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Generation of functional dendritic cells for potential use in the treatment of acute lymphoblastic leukemia

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Abstract Immunotherapy of malignant diseases mediated by dendritic cells (DC) pulsed with tumor antigens ex vivo is a promising new tool in the individual treatment of malignant diseases. The present study focuses on the problem of how to optimize in vitro culture conditions and induce the maturation of DC with the capacity to induce antitumor immunity toward leukemic cells. DC were generated from peripheral mononuclear cells by co-cultivation with granulocyte/macrophage–colony stimulating factor (GM-CSF) and interleukin-4 (IL-4). Tumor antigens were added for 2 h after 7 days in culture. Irradiated leukemic blasts, blast lysate, apoptotic cells from the Jurkat cell line (T ALL) and their lysate were used in various concentrations for antigen pulsing. Harvested DC were phenotyped by flow cytometry, and viability was assessed using trypan blue exclusion (Annexin test). After the cells had been pulsed with tumor antigens and co-cultured with autologous lymphocytes, the production of interferon-gamma (IFN-γ) and IL-12 was analyzed, and lymphocyte proliferative response and cytotoxicity against the target tumor cell line were assessed. The cultivation of monocytes under the described conditions led to the expression of surface markers typical of DC (i.e. CD83, CD86, HLA-DR, CD11c and CD40). Pulsation by antigens from leukemic cells further increased the cell populations expressing these markers. Antigen pulsation decreased the viability of generated DC depending on the increase in concentration of tumor antigens. Pulsed DC–lymphocyte interaction increased the proliferative response of lymphocytes and IFN-γ production depending on the type of tumor antigens used for pulsation. The highest proliferative response was detected with DC pulsed with Jurkat cell-line lysate. Similarly to the proliferation assay, cytotoxic testing showed the highest efficiency of DC pulsed with Jurkat cell-line lysate in killing the target malignant cells. Our results show that an appropriate antigen concentration used for DC pulsing is one of the crucial factors in an effective treatment strategy, as high concentrations of tumor antigens induce apoptosis of DC, thereby rendering them non-functional. Under optimal conditions, pulsation by lysate from leukemic blasts induced the maturation of DC and led to an increase in the proliferation of autologous lymphocytes, to the production of Th1 cytokines and to the induction of cytotoxicity toward the leukemic cell line. These results are encouraging for the possible application of pulsed DC in the therapy of acute lymphoblastic leukemia.

Keywords Dendritic cell · Immunotherapy · Jurkat cell line · Tumor antigen pulsation

Introduction

Hematological malignacies, especially acute lymphoblastic leukemia (ALL), represent the most common group of cancers in childhood. Although 75% of cases are now curable, 25% are still resistant to conventional chemotherapy. In the latter group, hematopoietic stem cell transplantation (HSCT) is the only and final therapeutic possibility. The complications associated with HSCT treatment, including the risk of relapse, have led to a search for new therapeutic strategies. In the last few years, cancer immunotherapy has therefore focused on the possible application of DC.
ALL immunotherapy during the phase of minimal residual disease (MRD) seems to be a possible and promising approach. The current methods used for the detection of MRD can be used to select a subset of patients with a very high risk of relapse.

DC are a heterogeneous population of antigen-presenting cells (APC) that play a central role in the induction of the primary immune reaction to specific antigens. The major problem of antitumor immunity is that, for various reasons, the immune system does not recognize the invasive tumor cells and therefore tolerates them. It has recently been found that this state of tolerance could be broken and immune reactivity against tumor antigens re-established in vitro if DC are cultured in a suitable culture environment with tumor antigens (antigen pulsing) [3, 4, 12]. The currently used methods for DC generation from peripheral blood monocytes have assisted in the design of a promising immunotherapeutic approach leading to the preparation of an individual cell vaccine. A number of clinical experiments investigating this kind of therapeutic approach in different malignant diseases have already been reported [9, 13, 17]. Nevertheless, a number of major methodological questions still remain unanswered. The main obstacles to the rapid introduction of this therapy in clinical practice involve two major areas. The first concerns the whole process of in vitro generation and pulsation of DC. In this respect, the major problem is that connected with the culture methodology for the generation of DC, including the type and concentration of tumor antigens used for pulsing. The second concerns preclinical trials and further clinical application. The timing of immunotherapy, the amount of generated DC required for administration, the interval between administration and verification of the effect of therapy in vivo are the most important issues that must be addressed in clinical trials.

