Intrinsic Radiation Resistance of Primary Clonogenic Blasts from Children with Newly Diagnosed B-Cell Precursor Acute Lymphoblastic Leukemia

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Abstract

The radiation sensitivity of primary clonogenic blasts from 44 children with newly diagnosed B-cell precursor acute lymphoblastic leukemia (ALL) was analyzed using leukemic progenitor cell (LPC) colony assays. The derived values for SF_2 (surviving fraction at 200 cGy) and α (initial slope of radiation survival curves constructed according to the linear quadratic model) indicated a marked interpatient heterogeneity in intrinsic radiation sensitivity of LPC populations. The SF_2 values ranged from 0.01 to 1.00 (median = 0.430; mean±SE = 0.47±0.04), and the α values ranged from 0.000 to 3.272 Gy^{-1} (median = 0.280 Gy^{-1}; mean±SE = 0.430±0.093 Gy^{-1}). When $CD19^+$ $CD34^+$ versus $CD19^+$ $CD34^-$ immunophenotypes were compared, a trend toward higher SF_2 and lower α values were observed in LPC from $CD34^+$ patients, consistent with greater radiation resistance. When patients were divided into three approximately equal groups based on increasing levels of CD34 expression, a clear ordering effect was observed indicating that increased CD34 expression levels are associated with significantly higher radiation resistance at the level of B-lineage LPC. The highest CD34 expression group (≥ 75% positivity) had 1.4-fold higher SF_2 ($P = 0.05$) and twofold lower α values ($P = 0.06$) than the lowest group (< 30% positivity). Furthermore, the CD34 positivity of radiation resistant ($\alpha \leq 0.2$ and $SF_2 \geq 0.5$) B-cell precursor ALL cases was greater than the CD34 positivity of radiation sensitive ($\alpha > 0.2$ and/or $SF_2 < 0.5$) cases (56±9% versus 34±9%, $P = 0.09$). Whereas only 6 of 16 (38%) of radiation sensitive cases were $CD34^+$, 11 of 15 (73%) of radiation resistant cases expressed CD34 ($P = 0.04$). Our results offer new insights into the inherent and/or acquired radiation resistance of primary clonogenic blasts from B-cell precursor ALL patients. (*J. Clin. Invest.* 1993. 91:1044–1051.) Key words: immunophenotype • leukemia • radiation resistance • clonogenic cells • leukemic progenitor cell

Introduction

High risk acute lymphoblastic leukemia (ALL)[1] patients typically suffer a poor outcome after conventional drug treatment protocols (1). Within the last decade, however, total body irradiation (TBI) and high dose chemotherapy have been applied before bone marrow transplantation (BMT) in aggressive regimens to enhance these patients' prospects for disease-free survival (2–9). Although improvements in long-term, disease-free survival have been reported, recurrence of leukemia within the first 6 mo after TBI and BMT continues to be a major obstacle to a more successful outcome after BMT for high risk ALL, and only < 20% of high risk ALL patients become long-term, disease-free survivors after autologous or allogeneic BMT (2–13). The inability of TBI to adequately destroy leukemia cells and prevent residual disease may involve several possibilities. Clonogenic blasts (i.e., leukemic progenitor cells [LPC] may (*a*) possess an intrinsic resistance to radiation; (*b*) contain a large fraction of noncycling dormant blasts; (*c*) effectively repair sublethal radiation injury; (*d*) be capable of rapid self-renewal and repopulation; and/or (*e*) survive in quantities sufficient to cause relapse as a result of a high leukemia burden at remission.

Until we learn more about the effects of TBI on LPC, the results of radiation based pre-BMT conditioning regimens are not likely to dramatically improve. A thorough radiobiologic analysis of primary clonogenic blasts from ALL patients is a requisite step toward greater understanding of clinical radiation resistance. The present study initiated this task by evaluating the radiation sensitivity of primary clonogenic blasts from 44 newly diagnosed B-cell precursor ALL patients. Two computer-based models of cell survival were used (14). The multitarget model of survival curve analysis (14–16) provides SF_2 values as parameters to describe intrinsic radiation sensitivity. The surviving fraction at 200 cGy (SF_2) value is the surviving fraction of clonogenic blasts after exposure to 200 cGy. The major parameter for intrinsic radiation sensitivity in the linear quadratic model (14–16) is the α value, representing the initial slope of the linear component of the continuously bending radiation dose survival curve. Clonogenic blasts are considered sensitive to radiation based on high α values and low SF_2 values.

