal for generation of B cells with DC morphology in terms of viability, yield and phenotype. Moreover, the use of CD40L was mandatory since IL-4 alone did not induce any DC features on B cells (data not shown). Therefore, this combination was subsequently used for phenotypic and functional characterization. Before culture, freshly isolated CD19+ B cells appeared as dispersed, small spherical cells with a smooth surface morphology (data not shown). After 3–4 days of culture, B cells appeared larger and were associated in non-adherent grape-like clusters with short projections emerging from the surface. Between days 5 and 8, like maturing Mo-DC, CD19+ cells displayed significant morphological changes with size increase. In clusters or dispersed, non-adherent cells with large cell bodies and long dendritic projections were the predominant population (Fig. 1A and D).
Next, we examined the capacity of B cells differentiated in the presence of IL-4 and CD40L for endocytosis by uptake of FITC–dextran. Like mature Mo-DC, B cells with DC morphology showed very little, if any, FITC–dextran uptake (data not shown). Hence, CD19+ B cells could be induced to display in vitro morphological and endocytic characteristics similar to mature Mo-DC. Such cultured B cells with DC-like characteristics will be referred to as B-DC hereinafter.

On the phenotypic level, before culture, CD19+ cells were negative for CD1a, CD80, CD83 and CD86, negative or dimly positive for CD54 and CD58, but positive for CD40, HLA-DR and HLA class I (data not shown). After culture, we investigated the expression of CD83 which is classically found on peripheral mature DC (21). For all normal donors, a CD83 expression could be obtained in immature Mo-DC after 2 days exposure to CD40L, as well as on all CD19+ cells after 5–6 days of culture in the presence of IL-4 and CD40L (Fig. 2A and B). Acquisition of CD83 on B-DC and mature Mo-DC was further confirmed by confocal microscopy staining (Fig. 1B and E). As expected, the monocytic marker CD14 was down-regulated on all of immature and mature Mo-DC, but B cells never expressed CD14 (Fig. 2A and B). CD1a, another lineage marker of DC, was expressed on Mo-DC, but never found on B-DC. We next analyzed on the CD19+/CD83+ B-DC fraction the expression of the adhesion molecules (CD54 and CD58), MHC molecules (MHC class I and HLA-DR) and co-stimulatory molecules (CD40, CD80 and CD86). All these molecules were up-regulated (CD40, CD54, CD58, MHC class I and HLA-DR) or induced (CD80 and CD86) (Fig. 2A and B). Confocal staining of B-DC indicated as for mature Mo-DC, a large expression and peripheral distribution of HLA-DR molecules comparable to that obtained in mature Mo-DC (Fig. 1C and F). B-DC did not express the myeloid markers CD13 and CD33. Mo-DC expressed GM-CSF receptor \( \alpha \) (CD116), which was not or dimly expressed on B-DC. B-DC did not express IL-3 receptor \( \alpha \) (CD123), which is a classical marker of the so-called plasmacytoid DC (19) (Fig. 2A and B).

To investigate their allo-stimulatory function, B-DC generated after 6–8 days of culture were used to stimulate naive CD4+/CD45RA+ T cells from an unrelated normal donor at different stimulator:responder ratios. B-DC were found to be potent allogeneic MLR stimulators. At all stimulator:responder ratios, the stimulating activity of B-DC was as powerful as that of mature Mo-DC. Furthermore, in comparison with immature Mo-DC, the stimulating activity of B-DC was 2-fold higher. In the same experiments, freshly isolated peripheral blood CD19+ B cells and resting monocytes did not induce any proliferation of naive allogeneic CD4+ T cells (Fig. 3). Thus, potent and sustained proliferation of naive CD4+ T cells is another prominent feature of B-DC.

