Presentation of Tumor Antigens by Phagocytic Dendritic Cell Clusters Generated from Human CD34+ Hematopoietic Progenitor Cells: Induction of Autologous Cytotoxic T Lymphocytes against Leukemic Cells in Acute Myelogenous Leukemia Patients

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ABSTRACT

The use of antigen-presenting dendritic cells (DCs) is currently proposed for tumor immunotherapy through generation of CTLs to tumor antigens in cancer patients. In this study, DCs were differentiated using granulocyte-macrophage colony-stimulating factor and tumor necrosis factor-α from CD34⁺ hematopoietic progenitor cells that had been mobilized into the peripheral blood. To use the phagocytic activity of DCs for processing and presentation of tumor antigens, we established DC clusters containing immature DCs by preserving proliferating cell clusters without mechanical disruption. After an 11-day culture, the developed clusters contained not only typical mature DCs but also immature DCs that showed active phagocytosis of latex particles, suggesting that the clusters consisted of DCs of different maturational stages. These heterogeneous clusters could present an exogenous protein antigen, keyhole limpet hemocyanin, to both CD4⁺ and CD8⁺ T lymphocytes. Furthermore, in three acute myelogenous leukemia patients, clusters pulsed with autologous irradiated leukemic cells could also induce antileukemic CTLs. The mechanical disruption of clusters abrogated the induction of CTLs to leukemic cells as well as to hemocyanin. This observation gives an important information for the use of heterogeneous DC clusters derived from autologous peripheral blood CD34⁺ cells in the case of immunotherapy for leukemia.

INTRODUCTION

Allogeneic bone marrow transplantation in leukemia is well established. Its efficacy may result from not only high-dose chemotherapy but also an allogeneic immune mechanism, known as the GVL effect (1, 2). Currently, autologous PBSCs are used as an alternative source for hematopoietic regeneration. When autologous PBSCT was performed in leukemic patients, however, it had a high relapse rate, which is principally connected with the lack of a GVL effect and with the presence of clonogenic malignant cells in the marrow graft (1). Attempts to develop the autologous immunogenic effect on PBSCT have been made, and some of them have been found to be effective. For example, modified immunotherapies together with PBSCT, such as administration of recombinant interleukin 2 or lymphokine-activated killer cells (3, 4), and cyclosporine-induced autologous graft-versus-host disease have been successfully treated patients with lymphoma and other malignancies (5). This implies that a proper immunological treatment of leukemic patients before or after PBSCT may induce a GVL effect, making PBSCT therapy in malignancy more successful and profitable.

Induction of tumor-specific CTLs usually requires in vitro priming with tumor cells. In fact, CTL effectors can be isolated from patients with AML or chronic myelocytic leukemia (6), but the generation of such cells in vitro from naive precursors has not been reported. DCs are the most potent professional APCs. DCs in the periphery capture and process antigens, migrate to lymphoid organs, and initiate immune responses (7). Therefore, DCs preloaded with leukemia antigens might be able to induce CTLs from autologous T cells. For processing particulate antigens, such as leukemia cells, phagocytic activity of DCs may be essential. Although DCs were regarded as nonphagocytic, DCs have been recently demonstrated to phagocytose and process antigens at the immature stage in mice and rats (8–12).

Here, we established a method to generate DC clusters containing immature cells with phagocytic activity from human HPCs and investigated whether or not they could induce specific CTLs against leukemic cells. (a) We developed DC clusters from CD34⁺ cell fraction of PBSCs by GM-CSF and TNF-α (13) and tested their phagocytic activity. (b) We examined whether the developed DCs possessed antigen-presenting capability using KLH as an exogenous protein antigen (14). (c) We investigated whether specific anti-leukemic CTLs could be induced by the DCs pulsed with irradiated autologous leukemic cells during their development. Our study demonstrates that immunization with in vitro generated DC clusters, which are preloaded with leukemia antigens, can induce CTLs. This strategy provides an attractive approach to developing novel vectors for cancer therapies.