We initially recorded a marked interpatient variation in the values obtained from survival curves generated by LPC. Clonogenic blasts from different B-cell precursor ALL patients varied substantially in their sensitivity to radiation. We examined possible relationships between LPC radiation responses and the standard diagnostic features of these patients, including age, sex, white blood cell count (WBC) at diagnosis, immunophenotype, and cell cycle distribution of bone marrow blasts. Our observations indicated that LPC are intrinsically more resistant to radiation when a high percentage of blasts are in the $G_{0/1}$

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1. *Abbreviations used in this paper:* ALL, acute lymphoblastic leukemia; BMT, bone marrow transplantation; L-BCGF, low molecular weight B-cell growth factor; LPC, leukemic progenitor cells; PKC, protein kinase C; SF_2, surviving fraction at 200 cGy; WBC, white blood cell count.

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phases of the cell cycle or a low percentage of blasts are in the S phase. Further results provided evidence that radiation resistance may be associated with a high level of CD34 antigen expression. To our knowledge, this report represents the first radiobiologic analysis comparing radiation responses of primary LPC in relation to diagnostic features that distinguish B-cell precursor ALL patients.

Methods

Patient selection and patient material. Fresh leukemic bone marrow samples from 44 newly diagnosed B-cell precursor ALL patients were used to study the radiobiologic features of primary clonogenic blast populations. Diagnosis of B-cell precursor ALL was based on the morphologic, cytochemical, and immunophenotypic features of marrow blasts. There were 17 females and 27 males with a median age of 4 yr (range: 0.1–17 yr; mean±SE = 5.5±0.7 yr; $n = 44$). 37 of 44 patients (84%) were < 10 yr of age. The initial WBC ranged from 4.0×10^9 to 999.0×10^9/liter (median = 74.0×10^9/liter; mean±SE = $138.1 \pm 33.6 \times 10^9$/liter; $n = 34$). The cell cycle distribution of leukemic blasts was evaluated by DNA flow cytometry in a total of 18 patients. The percentage of $G_{0/1}$ phase cells ranged from 72 to 95% (median-85%; mean±SE = 85±1%). The percentage of S phase cells ranged from 4 to 23% (median = 13%; mean±SE = 12±1%) and the proliferation index (PI = % S+ $\%G_2M$) ranged from 5 to 28% (median = 15%; mean±SE = 15±1%).

The immunophenotypic features of leukemic blasts were determined by immunofluorescence staining and flow cytometry using a broad panel of mAb reactive with lymphoid differentiation antigens. None of 44 patients expressed the pan-T cell antigens CD2, CD5, and CD7 or the mature B-cell marker surface immunoglobulin. In contrast, leukemic blasts from each of the 44 patients expressed high levels of CD19 and/or CD24 B-lineage differentiation antigens. 89±1% of leukemic blasts from 44 of 44 (100%) expressed CD19, 83±3% of leukemic blasts from 34 of 40 patients (85%) expressed CD24, and 57±4% of leukemic blasts from 10 of 28 patients (36%) expressed CD40 antigen. The lymphoid precursor cell antigen CD10 was expressed on 82±3% of leukemic blasts from 33 of 44 (75%) patients. The progenitor cell associated antigen CD34 was expressed on 76±5% of leukemic blasts from 17 of 31 patients (55%). Thus, leukemic blasts displayed a common composite immunophenotype ($CD2^-CD5^-CD7^-CD10^{+/-}CD19^+$ $CD24^+CD34^{+/-}CD40^{+/-}sIg^-$) consistent with B-cell precursor ALL. Fresh bone marrow aspirates were procured by conventional methods as part of clinical treatment protocols. Mononuclear cells highly enriched for leukemic blasts (i.e., containing > 90% blasts) were isolated from fresh bone marrow aspirates by density gradient separation on Ficoll-Hypaque gradients. All patient bone marrow samples were used following the guidelines of the University of Minnesota Committee on the Use of Human Subjects in Research for secondary use of pathologic or surgical tissue.

Immunophenotyping. The surface antigen profiles of freshly isolated B-cell precursor ALL blasts were analyzed by indirect immunofluorescence and flow cytometry using a panel of monoclonal antibodies (mAb) that define human leukocyte differentiation antigens as previously described (17–20). Specifically, we used the mAbs 13-B3/35.1 (anti-CD2), T101/10.2 (anti-CD5), G3.7 (anti-CD7), BA-3 (anti-CD10), B43 (anti-CD19), BA-1 (anti-CD24), MY10 (anti-CD34), and G28-5 (anti-CD40). Samples were considered positive if > 30% of cells bound the antibody used.

Analysis of cell cycle kinetics by quantitative DNA flow cytometry. DNA content and cell cycle distribution of leukemic marrow blasts were evaluated by flow cytometric DNA analysis of fixed cells stained with propidium iodide (Sigma Chemical Co., St. Louis, MO) after treatment with RNAse (21, 22). Flow cytometric data of at least 20,000 cells from each sample were acquired in linear mode using a flow cytometer (FACScan®; Becton Dickinson, Mountain View, CA). Data were analyzed using polynomial and broadened rectangle models of DNA-ploidy-cell cycle and software (DNA Cell-Cycle Analysis Software; Becton Dickinson).

