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Antithymocyte Globulin at Clinically Relevant Concentrations Kills Leukemic Blasts

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A B S T R A C T

In contrast to cyclosporine or methotrexate, rabbit antithymocyte globulin (ATG) used for graft-versus-host disease (GVHD) prophylaxis with myeloablative conditioning does not increase the risk of relapse after hematopoietic cell transplantation. The reason for this is unknown. We hypothesized that ATG at concentrations achieved with our standard ATG dose of 4.5 mg/kg exerts antileukemic activity. We measured ATG-induced killing of leukemic blasts via complement-dependent cytotoxicity (CDC) and via complement-independent cytotoxicity (CIC) in marrow or blood from 36 patients with newly diagnosed acute leukemia. The median percentage of blasts killed by CDC was 0.3% at 1 mg/L ATG, 2.8% at 10 mg/L ATG, 12.6% at 25 mg/L ATG, and 42.2% at 50 mg/L ATG. The median percentage of blasts killed by CIC after a 4-hour incubation with ATG was 1.9% at 1 mg/L ATG, 7.15% at 10 mg/L ATG, 12.1% at 25 mg/L ATG, and 13.9% at 50 mg/L ATG. CIC appeared to represent a direct induction of apoptosis by ATG. There was a high variability in the sensitivity of the blasts to ATG; at 50 mg/L, the percentage of blasts killed ranged from 2.6% to 97.2% via CDC and from 1.4% to 69.9% via CIC. In conclusion, ATG at clinically relevant concentrations kills leukemic blasts in vitro. Some acute leukemias are highly sensitive to ATG, whereas others are relatively resistant. This finding could lead to personalized administration of ATG.

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INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is a potentially curative treatment for hematologic malignancies, including acute leukemia [1,2]. Graft-versus-host disease (GVHD) and relapse of the original malignancy are the 2 most frequent causes of treatment failure and mortality [3,4]. Small-molecule immunosuppressive drugs like methotrexate or cyclosporine, although effective in decreasing the incidence of GVHD, increase the incidence of relapse [5-8].

Polyclonal rabbit anti-human thymocyte IgG (ATG; Thymoglobulin, extracted from serum of rabbits immunized with human thymocytes) and a similar polyclonal antibody, ATG-F (extracted from serum of rabbits immunized with the Jurkat T cell line) also decrease the incidence of GVHD; however, relapse in the setting of myeloablative conditioning is not increased [9-13]. This finding is counterintuitive, given that ATG is expected to interfere with both GVHD and the graft-versus-leukemia (GVL) effect. A potential explanation could be that ATG kills not only donor anti-host T cells mediating GVHD and GVL, but also leukemic cells. This is theoretically plausible, considering that ATG contains not only antibodies targeting antigens expressed by T cells, but also antigens expressed by leukemic cells, such as CD1a, CD2, CD4, CD5, CD6, CD7, CD11b, CD16, CD19, CD38, CD45, CD56, and HLA class I and II [14-19].

Recent studies have suggested that ATG can kill leukemic cells [20-23]. Those studies focused on determining whether ATG kills leukemic cell lines or primary cells from patients with myeloma or chronic lymphocytic leukemia, however, little information exists regarding primary cells from

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Table 1
Patient Characteristics

Characteristic	Value
Number of patients	36
Age, yr, median (range)	47.5 (20-98)
Acute leukemia type, ALL/AML, n	5/31
AML subtype, M0-2/M4-5, n	17/14
ALL subtype, B/T, n	5/0
Specimen type, marrow/blood, n*	20/16
Percent blasts, median (range)†	63 (0.8-94.9)
Cytogenetic risk status of AML, better/intermediate/poor/unknown, n‡	4/17/8/2
Cytogenetic plus molecular risk status of AML, better/intermediate/poor/unknown, n‡	3/5/14/9

* Included in main analyses.
† Among nucleated cells in the specimens used for the main analyses, that is, marrow if only marrow was available to us (n = 4), marrow if both marrow and blood were available to us (n = 15), blood if both marrow and blood were available to us (owing to temporary unavailability of CD41a and CD235a antibodies) (n = 2), and blood if only blood was available to us (n = 15). Blasts were counted microscopically on Giemsa-stained smears by a clinical hematology laboratory.
‡ National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology, acute myeloid leukemia, version 2.2014. Unknown cytogenetic and molecular risk status was defined as either cytogenetics not done/not available or minimum molecular test set (Flt3 internal tandem duplication and *NPM1* mutation) not done/not available.

patients with acute leukemia (the major indication for allogeneic HCT). Moreover, in the small number of patients with acute leukemia studied to date, only complement-independent cytotoxicity (CIC) was evaluated, and so the role of complement-dependent cytotoxicity (CDC), an important mechanism in the killing of T cells by ATG [20], remains unclear. Furthermore, in the small number of patients with acute leukemia studied to date, the concentration of ATG used for inducing cytotoxicity in vitro was higher than the serum concentration typically achieved in vivo in patients with conventional ATG doses (15 to 60 mg/kg ATG-F or 2.5 to 10 mg/kg Thymoglobulin [24]). Another limitation of the previous studies using primary cells is that cytotoxicity was measured among total marrow or blood mononuclear cells, which contain not only leukemic cells, but also other cells, including T cells, which are expected to be killed by ATG.