MATERIALS AND METHODS

Antigens and Reagents. KLH and ovalbumin were purchased from Sigma Chemical Co. (St. Louis, MO), and TNF-α was from Genzyme (Cambridge, MA). Recombinant human GM-CSF was kindly provided by Schering-Plough (Kenilworth, NJ). All cultures were performed in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) supplemented with 10% heat-inactivated pooled human AB serum, 2 mM L-glutamine, 100 mg/ml kanamycin, and 100 units/ml penicillin (hereafter designated as complete medium).

Source of Cells. CD34⁺ HPCs were isolated from PBSCs of recombinant human G-CSF-treated leukemia patients who were scheduled to undergo autologous stem cell transplantation after obtaining informed consent. All patients were in complete remission with stable clinical condition at the time of cell collection. After chemotherapy with Ara-C (100 mg/m² for 7 days), busulfan (4 mg/kg for 4 days), VP-16 (20 mg/kg for 2 days), and Ara-C (6 g/m² for 2 days), recombinant human G-CSF was administered at a dose of 10 μg/kg daily for 3–4 days. PBSCs were then harvested by leukapheresis using a Cobe...
Primary leukemia cells from each patient were stored in liquid nitrogen at de novo leukemia stage. In patient A [AML (M1)], leukemic cells comprised 80.8% of cells in BM with a translocation of t(9;11)(q22.23) and phenotype of CD38+, CD33+, c-kit+, HLA class I+, HLA class II+. In patient B [AML (M2)], leukemic cells comprised 61.0% of cells in BM with a normal chromosome of [46XY] and phenotypes of CD382+, CD33+, c-kit+, HLA class I+, HLA class II+. In patient C [AML (M2)], leukemic cells comprised 36.4% of cells in BM with a translocation of t(8;21) and phenotypes of CD38+, CD33+, c-kit+, CD7+, HLA class I+, HLA class II+.

Separation of CD34+ HPCs. An aliquot (3 ml) of harvested PBSCs, diluted 1:2 in RPMI 1640, was applied to Ficol-Hypeaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation. Cells obtained from interface were washed with serum-free medium and then allowed to adhere to culture dishes in complete medium for 2 h to remove monocytes. Nonadherent cells were then washed and resuspended in PBS supplemented with 1% BSA, 100 mg/ml kanamycin, and 100 units/ml penicillin (hereafter designated as isolation buffer). CD34+ cells were positively purified with anti-CD34 moAb 561-coated M-450 Dynabeads (Dynal, Oslo, Norway) by incubating 4 × 107 cells with 2.5 × 107–5 × 107 cells/ml for 60 min at 4°C. After removing rosetted cells with a magnet, and the beads were detached from the cells with DETACHaBEADs CD34 (Dynal) according to the manufacturer’s instructions. This yielded 50 ± 10% pure viable CD34+ cells.

Generation of DCs from CD34+ Cell Culture. DCs were generated from CD34+ cell populations of PBSCs (Fig. 1) in complete medium supplemented with GM-CSF (100 ng/ml) and TNF-α (2.5 ng/ml). CD34+ cells were cultured in six-well plates (Coster, Cambridge, MA) at a final concentration of 105 cells/ml for 60 min at 4°C. After removing adherent cells, was cryopreserved in liquid nitrogen. For T-cell isolation, CD34+ PBSCs were thawed and incubated with immunomagnetic beads coated with anti-CD4 or anti-CD8 moAbs (Dynabeads M-450, CD4, or CD8; Dynal) for 30 min on ice. After washing with isolation buffer, the positive cells were collected and beads were detached by incubation with CD4 or CD8 DETACHaBEADs (Dynal) at 25°C for 60 min. The purity for both CD4+ and CD8+ cells was 85 ± 5%.