Irradiation of cells. Primary B-cell precursor ALL blasts, 1×10^5 cells/ml in alpha-MEM supplemented with 5% FCS, were irradiated with 50–800 cGy gamma rays in a single exposure (100 cGy/min, 37°C) using a ^{137}Cs irradiator (model Mark I; J. L. Shephard and Associates, Glendale, CA), as previously described (22–25). After irradiation, 1×10^5 ALL blasts per sample were assayed in duplicate for LPC-derived B-cell precursor ALL blast colony formation, as described in the next paragraph. The surviving fractions of clonogenic blasts were determined using the formula: Percent survival = 100 × (mean number of colonies in irradiated samples)/(mean number of colonies in unirradiated control samples). The radiation survival curves were constructed using computer programs for the analysis of cell survival data according to the single-hit multitarget and linear quadratic models, as detailed under *Data analysis* hereinafter. Data were expressed as the mean±SE values for SF_2 as well as the α (initial slope reflecting the steepness of the linear component of cell killing) value of the linear quadratic model.

Colony assays for primary clonogenic blasts from B-cell precursor ALL patients. A small fraction of leukemic B-cell precursors from ALL patients proliferate in response to low molecular weight B-cell growth factor (L-BCGF; Cellular Products, Buffalo, NY) and form compact blast colonies (20, 25, 26, 27). Day 7 ALL blast colonies are very compact and have a small size containing 20–50 lymphoblasts (20, 27). Therefore, colony numbers up to 3,000 per Petri dish can easily be enumerated on a grid using an inverted phase microscope of high optical resolution (20, 27). In the present study, fresh ALL marrow blasts (1×10^5 blasts/ml, duplicate 1-ml samples) were irradiated and immediately plated in 35-mm Petri dishes for a 7-d culture at 37°C in a humidified 5% CO_2 atmosphere in alpha-MEM. Medium was supplemented with 0.9% methylcellulose, 50 μM 2-mercaptoethanol, 15% human plasma + 15% fetal bovine serum (or 30% calf bovine serum), and 10% (vol/vol) L-BCGF (20, 25–27). On day 7, blast colonies containing > 20 cells were counted using an inverted phase microscope with high optical resolution. After the enumeration of colonies in each petri dish, colony blasts were subjected to morphological and immunological analyses, as described (20, 25–30). In all experiments, culture dishes were examined immediately and 24 h after plating to exclude the possibility of clumping unrelated to proliferation, which might lead to wrong conclusions regarding the radiation sensitivity of clonogenic blasts.

Data analysis. The radiation survival curves were constructed using three computer programs for the MacIntosh, ENTER, DSUM, and FIT (kindly provided by Dr. N. Albright, Department of Radiation Oncology, University of California, San Francisco, CA), for the analysis of cell survival data according to the single-hit multitarget and linear quadratic models of cell survival (14, 22). The data in this study were expressed as SF_2 value representing the radiation sensitivity parameter of the single-hit multitarget model of cell survival, as well as the α value representing the radiation sensitivity parameter of the linear quadratic model of cell survival (14, 22). We used standard statistical methods, including Student's t tests, to evaluate the in vitro radiation survival data and to compare the radiobiologic features of primary clonogenic blasts from different patients, as previously described (22, 25). Chi square analyses were used to assess the degree of association between clinical/laboratory parameters and radiation resistance/sensitivity of LPC. All computations were performed using an updated statistics program for the MacIntosh (StatWorks; Cricket Software, Philadelphia, PA).

Results

Radiobiologic features of primary clonogenic blasts from newly diagnosed B-lineage ALL patients. Primary bone marrow blasts from 44 patients were exposed to ionizing radiation, cultured in the presence of L-BCGF, and assayed for LPC-derived

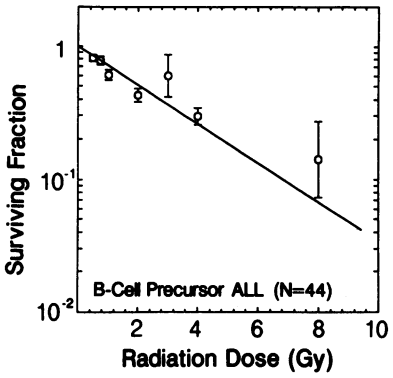

Figure 1. Composite radiation survival curve of primary clonogenic blasts from newly diagnosed B-cell precursor ALL patients. Survival of primary clonogenic blasts from 44 newly diagnosed B-cell precursor ALL patients is shown as a function of the radiation dose. Cells were irradiated, assayed for blast colony formation in vitro, and the composite radiation survival curve was generated using a computer program according to the linear quadratic model of cell survival as described in Methods. The calculated standard errors are also indicated. Each independent experiment was performed in duplicate.

blast colony formation. The median and mean numbers of blast colonies in unirradiated control cultures were 1,097 colonies/10^5 cells (1.1% plating efficiency) and 1,668±217 colonies/10^5 cells (1.7±0.2% plating efficiency), respectively.

