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In Vitro Cytodestruction of Leukemic Cells in Human Bone Marrow Using a Cocktail of Monoclonal Antibodies *

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Therapy for human leukemia is currently limited by toxicity of chemoradiotherapy to normal bone marrow stem cells. This limitation can be circumvented by the prior removal and subsequent reinfusion of autologous bone marrow [1, 2]. In order for successful engraftment to occur, reinfused bone marrow must be pruged of leukemic cells ex vivo while the viability of stem cells remains intact. Monoclonal antibodies have the potential of providing this selective destructive effect.

During the past several years our laboratory has produced and characterized a panel of monoclonal antibodies recognizing cell surface molecules *primarily* expressed on leukemic cells and B cells [3–7]. We now present preliminary experiments designed to test the feasibility of using monoclonal antibodies BA-1, BA-2, and BA-3 (anti-CALLA) for the ex vivo elimination of leukemic cells in autologous bone marrow transplantation.

A. Materials and Methods

1. Antibodies and Complement

The production, utilization, and characterization of monoclonal antibodies BA-1, BA-2, and BA-3 have been previously described in detail [3, 4, 7]. Baby rabbit complement was obtained from Pel-Freez Biologicals (Rogers, AR).

2. Source of Cells

The established leukemic cell lines NALM–6 (pre-B ALL), REH (non-T, non-B ALL), and KOPN-1 (pre-B ALL) were provided by Dr. Jun Minowada, Buffalo, NY, Fresh leukemic cells were obtained from patients seen on the Pediatric Oncology service at the University of Minnesota. Leukemic cells obtained from bone marrow were separated on Ficoll-Hypaque density gradients and cryopreserved in liquid nitrogen as previously described [8]. Normal bone marrow obtained from adult volunteers was separated on Ficoll-Hypaque density gradients.

3. Stem Cell Assays

Bone marrow stem cell assays to detect CFU-GEMM, CFU-GM, CFU-E, and BFU-E were conducted as previously described [9].

4. Cytotoxicity Assays

Cytotoxicity assays utilizing complement-dependent cytolysis were conducted by trypan blue exclusion [10], or chromium (^{51}Cr) release. Target cells were labeled with sodium chromate for 1 h at 37 °C, washed 3 times, and admixed with normal bone marrow to yield a final leukemic cell/bone marrow cell ratio of 1 : 10, 1 : 100, or 1 : 1000. All cell mixtures were adjusted to 5×10^7 cells (leukemic and normal) per milliliter in RPMI 5% human serum albu-

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min. The various mixtures were then incubated with monoclonal antibodies BA-1, BA-2, and BA-3, all at a final ascitic fluid dilution of 1 : 100, and corresponding to 1–10 μg antibody protein per milliliter of cells. Baby rabbit complement was added to a final dilution of 1 : 6. A negative control consisted of substituting control ascitic fluid for the monoclonal antibodies. All cell populations were incubated in triplicate in 12×75 Falcon polystyrene tubes for 1 h at 37 °C on a rocker platform. The cells were then centrifuged out and a volume of supernatant was removed from all tubes for quantitation of ^{51}Cr-release. Maximum release was accomplished by lysing the ^{51}Cr-labeled leukemic cell/bone marrow cell mixtures with 0.5% NP-40. Percent specific ^{51}Cr-release was then determined using the following equation:
(Exper. – Cont./Max. – Cont.)× 100.

B. Results and Discussion

Table 1 outlines the general characteristics of monoclonal antibodies BA-1, BA-2, and BA-3. The three antibodies recognize distinct cell surface molecules; BA-1 recognizing a 30K dalton protein (LeBien, et al., unpublished), BA-2 recognizing a 24K dalton protein [4], and BA-3 recognizing the 100K dalton glycoprotein common acute lymphoblastic leukemia antigen (CALLA) [7]. All three antibodies fix rabbit complement and effectively lyse target cells expressing the individual antigens in vitro. A prominent characteristic of BA-1 and BA-2 is their inability, in the presence of rabbit complement, to inhibited the growth of bone marrow stem cells (CFU-GEMM, CFU-GM, BFU-E, and CFU-E) in vitro [11, 12]. Using rabbit heteroantisera and monoclonal antibody J-5, CALLA has been shown to be absent from bone marrow stem cells [13–15]. Similar results have been obtained with the anti-CALLA monoclonal antibody BA-3 (Ash et al., unpublished). The three antibodies also bind to leukemic cells from the majority of patients with non-T ALL [16]. Thus, BA-1, BA-2, and BA-3 are excellent candidates for elimination of leukemic cells in autologous bone marrow transplantation.

