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ORIGINAL ARTICLE

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CD80 (B7-1) expression on human acute myeloid leukaemic cells cultured with GM-CSF, IL-3 and IL-6

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Abstract Acute myeloid leukaemia (AML) blasts rarely express the B7 family of co-stimulatory molecules and do not elicit a clinically significant autologous T-lymphocyte anti-tumour response. The aim of this study was the in vitro modification of AML blasts to an antigen-presenting cell phenotype characterised by upregulated expression of the co-stimulatory molecule CD80 (B7-1).

Circulating AML cells were induced to undergo partial differentiation in culture with the cytokines IL-3, IL-6 and GM-CSF; they exhibited variable upregulation of CD80 and continued to express MHC class I and II. These cells remained viable to day 20, in contrast with normal peripheral blood mononuclear cells (PBMNC), which did not survive under the culture conditions.

In contrast to unmanipulated blasts, cultured leukaemic cells expressed B7-1. Where initial cytogenetic abnormalities were present, they were also seen in flow-sorted CD80-expressing cells after culture in cytokines, indicating their malignant origin.

The immunogenic potential of these cultured cells was highlighted by allogeneic and autologous mixed lymphocyte reactions, in which both differentiated, but not unmanipulated, blasts produced expansion of T-lymphocyte numbers. Autologous cytotoxic T-lymphocyte (CTL) assays indicated specific killing of B7-1$^+$ leukaemic cells, which was greatly enhanced after priming of the T-lymphocytes by B7-1$^+$ blasts prior to the CTL assay, then enabling the CTL to lyse both unmanipulated and differentiated leukaemic cells.

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Introduction

Acute myeloid leukaemia (AML) is characterised by the accumulation of malignant myeloid cells, arrested at an early stage of development. Most leukaemic blasts express MHC class I and II, but it is unusual for them to express CD80 and other co-stimulatory molecules that are important in antigen presentation to T-cells. Terminally differentiated haemopoietic cells are among the most potent antigen-presenting cells (APCs). We have therefore attempted to determine whether AML blasts can be induced to differentiate and express B7-1, with a view to exploring their antigen-presenting capacity.

In vitro modification of leukaemic blasts to increase co-stimulatory molecule expression has been shown to augment T-cell numbers; and retroviral transduction with B7 in animal models has been associated with reduced tumorogenicity and specific CD4$^+$ or CD8$^+$ T-cell-mediated responses [2, 3, 8, 9, 16, 25, 32, 34, 35, 47]. Using an alternative strategy, IFN-γ has been used to increase B7-1 expression on a human monoblastic leukaemic cell line [18, 26]. Similarly, bone marrow chronic myelogenous leukaemia (CML) leukaemic cells cultured in the presence of IL-4, TNF and GM-CSF have been induced to express co-stimulatory molecules and exhibit a dendritic cell (DC) phenotype [17]. More recently, a number of groups have induced a DC phenotype and B7-1 expression in AML cells from some patients by incubation with cytokines [4, 10, 37].

We have investigated the in vitro immunogenic potential of AML peripheral blood leukaemic cells partially differentiated to express B7-1 by culture in medium containing the lineage-specific maturation cytokines, GM-CSF, IL-3 and IL-6 [39].

Materials and methods

Cell culture

Leukaemic blasts and peripheral blood mononuclear cells (PBMNC)

Leukaemic blasts were cultured from the peripheral blood of 12 patients with AML. Leukaemic blasts were obtained from patients treated in the Haematology Department of the Prince of Wales Hospital, Sydney. PBMNC were harvested by Ficoll-Hypaque density-gradient centrifugation. Blasts were cultured in RPMI–1640 medium (ICN, Ohio, USA) supplemented with 10% fetal calf serum (FCS) (Gibco BRL, Gaithersburg, Md., USA) and penicillin-streptomycin, in 5% CO_2 at 37 °C, in a humidified atmosphere. Adherent cells were discarded after overnight incubation of the isolated mononuclear cells. PBMNC were also harvested from the peripheral blood of 7 normal volunteers and cultured as described above.

Cell lines

The cell lines K562 (CML) [29], KG1 (AML) [27], HL-60 (acute promyelocytic leukaemia) [12], U937 (human histocytic lymphoma) [45] and Y79 (retinoblastoma) [36] were obtained from ATCC (Maryland, USA). Y79 cells were cultured in IMEM containing 10% FCS, and the other cell lines in RPMI–1640 containing 10% FCS.

Growth factors

Recombinant human IL-3, IL-6 and GM-CSF were obtained from Sandoz Pharmaceuticals (Camberley, UK) and used at a final concentration of 20 ng/ml.

