CASE REPORT

Derivation of Leukemic Plasmacytoid Dendritic Cells Coexpressing a Progenitor Cell Surface Antigen, CD117, without Interferon-α Production

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SUMMARY

CD4\textsuperscript{−}CD56\textsuperscript{−} lineage negative malignancy has recently been considered as plasmacytoid dendritic cell (PDC) leukemia/lymphoma. We investigated immunophenotypic and functional characterizations of PDC leukemic cells in one case. Lineage markers were all negative except partially positive CD11c and positive CD117, indicating malignant cells were leukemic PDCs coexpressing myeloid and progenitor cell surface antigens. Leukemic PDCs cultured with IL-3 increased in size and expression of CD11c, CD40 and HLA-DR, although the cells cultured with IL-2 or GM-CSF showed little proliferation. Furthermore, CD40 ligation after IL-3 stimulation yielded morphological changes such as expression of dendritic process. These findings showed that malignant cells were consistent with leukemic PDCs. However, secretion of interferon-α was not detected in leukemic PDCs with the stimulation of CpG ODN or inactivated herpes simplex virus-1.

Blastic NK lymphoma is included in precursor T-cell neoplasms, but described as neoplasms of uncertain lineage and stage of differentiation according to WHO classification of hematopoietic and lymphoid neoplasms. However, previous studies have revealed that blastic NK lymphoma/leukemia is identical to CD4\textsuperscript{+}CD56\textsuperscript{+} malignancies.\textsuperscript{1-5} CD4\textsuperscript{+}CD56\textsuperscript{+} malignancy is a rare neoplasm with typical clinical patterns of an aggressive course and high early relapse rate. Generally, it develops in elderly persons with some exceptions with a male predominance. Clinical presentation typically corresponds to skin lesions; cytopenia, lymphadenopathy, and/or hepatosplenomegaly.\textsuperscript{2,5,6} Blast cells are considered to be the leukemic counterpart of plasmacytoid dendritic cells (PDCs) or type 2 dendritic cells (DCs).\textsuperscript{4,6} PDCs play a major role in the human immune system. Appropriate control of PDC recruitment and activation is essential for defense against viral infection. High levels of type I interferon during this process produced by PDCs critically influence the outcome of viral infection. PDCs are responsible for the successful transition from innate to adaptive immune reactions.

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immunity for viral resistance, as well as play a central role in the nature of innate immunity. Furthermore, PDCs can differentiate into antigen presenting cells that may regulate tolerance to a given pathogen.

Although differential diagnosis of PDC malignancy may sometimes be difficult, recent studies have revealed that the specific leukemic PDC profile is CD4+, CD56+, HLA-DR+, CD116-, CD123+, CD45RA+, and CD45RO-. Such an immunophenotypic profile is useful for the diagnosis independently of the lineage profile, but the immunological properties of leukemic PDC (e.g., whether the neoplasm secretes type I interferon (IFN) are not fully elucidated. In this study, we performed a morphological, immunophenotypic and functional evaluation of a rare type of leukemic PDC coexpressing myeloid markers.

PATIENT AND METHODS

Patient

A 73-year-old female was referred to Haibara Town’s General Hospital for evaluation of anemia and skin lesions. The physical examination on admission showed purpuric or erythematous cutaneous lesions on the trunk. Splenomegaly and lymphadenopathy were both observed. Jaundice in the bulbar conjunctiva and anemia in the palpebral conjunctiva were also observed. Laboratory findings revealed anemia and thrombocytopenia: the hemoglobin was 3.7 g/dl, white blood cell count 5.1×10^9 cells/l (pathological cells 38%), and platelets 21×10^9 cells/l. Four weeks later, white blood cell count increased to be 74.8×10^9 cells/l with 98% pathological cells, and therefore leukemic transformation was indicated when written informed consent was obtained and blood samples were collected. Based on physical and laboratory findings, she was diagnosed as having PDC leukemia. This work was approved by the hospital medical ethical committee.

Isolation of tumor cells

To elucidate leukemic PDC surface antigen and cultivate the cells for further examination, whole blood was collected from the patient. Malignant cells were isolated by Ficoll-Paque (Pharmacia Biotech) density centrifugation. The number of peripheral mononuclear cells obtained was 2×10^6 cells/ml. The homogeneity of the isolated fractions of abnormal cells analyzed microscopically by their morphology was 98%. Aliquots of isolated tumor cells were stored in the serum-free freezing medium containing 10% DMSO (Banbanker®, Nippon Genetics, Tokyo, Japan), and frozen in liquid nitrogen for further investigation.

Staining tumor cells by the spinner method

For cell morphology examination, blood or cultured cell smears were prepared by the spinner methods using an automated slide preparation unit Cytospin 2 (Shandon, Pittsburgh, PA, USA), and observed under light microscopy. Cytochemical stain was performed with myeloperoxidase and esterase (Muto Pure Chemicals, Tokyo, Japan).