Table 1. General characteristics of BA-1, BA-2, and BA-3

Antibody	Ig class	Antigen detected	Complement fixation	Binding to stem cells	Binding to non-T ALL[a]
BA-1	M	p30 (?)	+	–	80%
BA-2	G3	p24	+	–	75%
BA-3	G2b	gp100/CALLA	+	–	70%

[a] Percentage of cases which were positive

Table 2. Monoclonal antibodies BA-1, BA-2, BA-3, and complement do not inhibit the growth of human hematopoietic stem cells[a]

Treatment[b]	CFU-GEMM	CFU-GM	BFU-E	CFU-E
Bone marrow alone	10.7±1.5	28.5±4	42.8±5.9	138±17
Bone marrow + complement	9.9±1.3	30.0±3.5	35 ±4.2	69± 9
Bone marrow + BA-1, BA-2, BA-3, and complement	11.3±1.2	33 ±2.1	40.5±3.7	85±14

[a] The four types of human hematopoietic stem cell colonies (CFU-GEMM, CFU-GM, BFU-E, CFU-E) were assayed in vitro using standard techniques. Data are expressed as the number of colonies per 10^5 cells plated from a single, normal bone marrow donor

[b] All three bone marrow groups were incubated for 1 h at 37° on a rocker platform prior to plating for the individual stem cell assays. BA-1, BA-2, and BA-3 were used at a final dilution of 1 : 100. Rabbit complement was used at a final dilution of 1 : 6

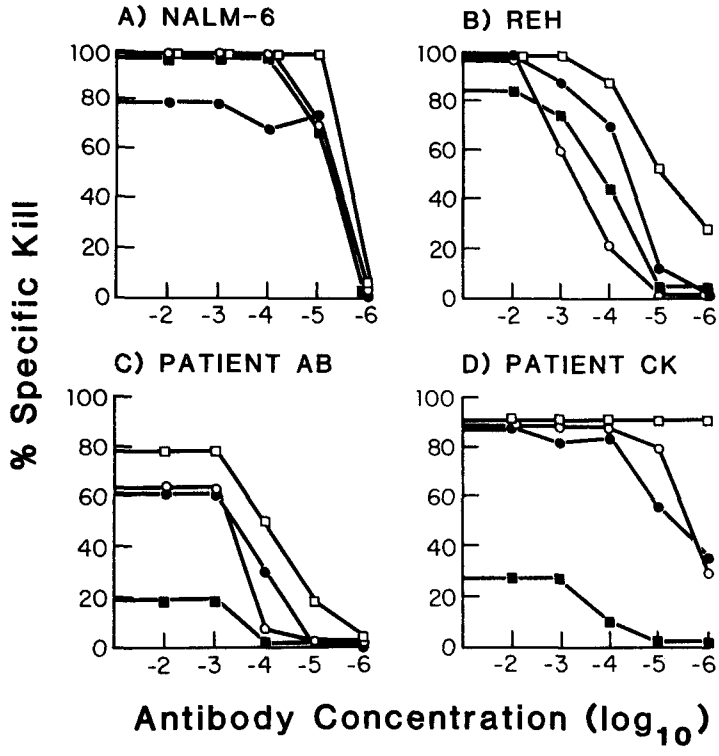

Fig. 1. Comparison of individual antibodies versus the antibody cocktail for cytolysis of leukemic cells. ●, BA-1 + complement; ○, BA-2 + complement; ■, BA-3 + complement; □, BA-1, BA-2, BA-3 + complement

Prior to utilizing BA-1, BA-2, and BA-3 for the ex vivo elimination of leukemic cells in autologous bone marrow transplantation, a series of experiments were undertaken to define the optimal conditions for their in vitro use.