Radiation survival curves of primary clonogenic blasts (i.e., LPC) were constructed for each of the 44 newly diagnosed B-cell precursor ALL patients using computer programs for the single-hit multitarget, as well as the linear quadratic models of cell survival. The computer-determined values for the radiobiologic parameters indicated a marked interpatient heterogeneity in intrinsic radiation sensitivity of LPC populations. The SF_2 values ranged from 0.01 to 1.00 (median = 0.430; mean±SE = 0.47±0.04), and the α values ranged from 0.000 to 3.272 Gy^{-1} (median = 0.280 Gy^{-1}; mean±SE = 0.430±0.093 Gy^{-1}). The SF_2, and α values of the composite radiation survival curve were 0.42 and 0.337 Gy^{-1}, respectively (Fig. 1). LPC from 17 of 44 patients (39%) had SF_2 values ≥ 0.50 and α values ≤ 0.2 Gy^{-1}, consistent with a marked radiation resistance at the level of LPC using the multitarget and linear quadratic models of cell survival. The mean SF_2 value for this subgroup of 17 patients was 0.75±0.04, which is significantly higher than the mean SF_2 value of 0.29±0.03 for the remaining 27 radiation sensitive patients ($P < 0.0001$). The mean α values were 0.063±0.016 Gy^{-1} for the radiation resistant group ($n = 17$) and 0.660±0.133 Gy^{-1} for the radiation sensitive group ($n = 27$) ($P < 0.0001$).

Correlation between radiobiologic features of primary B-lineage LPC and patients' characteristics. Patients were divided into groups according to sex, age, WBC at diagnosis, cell cycle distribution of leukemic blasts, and immunophenotype (Table I). Patients with a high percentage of blasts in $G_{0/1}$

Table I. Statistical Correlations

	n	SF_2	*P*	α (Gy^{-1})	*P*
All patients	44	0.47±0.04	—	0.430±0.093	—
M	27	0.46±0.05	NS	0.434±0.099	NS
F	17	0.48±0.07		0.423±0.185	
Age					
<4 yr	19	0.46±0.05		0.356±0.098	
≥4 yr	25	0.47±0.07	NS	0.486±0.146	NS
WBC					
<100,000/μl	20	0.49±0.06		0.327±0.064	
≥100,000/μl	14	0.39±0.08	NS	0.616±0.240	0.2
Plating efficiency (%)					
<1	22	0.48±0.06		0.409±0.117	
≥1	22	0.46±0.06	NS	0.451±0.147	NS
$\%G_{0/1}$					
<85	8	0.41±0.08		0.653±0.381	
≥85	10	0.73±0.07	0.006	0.112±0.061	0.1
%S					
<13	9	0.73±0.08		0.108±0.068	
≥13	9	0.45±0.08	0.017	0.598±0.340	0.1
$CD19^+CD10^+$	33	0.46±0.05		0.480±0.121	
$CD19^+CD10^-$	11	0.52±0.08	NS	0.108±0.068	NS
$CD19^+CD34^+$	17	0.60±0.06		0.261±0.116	
$CD19^+CD34^-$	14	0.46±0.06	0.09	0.300±0.058	0.2
$CD19^+CD40^+$	10	0.56±0.09		0.352±0.194	
$CD19^+CD40^-$	18	0.54±0.05	NS	0.252±0.050	NS
CD34 expression (%)					
<30	14	0.46±0.06		0.300±0.058	
30–74	8	0.57±0.09		0.388±0.241	
≥75	9	0.63±0.06	0.05	0.148±0.048	0.05

The SF_2 and α values (mean±SE) of B-lineage LPC from newly diagnosed B-cell precursor ALL patients were compared using two sample, two-sided Student's *t* tests.

phases of the cell cycle or a low percentage of blasts in S phase had higher SF_2 and smaller α values, consistent with a higher intrinsic radiation resistance at the level of LPC (Table I, Fig. 2). Whereas only 25% of cases with $G_{0/1} < 85\%$ had SF_2 values ≥ 0.5, consistent with radiation resistance according to single hit multitarget model of cell survival, 80% of cases with $G_{0/1} \geq 85\%$ had SF_2 values ≥ 0.5 (Fig. 2). Similarly, 90% of cases with $G_{0/1} \geq 85\%$ versus 37.5% of cases with $G_{0/1} < 85\%$ had α values ≤ 0.2, consistent with radiation resistance according to linear quadratic model of cell survival (Fig. 2). Furthermore, univariate analysis using $G_{0/1}$ and S phase percentages as continuous covariates established a significant association between $G_{0/1}$, as well as S phase percentages and SF_2 ($\%G_{0/1}$ versus SF_2: coefficient of correlation = 0.36, $P = 0.1$; %S phase versus SF_2: coefficient of correlation = 0.29, $P = 0.1$), as well as α values ($\%G_{0/1}$ versus α: coefficient of correlation = 0.29, $P = 0.1$; %S phase versus α: coefficient of correlation = 0.26, $P = 0.1$). In contrast, patient age, WBC, or in vitro plating efficiency as continuous covariates did not correlate with SF_2 or α values (P values all > 0.3). Similarly, when patients were divided into groups, patient sex, age, WBC at diagnosis, or in vitro plating efficiency did not have a significant impact on the radiation sensitivity of LPC (Table I).