Analysis of cell surface molecules

The fluorescence intensity distribution was determined by incubating the cells with monoclonal antibodies against cell surface antigens (listed in Table 1), followed by incubation with a FITC-labeled second antibody (Sheep anti-Mouse Ig [H+L] FITC-conjugated F(ab')2, Chemicon, Australia) and the single-color cytometric analysis with an EPICS XL flow cytometer (Beckman Coulter). Ten-thousand events were collected. Analysis was performed using EXPO32 software. Leukemic cells were gated electronically using the FSC/SSC dot plot. The percentage of positive cells was derived from the appropriate fluorescence histogram. An isotype matched negative control was used to set acceptable negative or positive discrimination.

Proliferation assay

Purified leukemic cells were cultured at 10^6 cells/ml in the RPMI-1640 medium containing 10% fetal calf serum and antibiotics supplemented with or without IL-2 (50 μg/ml, Shionogi, Osaka, Japan), IL-3 (400 μg/ml, Genzyme Diagnostics, Cambridge, MA, USA), or GM-CSF (500 μg/ml, Genzyme Diagnostics) for 4 days. Expression of cell surface molecules was analyzed. Subsequently, in order to assess maturation with CD40 ligation, the cells cultivated with IL-3 were further cultured with an agonistic monoclonal antibody to CD40 (MAB89; Beckman Coulter) for 2 days.
**Interferon-α secretion**

Purified cells were cultured at 10⁶ cells/ml/well in the RPMI-1640 medium containing 10% fetal calf serum, 2 mM L-glutamine (Gibco BRL, Carlsbad, CA, USA), antibiotics, and 10 mM HEPES (Nacalai Tesque, Kyoto, Japan), supplemented with or without IL-3 (10 ng/ml), phosphodiester/phosphorothioate oligonucleotide containing the CpG motif (CpG ODN2216: ggGGGACGATCG-TCggeggG, 6 μg/ml, Hokkaido System Science, Sapporo, Japan), or UV-irradiated herpes simplex virus (HSV)-1 (1 x 10⁶ pfu/ml, a gift from DNAX Research Institute, Palo Alto, CA, USA). The culture supernatants were collected after 48-hour incubation. IFN-α in the supernatants was measured using a human IFN-α ELISA development kit (Endogen, Woburn, MA, USA). As normal control, HSV-1-induced IFN-α secretion of normal peripheral mononuclear cells was assayed in a similar fashion.

**RESULTS**

Expression of cell surface molecules

As shown in Table 1, leukemic PDCs were found to highly express CD4, CD45RA, CD56, HLA-DR and CD123. On the other hand, their expression of CD2, CD40, CD303 (BDCA-2) and CD304 (BDCA-4) was weak and that of CD116 was very weak. Lineage markers were all negative except partially positive CD11c and positive CD117. These results indicated that the malignant cells were leukemic PDCs coexpressing myeloid markers.

Expression of cell surface molecules cultivated with IL-2, IL-3 or GM-CSF

Fig. 1A shows the forward/side scatter plots of primary cells and IL-3 cultured cells. Results of flow cytometric analysis of unstimulated leukemic PDCs from fresh blood and those after cultivation with or without IL-3 for 4 days are shown in Fig. 1B. Increase of fluorescence intensity of cultivated cells indicated the growth in size of those cells. Furthermore, expression level of HLA-DR, CD40, CD86, CD11c and BDCA-4 was markedly elevated, but that of BDCA-2 was not. On the other hand, leukemic PDCs had little response to both IL-2 and GM-CSF stimulation, and also showed morphologically apoptotic changes in a number of cells (data not shown).

**Morphological difference between tumor cells and proliferation cells**

The population of primary PDCs was slightly heterogeneous with that of a mixture of small to large cells (Fig. 1C, left). The malignant cells showed irregular nuclear configuration and faint basophilic cytoplasm. Nucleoli were prominently observed, and the chromatin was slightly lacy. The myeloperoxidase and esterase reactions were both negative by cytochemical stain (data not shown). After IL-3 stimulation followed by CD40 ligand stimulations using agonistic monoclonal antibody (MAB89), leukemic PDCs markedly increased in size compared to the primary cells, and expression of dendritic process or spines was observed in the spinner smear under optical microscopy (Fig. 1C, right).

**Expression of interferon-α secretion**

Leukemic PDCs were not induced to produce detectable levels of IFN-α by IL-3, CpG ODN 2216, or HSV-1, though normal mononuclear cells showed HSV-1-induced IFN-α secretion (Fig. 2).