As mentioned above, we had previously shown that when used individually with rabbit complement, neither BA-1, BA-2, nor BA-3 inhibited bone marrow stem cell growth in vitro. Thus, the first question we asked was whether BA-1, BA-2, and BA-3 *together* (antibody cocktail) inhibited stem cell growth. The results of one such experiment are shown in Table 2. The results clearly indicate that incubation of normal bone marrow with antibody cocktail plus rabbit complement did not inhibit the growth of CFU-GEMM, CFU-GM, and BFU-E. There was some suppression of CFU-E, but this also occurred with complement alone, suggesting that the effect was not antibody dependent.

We then asked whether antibody cocktail plus rabbit complement was more effective than any single antibody at lysing target cells. The results obtained with four individual target cells are shown in Fig. 1. It is apparent that the antibody cocktail plus rabbit complement effectively lysed all four targets. These targets included the cell lines NALM-6 and REH, and leukemic cells from two newly diagnosed ALL patients, AB and CK. Particularly noteworthy were the results obtained with leukemic cells from patient AB. Bone marrow from this patient had approximately 80% malignant cells by morphology. Immunologic phenotyping demonstrated 60% BA-1$^+$ cells, 60% BA-2$^+$ cells, and 20% BA-3$^+$ cells. The results show that treatment with the antibody cocktail lysed more cells than treatment with any single antibody. Most importantly, treatment with the antibody cocktail appeared to lyse all the morphologically malignant cells. Also, in no instance did the antibody cocktail result in less effective killing than any single antibody, thereby eliminating the possibility that use of the antibody cocktail would lead to steric hindrance of antibody binding at the cell surface. The data presented in Fig. 1 are based on analysis of cytotoxicity by trypan blue exclusion. Similar results were obtained using ^{51}Cr-release.

The next series of experiments were designed to determine if the BA-1, BA-2, and BA-3 antibody cocktail could effectively kill small numbers of leukemic cells in normal bone marrow. Table 3 summarizes the results of four preliminary experiments using three different leukemic cell lines. These experiments were conducted by incubating bone marrow for 1 h at 37 °C. In

data not shown, these conditions were found to be optimal for lysis of leukemic cells. The antibody cocktail was highly effective at eliminating ^{51}Cr-labeled leukemic cells in the presence of a 10-fold, 100-fold, or 1000-fold excess of normal bone marrow cells. In some instances we were not able to effectively lyse all ^{51}Cr-labeled leukemic cells (experiments 1 and 2). The reasons for this are not immediately apparent but may be explainable on the basis of variability inherent within the ^{51}Cr-release assay.

Table 3. In vitro cytodestruction of leukemic cells in human bone marrow using BA-1, BA-2, and BA-3, and complement

	% leukemic cells		
Cell line[a]	10	1	0.1
REH			
Exp. No. 1	100[b]	100	94.6
Exp. No. 2	99.8	100	97.9
NALM-6			
Exp. No. 1	100	100	100
KOPN-1			
Exp. No. 1	100	100	100

[a] REH = 99% BA-1$^+$, 50% BA-2$^+$ (weak), 95% BA-3$^+$; NALM-6 = 75% BA-1$^+$, 99% BA-2$^+$, 98% BA-3$^+$; KOPN-1 = 60% BA-1$^+$, 50% BA-2$^+$, 99% BA-3$^+$

[b] Values expressed as % specific ^{51}Cr-release using BA-1, 2, 3, and C

The experiments reported herein represent our initial efforts at optimizing the conditions necessary for the ex vivo elimination of residual leukemic cells with the BA-1, BA-2, and BA-3 antibody cocktail. Our decision to use all three antibodies is based on their binding to non-T ALL, and their inability to inhibit the in vitro growth of bone marrow stem cells in the presence of rabbit complement. Furthermore, we do not currently know whether the cell surface molecules recognized by BA-1, BA-2, and BA-3 are expressed on the *clonogenic* cell in any given case of ALL. An in vitro assay for the clonogenic ALL cell has recently been developed [17] and should assist in addressing the issue of the surface phenotype. For these reasons, we feel that the three antibodies together may be more effective than any single antibody alone.

We have recently initiated a phase I clinical trial to test the efficacy of the BA-1, BA-2, BA-3 antibody cocktail plus complement for the ex vivo elimination of leukemic cells in autologous bone marrow transplantation. Similar trials are underway using the J-5 anti-CALLA monoclonal antibody [18] and the anti-Leu 1 monoclonal antibody [19]. Hopefully, these trials will result in improved therapeutic results in patients who are currently not successfully treated with conventional chemotherapy.

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