Cell numbers

The proliferation of leukaemic blasts and normal PBMNC cultured in RPMI–1640/10% FCS, containing IL-3, IL-6 and GM-CSF, was assessed. Freshly isolated non-adherent mononuclear cells from leukaemic patients were cultured in 25-cm^2 flasks at a known concentration. Cell numbers were determined by counting viable cells (excluding trypan blue), using a haemocytometer. Medium containing cytokines was replaced every 4 days, at which time cell counts were performed.

Immunofluorescence assays

Cell surface antigen expression was assessed by flow cytometry, using FITC- or PE-conjugated monoclonal antibodies (MoAb). Cells were washed twice in PBS and resuspended in PBS containing 1% bovine serum albumin (BSA) prior to incubation with MoAb for 10 min at room temperature in the dark, followed by two washes in PBS/BSA. Cells were stained with 250 µl 0.1% propidium iodide, to enable non-viable cells to be excluded from analysis. Immunofluorescence positivity was determined by analysing 10,000 events on a FACScan flow cytometer (Becton Dickinson).

Phenotypic analysis

Leukaemic cells from each patient or normal non-adherent PBMNC from controls were studied after overnight incubation at 37 °C and after 16 days (for cells from normal controls) or 20 days (for cells from leukaemic patients) in culture with 20 ng/ml of IL-3, IL-6 and GM-CSF. The phenotype was determined using 20 µl of each of the following panel of antibodies (Becton Dickinson, Calif., USA): murine isotype control (FITC, PE); CD11b (PE); CD3/CD19 (FITC, PE); CD14/CD13 (FITC, PE); CD4/CD8 (FITC, PE); CD34/CD33 (FITC, PE), CD34/CD80 (FITC/PE), MHC class 11 (PE). All samples also included 20 µl CD45 (Cy-Chrome™ PharMingen, Calif., USA). MHC class I expression was evaluated using an MHC class I antibody (Sigma, St. Louis, Mo., USA) at a concentration of 5 µl per 10^6 cells and a secondary sheep anti-mouse IgG FITC-conjugated antibody (Silenus, Melbourne, Australia). An Immunotech IgG2a FITC-conjugated antibody [(Coulter, France) 10 µl per 10^6 cells] was used as a negative control.

Cell cycle analysis

Cell cycle analysis was performed on AML cells before and after culture in medium containing IL-3, IL-6 and GM-CSF, to further investigate their malignant nature. One million cells from each patient were washed in sample buffer (Ca^{++}/Mg^{++}-free PBS containing 0.1% glucose) and centrifuged for 10 min at 1000 rpm. Cells were fixed overnight at 4 °C in 70% ethanol and then centrifuged at 3000 rpm for 5 min. One millilitre of a staining solution containing 50 µg/ml propidium iodide and 100 U/ml RNase A (Sigma Chemical Co, St. Louis, Mo., USA) in sample buffer was added to the cell pellet. Proliferating Y79 cells and non-proliferating normal PBMNC were used as controls. Cell cycle analysis was performed using Verity MODFIT v2 software (Maine, USA).

Cytogenetics

Karyotypic analysis was performed at diagnosis in all patients with leukaemia. Where an abnormality was detectable, karyotyping was repeated following culture for 20 days in cytokines in order to assess the clonal origin of cells co-expressing CD80 and CD34. $CD34^+$/$CD80^+$ cells were sorted by flow cytometry into RPMI–1640 containing 10% FCS, incubated overnight and analysed for chromosomal abnormalities by standard techniques.

Mixed lymphocyte reactions (MLR)

PBMNC from normal individuals, or from patients with AML, were co-cultured with freshly isolated (unmanipulated) leukaemic blasts or blasts that had been cultured in cytokines for 20 days and then washed in RPMI–1640 containing 2% FCS. The cells were added at a 1:1 ratio in duplicate wells in a 96-well U-shaped tissue culture plate (Crown Corning, Calif., USA) in a total volume of 200 µl. The number of viable cells was evaluated on days 0, 4, and 7. Flow cytometry was used to quantitate the percentage of $CD3^+$ lymphocytes in the wells at the same time-points. PBMNC or leukaemic cells cultured alone in RPMI–1640/2% FCS provided negative controls, since neither was able to proliferate under these conditions. Both allogeneic and autologous MLR were performed.