For isolation of monocytes, PBSCs (2 × 107/ml) were cultured in complete medium at 37°C in Petri dishes (100 mm; Falcon, Lincoln Park, NJ). After 2 h, cells that remained adherent were dislodged by 1% trypsin-EDTA for 5 min and collected as a monocyte-enriched population, 70% of which displayed monocyte marker CD14. They were used for both antigen-presenting and target macrophages in CTL assay to KLH antigen.

Electron Microscopy. DCs were generated on a plastic sheet (Wako Pure-Chemical Ind., Tokyo, Japan) in the same culture condition as described above and harvested on day 11. After washing with PBS, cells on the plastic sheet were directly fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 18 h and postfixed in 2% osmium tetroxide for 1 h at 4°C. The cells were dehydrated through a graded series of ethanol, infiltrated in propyleneoxide/Epon mixtures, and then embedded in Epon 812. Ultrathin sections were cut and electron-stained with uranyl acetate and lead citrate and examined by a transmission electron microscope, JEM 100CX (Nihon Densi, Tokyo, Japan).

Flow Cytometric Analysis. Surface marker analysis of in vitro cultured cells was performed using a FACSscan and Lysis II software (Becton Dickenson, Mountain View, CA). We used the following FITC-conjugated moAbs to human CD1a, CD11c, CD34, and CD58 (Immunotech, Marseilles, France); CD4, CD14, HLA-DR, and CD80 (Becton Dickenson); MHC class I (DAKO, Glostrup, Denmark); MHC class II (Ancell Corporation, Bayport, MN); and CD40 and CD86 (PharMingen, Hamburg, Germany). moAbs to CD83 (Ref. 15; Dr. T. F. Tedder, Duke University, Durham, NC) and fcsin p55 (Ref. 16; Dr. E. Langhoff, Massachusetts General Hospital, Boston, MA) were kindly donated. Two-color analysis was performed by using phycoerythrin-conjugated moAbs to CD1a and CD83 (Immunotech) and fluorescein-conjugated moAbs to HLA-DR, CD14, CD40, and CD1a.

In Vitro Phagocytosis of Latex Particles. Fluospheres [carboxylate-modified microspheres which emit green fluorescence, 0.03% (v/v), 1-μm diameter; Molecular Probes, Eugene, OR] were applied to 3 × 106 DCs at day 5 culture in 200 μl of RPMI and were cultured in a chamber slide (eight chambers/slide, Lab-Tek; Nunc Inc., Naperville, IL) at 37°C in 5% CO2 for 6 days (Fig. 1). At day 11, the chambers were gently washed and adherent cells were air-dried and processed for immunostaining. Some cultures were subjected to two-color fluorescence-activated cell sorting analysis for phycoerythrin-conjugated anti-CD1a and Fluospheres.

Immunocytochemistry. Single immunostaining for cytoskears of the chamber slides was performed by the indirect immunofluoalcaline phosphate method and colored red with alkaline phosphate substrate kit I (Vector red; Vector Laboratories, Inc., Burlingame, CA), as described previously (17). Because the reaction product of Vector Red substrate emitted red fluorescence, both moAb (red) and latex (green) fluorescence could be examined simultaneously under epifluorescence microscope at a wavelength exciting FITC (490 nm). In addition, negative cells were visualized by a transillumination with a green filter.

