Polyclonal Rabbit Antithymocyte Globulin Induces Apoptosis and Has Cytotoxic Effects on Human Leukemic Cells

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Abstract

The possibility of antileukemic activity of antithymocyte globulin (ATG) was investigated in 8 human leukemic cell lines and primary leukemic cells from 15 leukemia patients. The study demonstrated that ATG induced apoptosis and reduced proliferation in both cell lines and primary leukemic cells, particularly in lymphatic origin cells, indicating that ATG has broad-spectrum antileukemic activity, especially for cells of lymphatic origin.

Background: Polyclonal ATGs are currently used to prevent graft-versus-host disease in allogeneic stem cell transplantation patients and to treat patients with severe aplastic anemia. It contains antibodies against antigens expressed on various hematopoietic cells, we hypothesized that it induces cell death not only in healthy cells but also in malignant hematopoietic cells. **Materials and Methods:** In this study, several human leukemic cell lines and primary leukemic cells from 15 patients with leukemia were used to investigate the ability of polyclonal ATGs to induce apoptosis and proliferation. **Results:** Polyclonal ATGs induced cell apoptosis in primary leukemic cells and in cell lines in a dose-dependent manner, and induced apoptosis in different populations through a variety of targets. Cell proliferation was significantly reduced in the presence of polyclonal ATGs; it arrested cells in the G0–G1 phase by cell cycle analysis. Treatment with polyclonal ATGs plus complement increased cytolysis of the leukemic cells; complement augments polyclonal ATG-induced leukemic cell death. **Conclusion:** These data show that polyclonal ATG has broad-spectrum antileukemic activity, especially for cells of lymphatic origin, as it induced cell death through a variety of targets. This study provides an experimental basis for the application of polyclonal ATGs in allogeneic hematopoietic stem cell transplantation and in patients with lymphatic leukemia.

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Introduction

Polyclonal antithymocyte globulin (ATG) is derived from the serum of rabbits inoculated with human thymocytes.[1-3] ATG contains a variety of antibodies that are specific for cell surface proteins, including CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD25, CD28, and human leukocyte antigen-class II.[4-6] ATG is generally used as an immunosuppressive agent for patients with severe aplastic anemia or organ transplantation. ATG-mediated depletion of lymphocytes may be related to some of these antigens. Leukemic cells share some antigens with normal lymphocytes and leukocytes. In this study, the effects of ATG on the proliferation and apoptosis of leukemia cell lines and primary leukemia cells obtained from 15 newly diagnosed leukemia patients were investigated. The results showed that ATG inhibits proliferation and induces apoptosis in leukemic cells. To explore the underlying mechanisms, cell cycle and apoptotic pathway analyses were conducted.

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Materials and Methods

Cell Culture and Treatments

Leukemic cell lines were maintained in RPMI-1640 supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, and 100 mg/mL streptomycin (Gibco) in a humidified atmosphere containing 5% CO_2 at 37°C. The cells were treated with rabbit ATG (Thymoglobulin; Genzyme) at doses ranging from 50 to 250