**DISCUSSION**

Leukemic cells in this study were confirmed to correspond to PDCs, but coexpressed unexpected antigens in a surface marker analysis. It is conceived that BDCA-2, BDCA-3 and BDCA-4 are the markers to distinguish subsets of dendritic cells. In our study, both BDCA-2 and BDCA-4 were expressed on primary cells, but BDCA-4 expression was further enhanced in the in vitro culture while BDCA-2 expression was not. These observations accord with a previous report describing that BDCA-2 and BDCA-4 expressions are both positive on PDCs and that expression of BDCA-4 is up-regulated on cultured PDCs although expression of BDCA-2 is completely down-regulated on PDCs once they have undergone IL-3-mediated maturation in culture.15

Although CD11c, a marker expressed by different subsets of myeloid DC, has been reported to be negative on leukemic PDCs, raw data obtained in flow cytometric analysis in the previous report has shown that there are 13.9% CD11c positive cells among primary leukemic PDCs.11 CD11c turned highly positive with IL-3 stimulation in this study,
Table 1 Surface phenotypes of purified fresh tumor cells

<table>
<thead>
<tr>
<th>Specific markers for</th>
<th>Percentage of positive cells</th>
<th>Antibody clone for flow cytometry</th>
<th>Source</th>
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<tr>
<td><strong>T or NK lymphocytes</strong></td>
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<tr>
<td>CD2</td>
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<td>MT910</td>
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BC indicates Beckman Coulter; BCI, Beckman Coulter Immunotech; CA, Caltag; BD, Beckton Dickinson; BDBIS, Becton Dickinson Biosciences Immunocytometry Systems; DC, DakoCytomation; MS, Miltenyi Biotec; NC, Nichirei; PH, Pharmingen

which was consistent with the previous report describing that CD11c was highly expressed in response to IL-3. Furthermore, in this study, leukemic PDCs expressed CD117 (c-kit) that was a transmembrane protein with receptor tyrosine kinase capacity and served as a receptor for steel factor. CD117 is expressed on almost all hematopoietic progenitor cells, and is also perceived to be negative on leukemic PDCs while positive on myeloid DCs. Two PDC leukemia cases of positive CD117 have been reported recently, but the interpretation of the positive myeloid markers was unclear yet.

PDCs have been demonstrated to be able to differentiate into mature DCs, and this maturation is characterized by an increased expression of HLA class II and costimulatory molecules such as CD80 and CD86. In our study, 4-day cultivation with IL-3 transformed leukemic PDCs into the large cells with abundant cytoplasm and vacuole. Further cul-
viation with CD40L made the leukemic cells have dendritic cell-like shape. In addition, IL-3-induced expression of HLA-DR and CD86 was further enhanced by CD40 stimulation. These findings were consistent with the behavior of leukemic PDCs as previously reported.

Recent studies have revealed that PDCs correspond to IFN producing cells (IPC) that can produce large amounts of type I-IFN by stimulating microbes or microbial antigens. Furthermore, PDCs have the ability to produce the large amount of IFN-α in response to CpG motif or HSV. Type I IFN plays an essential role in innate immunity, and is used for treating various types of malignancies as well as viral hepatitis. Much evidence supporting anti-tumor activities of type I IFN has been accumulated. These effects are likely to be due to the pleiotropic immunomodulating activity of type I IFN. Type I IFN will enhance the cytotoxic activity of NK cells and macrophages. Furthermore, type I IFN can induce T-cell activation, maintain the survival of activated T cells, induce the expression of TNF-related apoptosis-inducing ligand on T cells, and thereby enhance T-cell cytotoxicity. Therefore, PDCs play a critical role in linking innate and adaptive immune responses against malignant cells. Since reduction of IFN-α production may contribute to the escape of themselves from immune control, it is of interest whether malignant-altered PDCs still remain secretory capacity for IFN-α.

In this study, IFN-α from leukemic PDCs was not detected in the presence of inactivated HSV.
Results of our study seem to be inconsistent with those of two previous reports describing that leukemic PDCs secrete IFN-α by the stimulation of inactivated influenza virus.\textsuperscript{4,34} In an earlier report, IFN-α production by leukemic PDCs is observed, but far less than that by normal PDCs.\textsuperscript{4} The same authors report that some leukemic PDCs secrete vast amounts of IFN-α, but the production is again at very heterogeneous levels.\textsuperscript{34} In addition, a recently established...
Reduced production of IFN-α in response to CpG ODN and reduced frequencies of plasmacytoid dendritic cells were observed in peripheral blood mononuclear cells from HIV+ donors. These deficiencies were also related to levels of plasma HIV RNA. Thus, reduced production of IFN-α may contribute to diminished functional responses to a variety of stimuli such as CpG ODN in HIV disease. The drastically reduced secretion of IFN-α by leukemic PDCs would have serious consequences of the induction of immune responses against leukemia. Tumorigenic transformation of PDCs might reduce secretory capacity of normal PDC for IFN-α to a varying degree. Further investigation on secretion of IFN-α by leukemic PDCs will elucidate the pathophysiological aspects of the disease.

**REFERENCES**

12. Keller JR, Ortiz M, Ruscetti FW. Stem cell derived- factor (c-kit ligand) promotes the survival of hematopoietic