Allogeneic Mixed Leukocyte Reaction (MLR). PBSCs (5 × 106 cells/100 μl) were used as responder cells. As allogeneic stimulator cells, up to 2 × 108 cells/100 μl of CD34+ HPC-derived DCs (Fig. 1), peripheral blood mononuclear cells, or PBSCs from healthy volunteers were irradiated with 30 Gy. Both were cocultured for 6 days in complete medium in 96-well U-bottomed culture microplates (Costar, Cambridge, MA) and 3H-thymidine (Amersham, Amer- sham, United Kingdom) incorporation was measured after 8-h pulse with 1 μCi per well. Results are shown as mean cpm of triplicates.
Fig. 2. A and B, phase contrast micrographs of cultured CD34^+ cells with GM-CSF and TNF-α at day 3 (A) and day 11 (B). In the early stage, cells are fusiform and dispersed (A), but gradually they gather to form cell clusters (B). Scale bars, 100 μm. C–F, fluorescent microfluorographs of cytosmears of CD34^+ cell cultures in which fluorescent latex was applied to this culture at day 5 and examined at day 11. After immunostaining for CD1a (C), CD54 (D), MHC class II (E), and p55 (F), areas of low cellularity were photographed. Note some CD1a^+ , CD54^+ , MHC class II^+ , and p55^+ cells (red fluorescence, small arrows) ingested latex (green). Also note the stromal cell-like cells (large arrows), which ingested latex but were negative for CD1a (C) and CD54 (D). Scale bars, 20 μm. G and H, phase contrast micrographs of T cells cocultured with the DC cluster after 1 (G) and 7 (H) days when the cluster became very large. T cells were preferentially associated with the DC cluster and expanded in a vicinity of the cluster (arrows). Scale bars, 100 μm.
Antigen Pulsing of DCs. For induction of CTLs, antigens were added to the DC culture at days 3 and 7, when DCs were still at an immature stage, and then CD4+ and/or CD8+ T lymphocytes were added at day 11 when a considerable proportion of DCs had already matured (Fig. 1). As an exogenous protein antigen, KLH, a large protein (M, 3 x 10^7-7.5 x 10^8) to which most individuals have not been sensitized, was added to DC cultures (~1 x 10^6 cells/ml) of PBSCs of two healthy volunteers. As a control, autologous macrophages (PBSC-derived monocytes) into which KLH (5 mg/ml) was directly introduced by osmotic lysis of pinosomes were used as other APCs (14). As autologous leukemic antigens, AML cells from three patients were irradiated with 70 Gy and added to the corresponding autologous DC cultures at a cell ratio of 3:1.

Generation of CTLs. Autologous CD4+ or CD8+ T cells in PBSC fraction, which showed naive phenotype of CD45RA+ (>85%) and CD45RO- (>95%), were used as responder cells. At day 11, the antigen-pulsed DC cultures were gently pipetted without disruption of the cluster, and medium containing free cells was removed. To this, a total of 4 x 10^6 purified CD4+ or CD8+ T cells in 2 ml of complete medium containing 10 units/ml interleukin 2 was added (Fig. 1). After further culture for 7 days, these in vitro primed T cells were harvested and examined for CTL activity.

CTL Assay. 51Cr-release assays were performed as described (18). Briefly, 10,000 target cells were labeled with Na251CrO4 (100 μCi/2 x 10^6 cells) and were cultured with effector cells at various E:T ratios in triplicate in U-bottomed microtiter wells for 4 h at 37°C. As targets for KLH-specific CTLs, macrophages (PBSC-derived monocytes) were activated by preculture with macrophage colony-stimulating factor for 16 h and then pulsed with KLH (5 mg/ml) or ovalbumin (5 mg/ml) for another 16 h. Unpulsed macrophages with or without addition of free KLH (5 mg/ml) during CTL assay were also used. For antileukemic CTLs, autologous AML cells were treated as targets in a similar manner as above. The spontaneous release never exceeded 20%.

Blocking Experiment. To investigate CD8 and MHC class I or CD4 and MHC class II restriction in the recognition of the target cells, blocking studies were performed (14). Anti-CD4 or anti-CD8 mAbs (Becton Dickinson) were added at the final concentration of 20 μg/ml to effector cells for 30 min before coculture with target cells and to the coculture for the 51Cr release assay. Either anti-MHC class I (Becton Dickinson) or anti-class II (Ancell Corporation, Bayport, MN) mAbs were added to target cells in a same manner as above.