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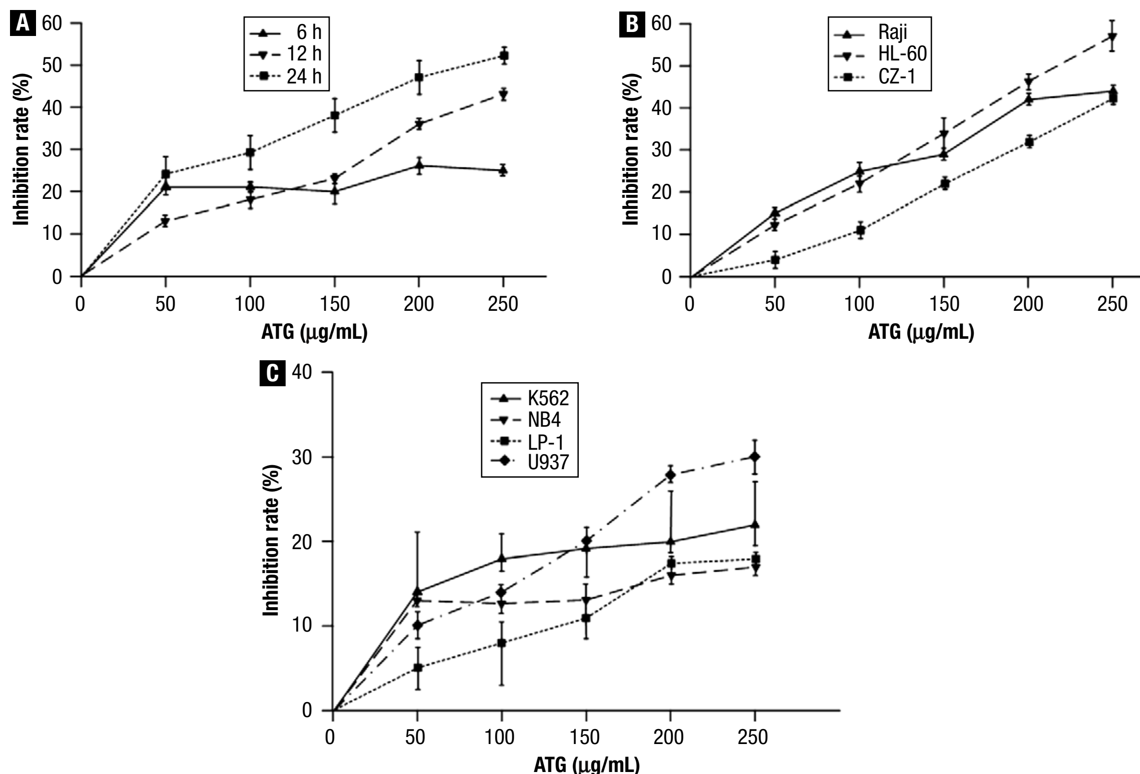
Figure 1 The Proliferation of Leukemia Cell Lines and Myeloma Cell Lines Was Inhibited in a Dose-Dependent Manner After Treatment With ATG. (A) The Inhibition of Proliferation of Jurkat Cells were Assessed by WST-8 Dye After 6, 12, or 24 Hours of Incubation With Antithymocyte Globulin (ATG). After 24 Hours of Incubation With ATG, the Inhibition of (B) HL-60, Raji, and CZ-1 Cells, and (C) K562, U937, NB4, and LP-1 Cells were Shown



μg/mL. The concentration of ATG was determined based on the blood levels observed in patients with aplastic anemia during ATG therapy.[7] Normal rabbit immunoglobulin G (rIgG) (Santa Cruz Biotechnology) was used as a control. Except for the CZ-1 (myeloma) cell line, which was a gift from Dr Hou at the Changzheng Hospital, Shanghai,[8] all cell lines, including Jurkat (T lymphoma), Raji (B lymphoma), HL-60 (myeloid leukemia), U937 (myeloid leukemia), K562 (myeloid leukemia), NB4 (myeloid leukemia), and LP-1 (myeloma), were purchased from the Shanghai Cell Institute, Chinese Academy of Sciences. Primary leukemic cells from heparinized bone marrow samples were obtained from 15 leukemia patients, who had signed informed consent forms for this study. The primary leukemic cells were isolated by Ficoll-Hypaque density centrifugation. All samples contained more than 90% leukemic cells as determined by evaluation of morphology and cell viability before experimentation. Cell viability was determined by trypan blue exclusion.

Cell Proliferation Assay

Cell proliferation was determined using WST-8 dye (Beyotime Inst Biotech, Shanghai, China) according to the manufacturer's instructions. Briefly, 1.5×10^4 cells per well were seeded in a 96-well flat-bottomed plate. The cells were treated with ATG and rIgG as negative control at increasing concentrations in the presence of 10% fetal bovine serum, and then cells were kept in a humidified atmosphere containing 5% CO_2 at 37°C for 6, 12, or 24 hours. A total of 10 μL of WST-8 dye was added to each well. The cells were incubated at 37°C for another 2 hours, and the absorbance was finally determined at a wavelength of 450 nm using an MRX microplate reader (Themo electron corporation). Proliferative inhibition was defined by the following equation:

Growth inhibition ratio (% control) = (1−100× [absorbance treated sample]/[absorbance of cells incubated with the normal rabbit IgG]).

Apoptosis Assay

To determine the apoptosis rate of the cell lines and primary leukemic cells after treatment with ATG, we used the AlexaFluor 488 annexin V/propidium iodide (PI) apoptosis detection kit (Invitrogen). Briefly, the cells were incubated with different concentrations of ATG (50, 150, or 250 μg/mL) in RPMI-1640 medium. Normal rabbit IgG was used as a control. The cells were harvested and washed in phosphate-buffered saline (PBS) after 24 hours. The washed cells were recentrifuged and resuspended with annexin V binding buffer. Annexin V and PI staining solutions were added for 15 minutes at

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