In our initial analyses of immunophenotype-radiation sensitivity associations, we used antigen expression on $\geq 30\%$ blasts as an arbitrary criterion for positivity. According to this conventional classification, 17 of 31 patients (55%) were $CD19^+CD34^+$, and 14 of 31 patients (45%) were $CD19^+CD34^-$. When $CD19^+$-$CD34^+$ versus $CD19^+CD34^-$ patients were compared, a trend towards higher SF_2 and lower α values was observed in LPC from $CD34^+$ patients, consistent with greater radiation resistance (Table I). By comparison, a trend towards lower SF_2 and higher α values was observed for $CD19^+CD10^+$ patients and CD40 expression was not associated with radiation resistance or sensitivity of LPC from $CD19^+$ B-lineage ALL patients (Table I). There were not enough $CD19^-$ or $CD24^-$ patients to examine the potential influence of lack or presence of CD19 or CD24 on LPC radiation sensitivity. To further investigate the influence of CD34 expression on radiation sensitivity at the level of LPC, patients were divided into three approximately equal groups based on increasing levels of CD34 expression (Table I). A clear ordering effect was observed, indicating that increased CD34 expression levels are associated with significantly higher radiation resistance at the level of B-lineage LPC. The highest CD34 expression group ($\geq 75\%$ positivity) had 1.4-fold higher SF_2 ($P = 0.05$) and two-fold lower α values ($P = 0.05$) than the lowest CD34 expression group ($< 30\%$ positivity). As shown in Fig. 3, increased CD34 expression levels were associated with a higher radiation resistant fraction. Whereas only 35.7% of $CD34^-$ cases ($< 30\%$ positivity) had LPC with $SF_2 \geq 0.5$, 62.5% of patients with intermediate ($< 75\%$, $\geq 30\%$) CD34 positivity, and 66.6% of patients with high ($\geq 75\%$) CD34 positivity had LPC with $SF_2 \geq 0.5$ ($\chi^2 = 2.10$, $P = 0.15$). Similarly, only 35.7% of $CD34^-$ cases had $\alpha \leq 0.2$, whereas 62.5% of cases with intermediate CD34 positivity and 77.8% of cases with high CD34 positivity had $\alpha \leq 0.2$ ($\chi^2 = 3.88$, $P < 0.05$).

Presenting features of B-cell precursor ALL patients with radiation-resistant LPC. LPC from 17 patients had SF_2 values ≥ 0.50 and α values ≤ 0.2 Gy^{-1}. The radiation resistant group was composed of slightly younger children than the radiation-sensitive group. Of 17 radiation-resistant cases, 16 (94%) were children < 10 yr of age. By comparison, 21 of 27 (78%) radiation sensitive cases were < 10 yr of age. The mean age was 4.5±0.8 yr for the radiation resistant group and 6.2±0.9 yr for the radiation-sensitive group ($P = 0.2$). A trend towards lower WBC values was noted among radiation-resistant cases (Table II). Whereas only 25% of radiation resistant cases had WBC > 100,000, some 50% of radiation-sensitive cases had WBC > 100,000. The mean WBC values were $91 \pm 39 \times 10^9$/liter for radiation-resistant cases and $170 \pm 46 \times 10^9$/liter for radiation-sensitive cases ($P = 0.2$). The sex distribution of patients with radiation-resistant versus radiation-sensitive LPC were not significantly different (Table II). Notably, a greater fraction of radiation-resistant cases had high $G_{0/1}$ phase percentages ($\geq 85\%$) (80% versus 25%, $P = 0.02$) and low S phase percentages ($< 13\%$) (70% versus 25%, $P = 0.06$) than radiation sensitive cases (Table II), in accordance with the association between high $G_{0/1}$ phase percentage or low S phase percentage and radiation resistance shown in Table I and Fig. 3. 70% of