Autologous CTL assays

Mononuclear cells were harvested from peripheral blood by density gradient separation, and effector T-cells were grown selectively in medium containing 50 U/ml IL-2 (Sigma Chemical Co., St. Louis, Mo., USA) (>95% $CD3^+$, as determined by FACs analysis). T-cells were exposed to unmodified blasts or B7-1^+ blasts (grown in cytokines for 20 days) for 3 days prior to the CTL assay at an effector stimulator ratio of 3:1. Target cells included the natural killer cell line K562 and freshly isolated (unmodified) or cytokine-modified (B7-1^+) autologous leukaemic blast cells. Cells were co-cultured at an effector to target ratio of 10:1 in 200 µl phenol-red free RPMI–1640 containing 2% FCS for 6 h in round-bottomed 96-well plates. Spontaneous release by effector and target cells were controlled by separate incubation of the respective populations. The harvested cell-free supernatants were incubated with lactate dehydrogenase (LDH) substrate and absorbance measured [49]. The results were calculated as follows:

$$\% \text{ cytotoxicity} = \frac{\text{test release} - \text{non-specific release}}{\text{total LDH release} - \text{non-specific release}} \times 100$$

Results

Cell numbers

PBMNC from normal individuals were cultured in medium containing IL-3, IL-6 and GM-CSF for up to 20 days. As indicated in Fig. 1, there was a gradual decrease in the number of remaining viable cells in all cases ($n = 7$, +/− SD), so that by day 16, the number of remaining cells was reduced to less than 20%. In contrast, the number of viable cells in culture when leukaemic blasts were incubated under the same conditions, at day 16, was 87% (+/− 10%) ($n = 8$, +/− SD). By day 20, no viable cells remained in normal controls, whereas flasks containing leukaemic cells had between 42 and 100% of starting cell numbers.

Phenotypic analysis

The phenotype of the total mononuclear cell fraction from patients with circulating leukaemic cells and normal controls was determined by flow cytometry on days 0 and 20 (for blasts) or days 0 and 16 (for normal mononuclear cells), of culture with cytokines. Only a limited panel of antibodies (isotype control, CD11b, CD33, CD34 and CD80) was used for normal PBMNC-derived cells on day 16 because of low cell numbers (Table 1). In five normal controls, CD11b was expressed on about 15% of cells on day 0, while corresponding expression of CD33, CD34 and CD80 was approximately 23%, 0.6% and 0.8%, respectively. By day 16, no cells expressed CD11b, but approximately 60% of the few remaining cells expressed CD33, ~4% CD34 and

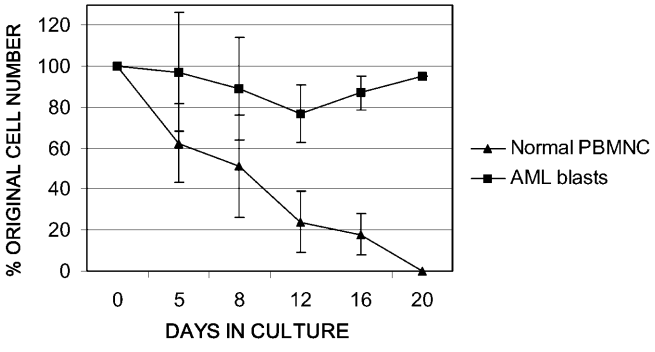

Fig. 1 Proliferation assays representing the change in PBMNC number from normal (▲) or AML patients (■) after culture in RPMI–1640 containing 10% FCS and 20 ng/ml IL-3, IL-6 and GM-CSF, for a period of 20 days, expressed as the percentage of original cell numbers. Cells were plated into 25-cm^2 TC flasks and the number of viable cells counted, using a haemocytometer, on days 0, 5, 8, 12, 16 and 20. Viability was assessed by trypan blue exclusion. Each value is the mean of seven patients ± SD

Table 1 Phenotype analysis of normal PBMNC, freshly isolated and after culture in IL-3, IL-6 and GM-CSF

CD antigen	Cells stained via antibody reactivity (% ± SD)	
	Fresh cells (day 0)	Day 16
Isotype control	0.5 ± 0.1	2.3 ± 0.1
CD11b	15 ± 6	0
CD33	23 ± 1	60 ± 18
CD34	0.6 ± 0.1	4 ± 3
CD80	0.8 ± 1	40 ± 3
CD80/CD34	0.3 ± 0.1	2.3 ± 1

~40% CD80. No, or very few, cells co-expressed CD34 and CD80 on day 0 and day 16. In contrast to samples of leukaemic origin, no PBMNC from normal patients remained by day 20.

Antigen expression on freshly isolated leukaemic blasts was heterogeneous (as described in Table 2). Although each AML blast profile varied, trends over time in culture were evident. These included an increase in the percentage of cells stained for CD33 and measurable co-expression of CD80/34 after 20 days in culture. There was little or no expression of CD80 on unmanipulated leukaemic cells (range 0–12%, see Table 2). Leukaemic blasts from all patients showed increased expression of CD80 after culture for 20 days (range 9–80%). Figure 2 shows a representative dot plot for uncultured and cytokine-modified AML cells, with the subsequent increase in the percentage of cells stained for B7-1. Similar findings were seen for the haemopoietic cell lines HL-60 and K562 after culture in media containing IL-3, IL-6 and GM-CSF, (CD80 expression not detected in either line prior to cytokine exposure, rising to 10 and 35% for HL-60 and K562, respectively, by day 9).