RESULTS

Characterization of DCs Generated from CD34+ HPCs of PBSC Fraction in the Presence of GM-CSF and TNF-α. When CD34+ HPCs were cultured with GM-CSF plus TNF-α for 11 days, the total number of cells increased ~20-fold, ranging from 10- to 50-fold (10 independent cell cultures). The extent of cell growth strongly correlated with the degree of purity of the starting CD34+ cell population. Culture of CD34+ HPCs produced large cells with polygonal, fusiform, or dendritic shape, which formed cell clusters by day 11 (Figs. 2, A and B, and 3). As shown by a transmission electron microscopy (Fig. 3), cells in the cluster generally have an irregular euchromatic nucleus and a cytoplasm with extremely long ruffled projections. However, no distinct junctional specializations have as yet been noted at the contact points through these projections in these cell clusters. Mitotic cells were often observed within the clusters, and the number and size of the clusters increased with time.

Because cell clusters could be dispersed by pipetting with relative ease, isolated cells were analyzed by flow cytometry (Fig. 4). Developed cells contained different proportions of cells positive for DC markers, namely, CD1a, CD4, CD54, CD86, and HLA-DR (MHC Class II). Although some macrophages (CD14+) were present as well, very few cells displayed cell surface markers of HPCs (CD34), B cells (CD19), T cells (CD3, CD8), or natural killer cells (CD56). In this experiment, 1.5 x 10^6 ± 0.2 x 10^6 CD1a+ DCs were derived from a starting cell number of 2 x 10^5 CD34+ HPCs. By double-color flow cytometric analyses using CD1a as a reference molecule (Fig. 5), it was found that 16–40% of CD1a- cells coexpressed HLA-DR, CD40, and CD83, indicating that these clustering cells contained typical mature DCs.

When the phagocytic activity of the DC clusters was examined, ~25% of the cells showed active ingestion of latex particles. By immunostaining, phagocytic cells were found to be mostly CD1a- or CD1a+ but CD11c+, CD54+, MHC class II+/− or MHC class II+, and fascin+ (Fig. 2, C–F). FACS analysis showed that 25.9 ± 2.5% of cells in the DC clusters were actively phagocytic and 19.9 ± 3.2% of CD1a+ cells...
ingested latex particles. These results indicated that phagocytic cells corresponded to the relatively immature DC population. The clusters seemed to grow upon a sheet of the flat cells spreading on the plastic substratum, like stromal cells in bone marrow cell cultures (Fig. 3). They ingested a small amount of latex particles that accumulated in the cytocenter (Fig. 2, C and D). By immunostaining, they were negative or very weakly positive for CD1a (Fig. 2C), CD4, CD11c, CD14, CD54 (Fig. 2D), or CD80. We assume that these stromal cell-like cells do not belong to either lymphoid, macrophage, or DC lineages. These results revealed that cells forming the clusters were heterogeneous and mainly consisted of DCs in different maturational stages.

Capacity of the Cultured DCs to Stimulate the MLR. As shown in Fig. 6, CD34+ PBSC-derived cells were potent stimulators against resting T lymphocytes in primary allogeneic MLR, being more potent than the allogeneic mononuclear cells or allogeneic PBSCs. These cells therefore have functional as well as phenotypic characteristics of DC.

Induction of CTLs by DCs Pulsed with a Complex Protein Antigen in Vitro. DCs but not macrophages pulsed with KLH could induce CD4+ or CD8+ T cells that efficiently lysed target KLH-pulsed macrophages (Fig. 7a). Nonpulsed DCs did not induce specific CTL activity (<5% at an E:T ratio of 50:1). Supernatants from cocultures of KLH-pulsed DC and T cells were not specifically