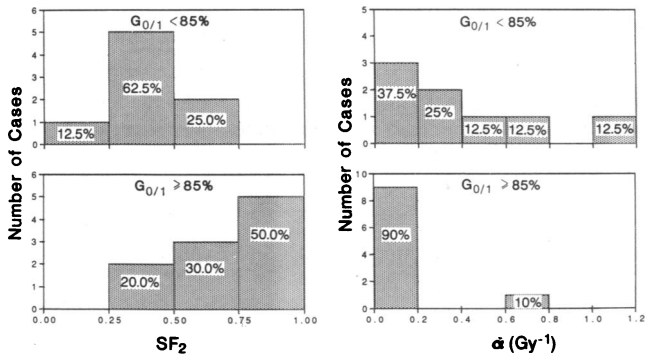

Figure 2. Radiation sensitivity of primary clonogenic blasts from newly diagnosed B-cell precursor ALL patients according to the $G_{0/1}$ percentage. The distribution of SF_2 and α values is shown for patients with $G_{0/1} \geq 85\%$ or $G_{0/1} < 85\%$.

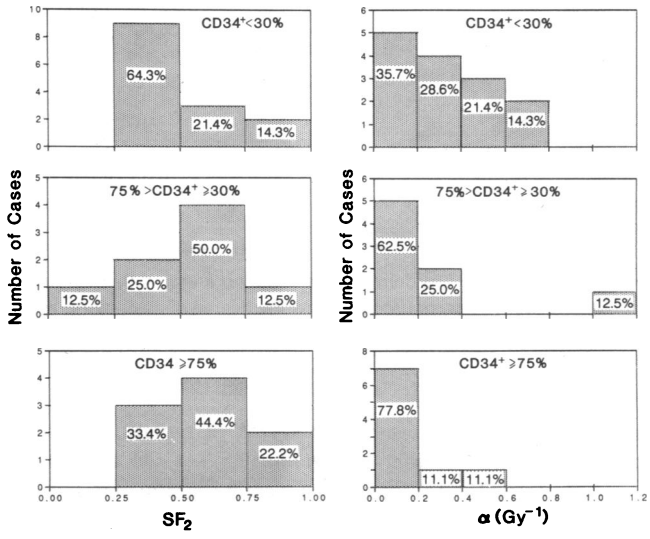

Figure 3. Radiation sensitivity of primary clonogenic blasts from newly diagnosed B-cell precursor ALL patients according to CD34 antigen expression. The distributions of SF_2 and α values from $CD34^+$ and $CD34^-$ patients are compared.

Table II. Presenting Features of B-Cell Precursor ALL Patients According to Radiation Sensitivity of the LPC

	Radiation resistant LPC $n = 17$	Radiation sensitive LPC $n = 27$	P value
D_0 value	353.9±65.5	216.2±32.8	0.04
SF_2 value	0.75±0.04	0.29±0.03	<0.001
α Value	0.063±0.016	0.660±0.133	<0.001
Sex			
Male	11/17 (65%)	16/27 (59%)	
Female	6/17 (35%)	11/27 (41%)	
Age			
<10 yr	16/17 (94%)	21/27 (78%)	
≥10 yr	1/17 (6%)	6/27 (22%)	
Mean±SE yr	4.5±0.8	6.2±0.9	
WBC			
<100,000/μl	9/12 (75%)	11/22 (50%)	
≥100,000/μl	3/12 (25%)	11/22 (50%)	
Mean±SE ($\times 10^3/\mu l$)	91±39	170±46	
CD10			
Positive cases	13/17 (76%)	20/27 (74%)	
Negative cases	4/17 (24%)	7/27 (26%)	
Mean % positivity	65±9	63±7	
CD19			
Positive cases	17/17 (100%)	27/27 (100%)	
Negative cases	0/17 (0%)	0/27 (0%)	
Mean % positivity	90±2	88±2	
CD24			
Positive cases	15/17 (88%)	21/24 (88%)	
Negative cases	2/17 (12%)	3/24 (12%)	
Mean % positivity	70±7	76±5	
CD34			
Positive cases	11/15 (73%)	6/16 (38%)	0.04 ($\chi^2 = 4.0$)
Negative cases	4/15 (27%)	10/16 (63%)	
Mean % positivity	56±9	34±9	0.09
CD40			
Positive cases	6/14 (43%)	4/14 (29%)	
Negative cases	8/14 (57%)	10/14 (71%)	
Mean % positivity	31±6	21±7	
$G_{0/1}$ phase			
<85%	2/10 (20%)	6/8 (75%)	
≥85%	8/10 (80%)	2/8 (25%)	0.02 ($\chi^2 = 5.4$)
S phase			
<13%	7/10 (70%)	2/8 (25%)	
≥13%	3/10 (30%)	6/8 (75%)	0.06 ($\chi^2 = 3.6$)
S + G_2M phase			
<16	9/11 (82%)	3/10 (30%)	
≥16	2/11 (18%)	7/10 (70%)	0.02 ($\chi^2 = 5.7$)