Our study focused on an in vitro methodology for the generation of DC pulsed with tumor antigens from leukemic cells. In a series of experiments we determined the optimal conditions for induction of the immune response directed against leukemic cells. We described the experimental design and the different culture conditions for the pulsation of DC by tumor antigens, and defined under which culture conditions generated DC showed a mature phenotype, and which form and concentration of tumor antigens were suitable for DC pulsing in order to induce lymphocyte proliferation and cytotoxicity against tumor cells. The method we have proposed could constitute a starting point for the preparation of an individual cell vaccine for ALL patients.

Materials and methods

Generation of DC

Dendritic cells were obtained with informed consent from healthy volunteers and generated from a peripheral mononuclear fraction using Ficoll-Paque density centrifugation. PBMCs were washed and then plated in 24-well culture plates (Nunc) at a concentration of $1.5 \times 10^6$ ml in RPMI 1640 containing 10% fetal calf serum (FCS), 1% penicillin (PNC) and 2 mM l-glutamine. The cells were incubated in 5% CO$_2$ at 37°C for 24 h. The non-adherent cells were gently resuspended and removed. The adherent fraction containing monocytes/macrophages (mo/mf) was cultured in complete medium supplemented with 100 ng/ml granulocyte/macrophage-colony stimulating factor (GM-CSF) and 50 ng/ml interleukin-4 (IL-4). After 3–4 days of culture, the media including the supplements were replaced.

Study design

The total number of DC was counted after 7 days in culture using light microscopy, viability was assessed using trypan blue exclusion, and cell surface analysis was carried out by flow cytometry. DC were washed, pulsed for 2 h with tumor antigen with subsequent repeated phenotypic cell surface analysis. Pulsed DC were added to lymphocytes at varying ratios in triplicate. Co-incubation of pulsed DC with lymphocytes was continued for an additional 7 days and then cell viability was assessed using the Annexin test. Proliferative activity of the autologous lymphocyte, IFN-γ and IL-12 production were assessed, and cytotoxicity assays were performed.

Phenotypic analysis

For cell surface marker analysis, the DC were washed twice in phosphate-buffered saline (PBS) with 5 mM ethylene diamine tetra-acetic acid (EDTA) and then incubated with mouse monoclonal antibodies conjugated with either isothiocyanate (FITC) or phycoerythrin (PE): CD14, CD3, HLA-DR, CD40, CD11c, CD80, CD56 and CD83 for 20 min at room temperature. Mouse immunoglobulin-1 (IgG1) FITC/IgG2 PE were used as isotypic control. After washing in PBS, the cells were analyzed using a FACSCalibur flow cytometer and CellQuest software (Becton Dickinson, Mountain View, Calif.). Typically, 5,000–10,000 events were collected, and cells that displayed a typical DC scatter were gated and analyzed.

Cell viability

The viability of generated DC was assessed using trypan blue exclusion under light microscopy. The viability of pulsed DC cultured with lymphocytes was assessed after 7 days in culture using the Annexin test (Annexin V–FITC and propidium iodide; Immunotech). Cells were analyzed using FACSCalibur and CellQuest software.

Generation of tumor antigens

Leukemic blasts from ALL patients were obtained after informed consent from the remaining diagnostic bone marrow sample, and Jurkat T cell-line blasts (T-ALL; Institute of Hematology and Blood Transfusion, Prague) were used as a source of tumor antigens. DC were pulsed with irradiated leukemic blasts (25–30 Gy) or their lysate. The Jurkat cell line was used as lysate or apoptotic cells after the induction of apoptosis, as detailed below.

For the tumor lysate, Jurkat cells were washed, resuspended in RPMI medium at a concentration of $1 \times 10^7$/ml, and lysed by three freeze ($-70^\circ$C)–thaw (room temperature) cycles. The same method was used for the generation of tumor antigens from leukemic blasts after they had been isolated on Ficoll-Paque gradient, washed and diluted to a concentration of $1 \times 10^7$/ml.

For the apoptotic Jurkat cell line and the induction of apoptosis, the cells were cultured in RPMI 1640 with 10% FCS and 5 mM sodium butyrate in culture flasks for 3 days at a concentration of $1 \times 10^7$/ml. Apoptotic cells floating on the surface were repeatedly removed from the supernatant every 24 h and stored at...