Fig. 4. Surface phenotype of cells in clusters generated from CD34+ cell cultures at day 11. They contained different proportions of cells positive for DC markers, namely, CD1a, CD4, CD54, CD86, and HLA-DR. Although some macrophages (CD14+) were present as well, very few cells had markers for HPCs (CD34), B cells (CD19), T cells (CD3, CD8), or natural killer cells (CD56).
cytotoxic (<5% at an E:T ratio of 50:1) indicating that target cells were directly lysed by activated T cells but not by soluble factors (data not shown). T cells activated by KLH-pulsed DCs could lyse KLH-pulsed macrophages but not unpulsed macrophages regardless of the presence or absence of soluble KLH during the CTL assays (Fig. 7b). In addition, CD8^+ and CD4^+ (partly) T cells activated by KLH-pulsed DCs could lyse KLH-pulsed macrophages but not macrophages pulsed with a different antigen, ovalbumin (Fig. 7c). Cytolysis was significantly blocked by anti-MHC class II moAb and partly by anti-CD4, anti-CD8, and anti-MHC class I (Fig. 7d). These results imply that KLH-pulsed DCs have induced specific CTLs that can lyse target cells via cognate interaction.

**Induction of Antileukemic CTLs by DCs Pulsed with Irradiated Autologous Leukemic Cells in Vitro.** On the basis of the above findings, we finally tried to induce specific CTLs against autologous leukemic cells using DCs in three patients with AML. By coculturing with antigen-pulsed DCs for 7 days, autologous CD4^+ and CD8^+ cells proliferated significantly. They expanded preferentially in the vicinity of DC clusters (Fig. 2, G and H). As the number of DCs per well increased, proliferative responses of T lymphocytes were elevated. When harvested at day 18, sensitized T lymphocytes lysed autologous leukemic cells from all three patients (Fig. 8, a–c). In patient A, the cytotoxicity of sensitized T cells was specific for autologous leukemic cell (percentage cytotoxicity, 62.88 ± 8.25% at 50:1 E:T ratio), with no effect on allogeneic leukemic cell (percentage cytotoxicity, 4.53 ± 1.25% at 50:1 E:T ratio) or EBV-transformed lymphoblastoid cell lines (percentage cytotoxicity, 1.33 ± 1.11% at 50:1 E:T ratio; Fig. 8d). The results imply that DCs developed from CD34^+ PBSCs possessed the capacity to present leukemia antigens to autologous T cells and to induce specific CTLs.

**Induction of CTLs Is Dependent upon Cluster Formation by DCs.** When the DC cluster formation was interrupted by frequent pipetting, specific CTL induction to KLH (Fig. 9) or to leukemia cells (Fig. 8d) was greatly retarded.

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**Fig. 5. Double-color flow cytometric analyses of CD1a^+ cells generated from CD34^+ cell culture at day 11. Sixteen to 40% of CD1a^+ cells coexpressed HLA-DR, CD40, and CD83.**

**Fig. 6. Immunostimulatory capacity of developed DCs in a primary allogeneic MLR.** Note an intense alloantigen-presenting capacity of DCs (■) compared to PBSCs (○) or mononuclear cells (□).
DISCUSSION

Here, we could generate DC clusters containing phagocytic cells from CD34^+ cell fraction of human PBSCs. The developed cells have been proven to be of DC lineage by their characteristic phenotype and strong stimulating capacity in primary allogeneic MLR (19). Although PBSC fraction-derived CD34^+ HPCs were relatively less pure after positive selection and expanded to a lesser degree than bone marrow-derived HPCs, PBSC-derived HPCs produced more DCs, suggesting that they contained more committed DC precursor cells (20). Currently, many laboratories develop DCs from peripheral blood CD14^+ cells. However, it is not easy to obtain large numbers of DCs enough for immunotherapy by this method because generated DCs usually do not expand and usually do not survive for long time (21). Therefore, we suggest that PBSCs collected through leukapheresis after G-CSF treatment are an efficient and economical source for developing a large number of viable DCs from CD34^+ HPCs.