**In vitro generation of leukemic B-DC from patients with LM**

Due to the lack of ability of tumors to behave as APC, presentation of tumor antigens in vivo would proceed mainly by cross-priming with capture and processing of tumor antigens by DC (22). Thus, we investigated whether malignant B cells such as in ALL, and other LM such as CLL and PCL could also be inducible into B-DC. Leukemic cells positive for the B cell marker CD19 and negative for the monocytic marker CD14 (except for patient LM177 with a 14% population of CD14+ cells), from 10 patients with various LM (Table 1) were cultured in the presence of IL-4 and CD40L. As soon as 4–5 days of culture, cells consistently displayed an increase in cell size and showed cell clusters with dendritic morphology (Fig. 4A). Before culture, leukemic cells were CD1a, CD80, CD83 and CD86 negative, but were CD40, HLA class I and HLA-DR positive (data not shown). After culture, among the 10 cases, CD83+ cells reached a mean of 40% (SD 22%; range 5–76%). Interestingly, although malignant B cells were heterogeneous, ranging from B cell progenitors/precursors to plasma cells, a significant percentage of malignant B-DC could always be obtained. These CD83+ cells remained CD19+. CD1a expression remained negative, but we observed an important up-regulation of CD40, CD54, CD58, HLA class I and HLA-DR molecules. Also, leukemic cells acquired co-stimulatory molecules CD80 and CD86 (Fig. 5). Acquisition and distribution of CD83 and HLA-DR molecules were confirmed by confocal microscopy staining (Fig. 4B and C).
Leukemic origin of leukemia-derived B-DC

The leukemic origin of CD19+/CD83+ cells arising after culture was investigated by FISH experiments. Purified CD19+/CD83+ cells from patients LM4 and LM274 were analyzed for the persistence of the initial bcr-abl fusion gene. After culture, the percent of CD83+ cells that were positive for bcr-abl was comparable to the percent of unfractonated blasts that were positive for bcr-abl. An example from LM4 bearing the bcr-abl fusion gene is depicted in Fig. 4(D). In addition, a unique property of B lymphocytes in CLL is the presence of the CD5 molecule (23). The expression of CD5 on CLL B cells is so unique that many consider its absence to argue against a diagnosis of B cell CLL (24). Thus, the expression of CD5 (Fig. 5) on the whole culture-differentiated leukemic cells in both patients with CLL in our series (LM8 and LM283) further confirmed the leukemic origin of DC-like cells obtained by differentiation of LM cells in vitro.

Profile of chemokine receptor expression on leukemic B-DC

As a way to understand the regulation of leukemic B-DC traffic, we examined the chemokine receptor expression on their surface in comparison with immature and mature Mo-DC. Flow cytometric analysis revealed, as previously described (25), that immature Mo-DC expressed CCR5, which was down-regulated upon maturation. In contrast, mature Mo-DC were found to express CCR7, which is usually induced on mature Mo-DC (25). Normal and leukemic B-DC did not express CCR5 at any stage of their differentiation process, but could acquire the expression of CCR7. In addition, B-DC, but not Mo-DC, expressed CD62L (L-selectin), a molecule which is known to mediate adhesion and ‘rolling’ on high endothelial venules (HEV) (26) (Table 2).

CD4+ naive T cells proliferative response in the presence of leukemic B-DC

The stimulatory activity for allogeneic naive CD4+/CD45RA+ T cells in MLR assay was compared among the different normal and leukemic DC fractions as shown in Fig. 6(A). Leukemic B-DC showed a potent allo-stimulatory potential as compared to B-DC generated from healthy volunteers. The leukemic B-DC elicited higher proliferation than did the fresh/uncultured...
leukemic population. Among the six cases tested in allogeneic MLR, the proliferation induced was at least 3-fold higher than the magnitude of that induced by freshly isolated leukemic cells (Fig. 6B) irrespective of the stimulator:responder ratio in culture (data not shown). Interestingly, the least potent allo-stimulatory effect (which was still greater than that of fresh leukemic cells) was obtained with blasts from patient LM240, who had the lowest percentage of B-DC after culture (Fig. 6B). Thus, the superior stimulatory activity of cultured leukemic B cells compared with the freshly isolated blasts was established.