In the present study, we set out to conclusively determine, without the foregoing limitations, whether ATG at clinically relevant concentrations kills primary leukemic cells via CDC or CIC in patients with acute leukemia.

PATIENTS AND METHODS

Patients and Healthy Volunteers

Blood and bone marrow from patients with newly diagnosed acute myelogenous leukemia (AML; n = 31) or acute lymphoblastic leukemia (ALL; n = 5) served as the sources of primary leukemic cells for these experiments investigating ATG-induced cytotoxicity. Blood or marrow specimens were obtained before induction chemotherapy. Characteristics of the study population are specified in Table 1.

We used blood from 26 healthy volunteers (not screened for a disease) to assay ATG-induced killing of normal T cells and other leukocyte subsets. To determine the clinically relevant concentration of ATG, we also studied

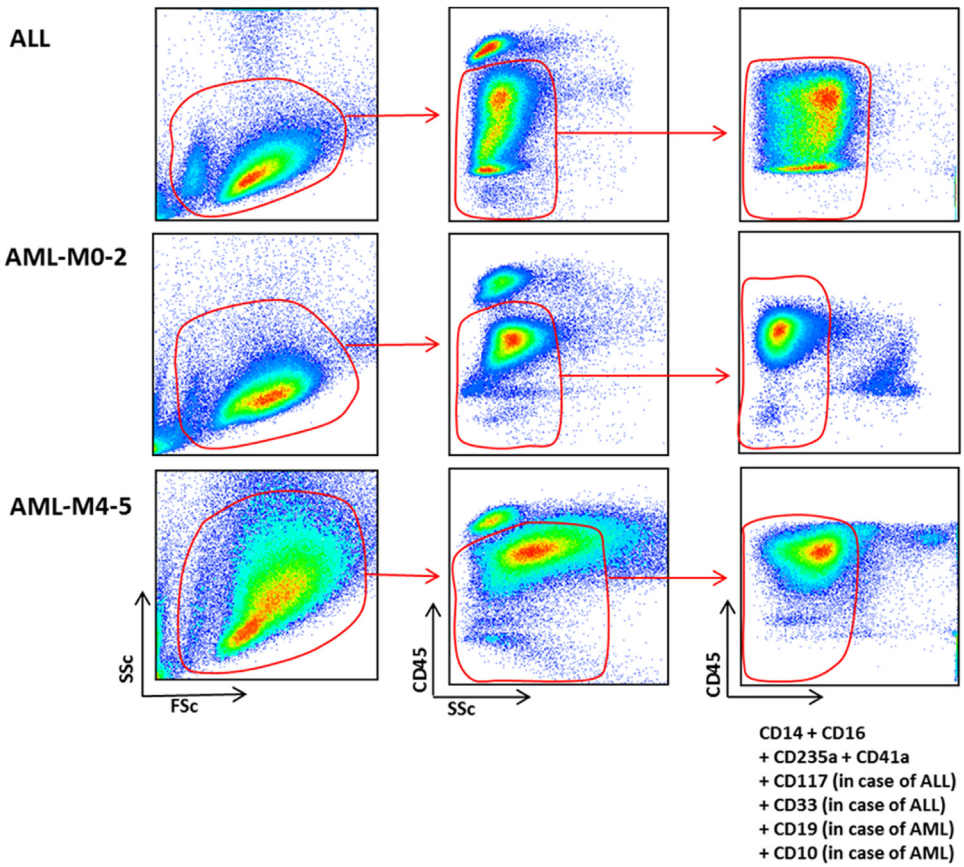

Figure 1. Gating for identification of leukemic blasts. Leukemic blasts were defined as mononuclear cells that were $CD45^{dim/neg}$ and SSc^{low} (or $SSc^{low/intermediate}$ for M4/M5) and did not express dump channel antigens. The dump channel antigens are listed on the *x*-axis of the right dot plots. ALL indicates acute lymphogenous leukemia; AML, acute myelogenous leukemia, FSc, forward scatter; SSc, side scatter.

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