Phagocytic DCs in the DC clusters showed a relatively immature phenotype. Immature DCs can take up antigens during a definite period (8, 9), and in some conditions, they can avidly internalize large particulates including microorganisms (10–12) and apoptotic cells (22). For DCs to obtain antigen-presenting ability, two sequential events may be necessary: (a) capturing and processing of particulate antigens by immature DCs together with active biosynthesis of abundant MHC class II molecules and invariant chain (23, 24) and numerous acidic endocytic vacuoles (25); and (b) antigen presentation by mature DCs through enhanced accessory or immunostimulatory functions, namely, adjuvant effects (7). An example of this is that seen in the handling of soluble proteins (23, 24) and particles (12) by epidermal Langerhans cells. In fact, isolated DCs from human peripheral blood, which mainly consisted of DCs without phagocytic activity (26), could not induce CTLs to KLH unless antigen was directly introduced by osmotic lysis (14). Therefore, phagocytic DC may be critical in our study for CTL induction to leukemic cells as well as protein antigens, possibly through phagocytosing them before processing.

The DC clusters could present an exogenous protein antigen, KLH, to not only CD4^+ but also CD8^+ T lymphocytes, which, in turn, acquire the ability to proliferate and kill the target KLH-pulsed macrophages through antigen-specific cognate interactions. It was generally believed that exogenous antigens were handled by endocytic pathway, loaded on MHC class II molecules and not on class I molecules of APCs, and were recognized only by CD8^+ T lymphocytes (27, 28).
However, when exogenous antigens are internalized into phagosomes of APCs, they can be loaded on MHC class I molecules (29, 30), thus stimulating CD8\(^+\) T cells as well. Therefore, we suggest that phagocytic activity of the DC clusters may be essential for processing exogenous antigens for binding to MHC class I as well as class II molecules by which both CD8\(^+\) and CD4\(^+\) CTLs can be generated. Because immunogenic peptides loaded on MHC molecules are long lived on the surface of DCs, compared to those on macrophages and B cells, T cells may be stimulated by DCs even if they are added 4 days after antigen pulse to the DC culture.

We could induce autologous antileukemic CTLs in three AML patients using the DC clusters pulsed with irradiated leukemic cells. It may be important that leukemic cells receive irradiation, which leads cells to commit apoptosis because DCs are suggested to induce CTLs by acquiring antigens from apoptotic cells but not necrotic cells (22). Once pulsed during immature phase, mature DCs may be able to activate both CD8\(^+\) and CD4\(^+\) T lymphocytes against tumor cells in a similar fashion as KLH antigens. Although most of other laboratories use leukemia-derived peptides as antigens, it is not always easy to obtain antigenic peptides from different types of leukemia patients. This study should overcome this problem because our DC clusters can be pulsed with a whole leukemia cell body. Similarly, we could induce allogeneic CTLs to a leukemia cell line using cord blood CD34\(^+\) cell-derived DCs in a preliminary study (18). Therefore, we suggest that manipulation of the immunogenic potential of PBSCs to produce antileukemic effect in autologous PBSCT (1, 3) is quite feasible.

The DC clusters contained DCs of different maturational stages because we did not enrich mature DCs. Because the mechanical disruption of the clusters abrogated CTL induction to hemocyanin or leukemic cells, these DC clusters seem to be the most essential for developing T-cell responses against target cells. The stromal cell-like cells forming the foundation of the clusters may efficiently support development of DC for antigen uptake and presentation. In this respect, putative endothelial cell progenitors were recently isolated from adult human peripheral blood CD34\(^+\) cells (31), and fibroblast-like cells with endothelial phenotype derived from CD34\(^+\) cells of umbilical cord blood are reported to support hematopoiesis (32). Similarly, it seems to be essential for T cells to make cell clusters (rosettes) with DC for effective induction of committed CD4\(^+\) and CD8\(^+\) T cells. DCs form rosettes with antigen-specific T cells, creating a microenvironment in which immunity can develop (33, 34).

In conclusion, this study has demonstrated the successful induction of antileukemic CTLs in vitro using an autologous DC culture. We
suggested that, to develop the phagocytic DC clusters from CD34+ cells may be very critical for efficient CTL induction, not only in vitro but also in vivo, in case of the leukemia immunotherapy together with autologous PBSCT. The study on the interrelationship between DC and T lymphocytes will become more and more important for active immunotherapy in vivo.

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