_Th1 polarization capacity of B-DC_

We next examined the nature of primary allogeneic T cell responses induced by normal and leukemic B-DC. Naïve CD4+ CD45RA+ T cells isolated from human peripheral blood were co-cultured for 7 days with immature Mo-DC, mature Mo-DC and B-DC generated from six patients in our series. The cultured cells were counted and re-stimulated with phorbol myristate acetate and ionomycin for either 5 h for single-cell cytokine analyses by flow cytometry (Fig. 7A) or 48 h for cytokine secretion analyses (Fig. 7B). T cells originally cultured with normal and leukemic B-DC secreted IFN-γ (Fig. 7B), but little or undetectable IL-4 and IL-10. T cells cultured with mature Mo-DC secreted the highest amounts of IFN-γ (1-fold higher) (Fig. 7B). These polarized cytokine production profiles were confirmed by single-cell

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Table 2. Chemokine receptor and CD62L expression on Mo-DC and B-DC

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<tr>
<th></th>
<th>CD62L</th>
<th>CCR5</th>
<th>CCR7</th>
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<tbody>
<tr>
<td>Immature Mo-DC</td>
<td>–</td>
<td>+ (16)</td>
<td>–</td>
</tr>
<tr>
<td>Mature Mo-DC</td>
<td>–</td>
<td>–</td>
<td>+ (77)</td>
</tr>
<tr>
<td>Normal B-DC</td>
<td>+ (63)</td>
<td>–</td>
<td>+ (69)</td>
</tr>
<tr>
<td>Leukemic B-DC</td>
<td>+ (24)</td>
<td>–</td>
<td>+ (69)</td>
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Mo-DC and B-DC were obtained as described in Methods. The expression of individual markers was monitored by flow cytometry and scored as negative or positive. The mean fluorescence intensity values are provided for a representative experiment out of five independent experiments from healthy donors and patients.
cytokine analysis using intracellular immuno¯uorescence flow cytometry (Fig. 7A). Thus, leukemic B-DC can drive naive T cells towards a Th1 response profile.

Discussion

In the present report, we have demonstrated that peripheral blood CD19+ B lymphocytes and leukemic B cells could be induced in the presence of IL-4 and CD40L to differentiate into cells with DC features. B-DC exhibited DC morphology, had a phenotype of mature APC and could induce a potent proliferative response in naive CD4+ T cells. All these features are in accordance with DC properties currently accepted for the characterization of DC subsets (27±29). In addition, B-DC expressed chemokine receptor CCR7 and CD62L, and could drive T cells towards a Th1 response with secretion of IFN-g.

These findings raise the question whether CD19+ B cells could have a B/DC bipotential lineage capacity. DC were originally thought to be only of myeloid origin. However, different observations indicated that DC might be of diverse origins (30). Recent investigations showed that early T lineage cells can differentiate into B cells, NK cells and DC (31). In this respect, Galy et al. showed that a multipotential hematopoietic stem cell can give rise to lymphocytes, NK cells and DC (32). In addition, B lymphocyte lineage cell lines can convert to macrophage-like cells spontaneously or through experimental manipulation (33). Using a murine model, CD19+ pro-B cells were shown to be able to give rise to DC in vitro (34). In a large study by Shultz et al., CD40-activated B cells were found to be efficient APC to generate autologous antigen-specific T cells (35). Moreover, in an attempt to generate a novel anti-B cell mAb, Zhong et al. isolated a B lymphocyte subset with DC morphology and efficient APC function in the first 24 h after isolation (36). On the pathological level, although malignancies involving DC are rare, some Hodgkin’s lymphomas can have DC features and phenotypic markers (37). Furthermore, some malignant B cells from non-Hodgkin lymphomas were found to be able to express functional co-stimulatory molecules (CD80 and CD86) and to be fully competent APC (38). Taken together, all these findings suggest a close relationship between DC and B lymphocytes.