Radiation resistance was defined as $SF_2 \geq 0.50$ and $\alpha \leq 0.2$. Continuous covariates were compared using two-sample, two-sided Student's t tests. Chi-square analyses were used to assess the degree of association between clinical/laboratory parameters and radiation resistance/sensitivity of LPC. P values are given only for significant or nearly significant ($P < 0.1$) differences/associations.

radiation-sensitive cases but only 18% of radiation-resistant cases had a high proliferative index (%S + G_2M ≥ 16%) ($P = 0.02$) (Table II). Importantly, a greater fraction of radiation-resistant cases were CD34$^+$ (73% versus 38%, $P = 0.04$) and the mean CD34 positivity of radiation-resistant B-cell precursor ALL cases was greater than the mean CD34 positivity of radiation-sensitive cases (56±9% versus 34±9%, $P = 0.09$) (Table II). No differences between radiation-resistant and radiation-sensitive groups were noted relative to expression of CD10, CD19, CD24, or CD40 antigens.

Discussion

Until recently, very little was known regarding the radiobiologic features of primary leukemic blasts from ALL patients. This paucity of knowledge was caused by historic difficulties in

cloning freshly isolated primary ALL blasts in vitro. Within the past 6 yr, we have refined in vitro colony assay systems to culture primary blasts from T-lineage ALL (10, 18, 28, 29), as well as B-lineage ALL (11, 20, 27, 30, 31) patients. Subsequent studies using these assay systems provided strong evidence that in vitro clonogenic ALL blasts, referred to as LPC, likely represent counterparts of in vivo clonogenic ALL blasts (10, 11, 22, 28). In the present study, we used the B-lineage ALL LPC colony assay system to elucidate the radiobiologic features of primary clonogenic blasts from 44 newly diagnosed B-cell precursor ALL patients.

This report extends our earlier work on the radiobiologic features of primary leukemic blasts from ALL patients (22, 25, 28), amplifies our knowledge of the radiobiologic features of human tumor cells (32–46), and provides novel insights into possible associations between cell cycle kinetics, immunophenotype, and radiation sensitivity. The α value, reflecting the initial slope of radiation survival curves constructed according to the linear quadratic model of cell survival, is one predictor of the sensitivity of human tumors to clinical radiation (44). The reported α values for human tumor cells range from 0.2 to 0.6 Gy^{-1} (16, 32, 51). The mean α value for B-lineage LPC in the present study is 0.430 ± 0.093 Gy^{-1}. Notably, 17 of the 44 (39%) newly diagnosed B-cell precursor ALL patients had SF_2 values ≥ 0.50, which are equivalent to the reported SF_2 values for the least radiation-responsive tumors in clinical radiation therapy (16, 42). In conjunction with α values ≤ 0.2 Gy^{-1}, our results are consistent with marked intrinsic radiation resistance at the level of clonogenic blasts using the multitarget and linear quadratic models of cell survival. Thus, primary clonogenic blasts from some B-cell precursor ALL patients are clearly among the most radiation resistant human tumor cells reported to date. Early and frequent relapses experienced by B-cell precursor ALL patients within the first 6 mo after 800 cGy single dose or 1,375 cGy hyperfractionated TBI and BMT with only 10–15% disease-free survival at 2 yr after BMT are in accordance with this conclusion (2, 6, 11, 13). Taken together, these preclinical and clinical observations in B-cell precursor ALL emphasize the need for therapeutic innovation and recommend a reevaluation of the role of TBI in BMT.

The observed radiobiologic heterogeneity among B-cell precursor ALL patients encouraged us to assess the radiobiologic features of primary B-lineage LPC in relation to the more frequently measured diagnostic parameters of age, sex, WBC at diagnosis, cell cycle distribution, proliferation index of leukemic blasts, and immunophenotype. Among these diagnostic parameters, only cell cycle distribution and immunophenotype showed a significant correlation with the intrinsic radiation sensitivity of B-lineage LPC. A trend towards lower SF_2 and higher α values was observed for $CD19^+CD10^+$ patients, consistent with greater radiation sensitivity. By comparison, an opposite relationship existed between CD34 expression and radiation sensitivity of LPC. LPC from $CD19^+CD34^+$ patients appeared to be more radiation resistant than LPC from $CD19^+CD34^-$ patients, as reflected by higher SF_2 and lower α values. When patients were divided into three approximately equal groups based on increasing levels of CD34 expression, a clear ordering effect was observed indicating that increased CD34 expression levels are associated with significantly higher radiation resistance at the level of B-lineage LPC. The highest CD34 expression group ($\geq$ 75% positivity) had 1.4-fold higher SF_2 ($P = 0.05$) and 2.0-fold lower α values ($P = 0.05$) than the lowest group (< 30% positivity). Importantly, a greater fraction of radiation resistant cases were $CD34^+$ (73% versus 38%, $P = 0.04$) and the mean CD34 positivity of radiation resistant B-cell precursor ALL cases was greater than the mean CD34 positivity of radiation sensitive cases ($56 \pm 9\%$ versus $34 \pm 9\%$, $P = 0.09$). Taken together, these results prompt the hypothesis that high level CD34 expression is associated with radiation resistance in B-cell precursor ALL. CD34 expression has also been associated with resistance to chemotherapy in myeloid malignancies (52, 53) and may be related to poor treatment outcome in B-cell precursor ALL (based on unpublished observations in 1992 by F. M. Uckun, H. Sather, and D. Hammond of the Children's Cancer Study Group).