Our results show that the simultaneous association of IL-4 and CD40L can induce normal and leukemic B cells towards cells with striking DC features. Although CD40L was already proved to induce proliferation and up-regulation of co-stimulatory molecules on normal and malignant B cells (20,39,40), the addition of IL-4 was mandatory to obtain the highest yields of fully functional B-DC. Furthermore, the role of IL-4 is essential since B lymphocytes triggered through their CD40 differentiate into plasma cells in response to IL-10, but not IL-4 (41). Moreover, signaling through CD40 can abrogate the tolerogenic capacity which has been sometimes associated with resting B lymphocytes and make them therefore potential adjuvants for immunotherapy. In our experiments, although we did not distinguish between peripheral blood naive and memory B cells for B-DC generation, our culture system did not direct differentiation either towards plasma cells, or secretion of Ig (data not shown), but induced morphologic, phenotypic and functional changes. These properties, including the expression of the CD83 DC-associated marker, might help to define the maturation pattern of these cells towards the DC lineage. The crucial role of co-stimulatory molecules in the generation of an antileukemic response has been shown in murine leukemia (44,45). Along with the acquisition of adhesion and co-stimulatory molecules, the immune response requires a timely interaction among
different cell types within distinct microenvironments. The migration of DC from the tumor site to the secondary lymphoid organs is believed to be one of the critical events (46). CCR7 is an important player in the mechanism by which T lymphocytes and DC enter secondary lymphoid organs through HEV. In the same manner, CD62L is known to mediate adhesion and ‘rolling’ on HEV (26). Indeed CD62L is expressed at high levels by naive T cells that reach the lymph node through HEV. In this study, leukemic B-DC expressed CCR7 and CD62L. After injection in vivo, it could therefore be hypothesized that a high proportion of these cells would be trapped in T cell areas of lymph nodes. B-DC never expressed CCR5, a chemokine receptor which favors the redirection of DC into inflammatory and tumor sites (47). This finding is in accordance with the absence of detection of an immature state in B-DC which were also devoid of dextran endocytic activity. Therefore, for optimal efficiency, one could assume that B-DC must already retain at least some leukemia-related proteins, avoiding the antigen capture step and homing directly into lymph nodes where they would be able to initiate an efficient antitumor immune response. In this respect, a critical parameter is to retain part or all of the tumoral antigens during in vitro differentiation. FISH analysis and phenotypic markers confirmed that malignant B-DC in our study still retain the same cytogenetic abnormalities and phenotypic markers expressed by freshly isolated leukemic cells. Therefore, B-DC in leukemic patients may retain at least some features of the malignant clone, like some leukemia-related proteins associated with the cytogenetic abnormality. Thus, we can assume that leukemic B-DC, while directing a Th1 response profile, will help in generating antileukemic cytokotoxic responses better than fresh tumoral cells. It has been already demonstrated that the immune balance (Th1/Th2 balance) controlled by cytokines produced by Th1 and Th2 cells plays an important role in immunoregulation, including antitumor immunity (48). Th1 and Th2 cells are cross-regulatory in vitro, and the balance of these cells in vivo determines the character of cell-mediated immune and inflammatory responses (49). The Th1 cells that produce IFN-γ have been shown to exert a powerful antitumor effect, whereas a Th2 profile may have an opposite effect, i.e. down-regulation of innate and acquired antitumor immunity (50). Our strategy leading to induced or increased LM cell immunogenicity while acquiring essential chemokine receptors for trafficking into secondary lymphoid organs may have potential clinical applications like vaccination in vivo to generate antileukemia cytokotoxic T effectors or identification of LM tumor antigens. Moreover, leukemic B-DC might be used in vitro for activating antileukemic T cells for use in an allogeneic or autologous setting. Therefore DC-like cells generated from patients with LM, while driving a potent Th1 profile, appear to be attractive candidates for adjuvant vaccination and adoptive immunotherapy.

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**Abbreviations**

- ALL: acute lymphoblastic leukemia
- APC: antigen-presenting cell
- B-DC: B lymphocyte-derived DC-like cell
- CLL: chronic lymphocytic leukemia
- CTL: cytotoxic T lymphocyte
- DC: dendritic cell
- FISH: fluorescence in situ hybridization analysis
- GM-CSF: granulocyte macrophage colony stimulating factor
- HEV: high endothelial venule
- LM: lymphoid malignancies
- Mo-DC: monocyte-derived DC
- PBMC: peripheral blood mononuclear cell
- PCL: plasma cell leukemia
- TNF: tumor necrosis factor

![Fig. 7.](image)  
**Fig. 7.** Th1-polarizing capacity of in vitro differentiated leukemic cells. Production of IFN-γ, IL-4 and IL-10 was measured by intracellular staining of CD4+ T cells stimulated with the in vitro differentiated leukemic cells (patient LM90) and B lymphocytes from a healthy donor (A). Results are representative of six experiments with different patients and healthy donors. IFN-γ content in the supernatant of co-culture of T cells with the indicated stimulating cells was measured by ELISA (B). Results are represented as the mean + SD obtained from six patients and three different healthy donors.
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