Human CD34 is a 110-kD lymphohematopoietic progenitor cell associated surface sialomucin antigen that is expressed on normal as well as leukemic progenitor cell populations corresponding to the earliest stages of differentiation (54–58). The gene for CD34 has been mapped to band 1q32 of the long arm of chromosome 1 (59, 60). CD34 cDNA predicts a 373-amino acid polypeptide that is a type I integral membrane protein and has no sequence homology to any known protein (58). Notably, > 30% of the predicted amino acids in the NH_2-terminal domain of this antigen are serine or threonine residues and CD34 antigen is a substrate for protein kinase C (PKC) (58, 61). CD34 antigen can be phosphorylated by PKC in $CD34^+$ leukemic B-cell precursors (58). Fackler et al. have recently shown that multiple serine kinases including glycogen synthase kinase and casein kinase II can phosphorylate CD34 antigen (62). Greaves et al. proposed that the currently undefined function of CD34 is likely to be modulated by signals that stimulate the activation of PKC (58). Intriguingly, more recent studies by Weichselbaum and colleagues demonstrated that ionizing radiation activates PKC in irradiated myeloid cells, including KG-1 cells, which are strongly $CD34^+$ (63). Similarly, Uckun et al. reported that in normal and leukemic B-cell precursors, ionizing radiation activates multiple serine kinases including PKC (64). It is therefore likely that CD34 becomes phosphorylated in irradiated cells. The relationship between CD34 positivity and radiation resistance, as presently reported, suggests a possible role for phosphorylated CD34 in intrinsic radiation sensitivity.

Several investigators have noted cell cycle dependent variations in radiation sensitivity of mammalian cells, with the highest levels of sensitivity usually appearing in the G_2- and M-phases of the cell cycle (65–69). By comparison, quiescent cells and cells in early and mid-G_1 phase are relatively radiation resistant (65–69). Kimler and Anderson reported that 9L rat brain tumor cells are most resistant to radiation while in G1 phase (70). Weichselbaum explained that the repair of radiation-induced molecular damage may be facilitated if DNA replication is delayed by holding cells in G1 (71). Potmesil and Goldfeder reported that nonproliferating G_1 confined cells from mouse mammary adenocarcinoma cell lines DBAH and MT2 persist in irradiated tumors of mice and a transition of these nonproliferating cells to the proliferating pool takes place at the start of tumor recurrence (67). In contrast, Wallen et al. reported that quiescent cells from the murine mammary carcinoma cell lines 66 and 67 are significantly more sensitive than proliferating cells from the same cell lines (72). Similarly, Madoc-Jones reported that rat sarcoma cells are most sensitive to ionizing radiation during the G_1 phase of the cell cycle (73). These reports regarding the radiation sensitivity of quiescent

G_1 cells indicate that the most sensitive and resistant phases of the cell cycle may be different for each cell type. Alternatively, not only the exact time of radiation exposure in relation to cell cycle, but also the length of a given cell cycle phase during which the radiation exposure occurred may determine the biological outcome of radiation exposure. In the present study, a high $G_{0/1}$ percentage or low S phase percentage in leukemic blast populations from newly diagnosed B-cell precursor ALL patients was associated with radiation resistance at the level of LPC. The observed relationship between cell cycle kinetic features and radiation sensitivity recommends agents that can stimulate S phase entry of quiescent ALL blast populations, such as mitogenic cytokines as potentially useful adjuncts to current TBI/BMT regimens for high risk B-cell precursor ALL.

Notably, high WBC at diagnosis was not associated with radiation resistance at the level of LPC. Therefore, the published ability of this factor to predict relapse after TBI and BMT in B-cell precursor ALL (2–9) cannot be explained by the level of radiation resistance.

In summary, we have used in vitro colony assays to study and compare the radiobiologic features of B-lineage LPC from newly diagnosed B-cell precursor ALL patients. Our findings demonstrate a marked interpatient variation in the radiation sensitivity of LPC. We postulate that clonogenic blasts with an inherent and/or acquired resistance to radiation contribute to the high relapse rate after BMT for B-cell precursor ALL. Differences in radiation sensitivity may partially explain the inconsistent responses of B-cell precursor ALL patients to TBI and BMT. The insights acquired from this study should promote sequential comparative analyses of novel radiosensitizing agents, which may ultimately provide more effective conditioning regimens.

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