All leukaemic blasts expressed MHC antigens, with 82–97% (mean 89%) of freshly isolated cells expressing MHC class I and 22–94% cells positive for MHC class II (mean 58%). After 2 weeks in cytokines, the staining results were: MHC class I, 93–99% (mean 96%); MHC class II, 47–97% (mean 73%).

Cell cycle analysis

The cell cycle of leukaemic cells was determined at day 1 and day 10 of culture in medium containing cytokines ($n = 5$). For comparative purposes, PBMNC from normal patients were also analysed ($n = 3$). The retinoblastoma cell line Y79 was included as a positive control for proliferation. Table 3 describes the percentage of the total cell population in each stage of the cell cycle at day1 and day 10, (mean ± SE). The proportion of leukaemic cells in G2/M and S phase was higher for each time-point than for the PBMNC from normal patients. Further, the percentage of cells in these phases remained approximately the same over the 10-day period in culture. Approximately 10% of blasts remained in S phase. A drop in the percentage of PBMNC in G0/G1 over the time period was attributable to an increase in the proportion

Table 2 Phenotypic analysis of leukaemic cells from AML patients, freshly isolated or cultured with IL-3, IL-6 and GM-CSF for 20 days. *NA*, results not available; + +, strongly positive

Patient number	FAB sub-type	% blasts on day 0	CD13		CD14		CD11B		CD33		CD34		CD80	
			Day 0	Day 20	Day 0	Day 20	Day 0	Day 20	Day 0	Day 20	Day 0	Day 20	Day 0	Day 20
1	RAEB-T	9	14	NA	32	9	83	61	43	74	13	3	1	20
2	M4	10	73	NA	2	11	9	25	3	52	12	7	3	17
3	M4	2	89	NA	1	14	20	33	73	83	7	3	5	58
4	M2	85	88	NA	2	12	1	79	3	92	81	33	3	80
6	M0	14	NA	NA	0	4	1	33	5	45	82	64	1	77
7	RAEB-T	68	94	NA	2	32	7	18	68	98	70	9	3	17
8	M2	65	87	96	6	51	24	80	82	92	34	5	12	21
9	M2	49	79	98	0	35	4	60	5	89	75	25	0	9
10	M5	90	0	*	7	*	22	*	99	*	0	*	0	+ +
11	RAEB-T	3	21	42	12	25	12	31	7	34	5	1	1	22

* Autofluorescence too bright for flow cytometry; CD 80 determined by APAAP staining

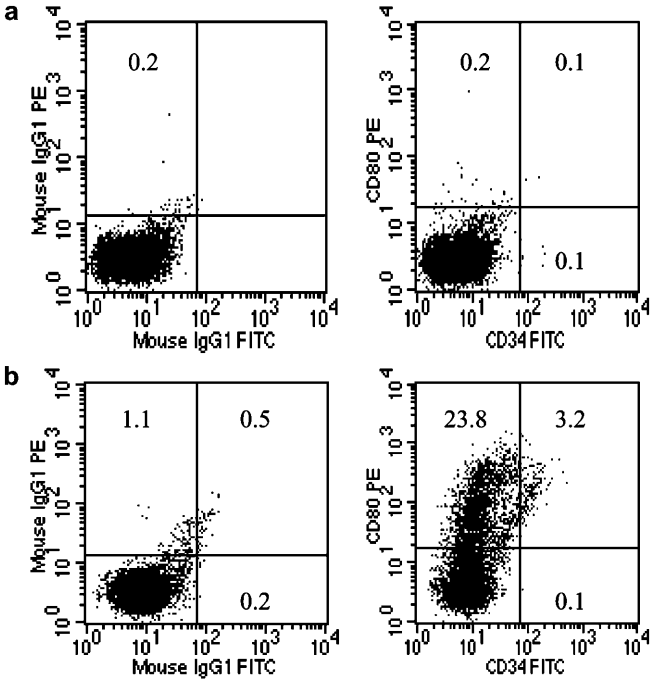

Fig. 2a, b AML blasts cultured in medium containing the cytokine combination IL-3, IL-6 and GM-CSF display upregulated expression of CD80 (B7-1). Ficolled peripheral blood from a patient with AML was cultured in RPMI–1640/10% FCS supplemented with 20 ng/ml IL-3, IL-6 and GM-CSF for 20 days. FACs analysis was performed on non-cultured (**a**, unmodified) or cytokine-cultured (**b**, IL-3, IL-6 + GM-CSF) AML cells from the same patient, after staining with conjugated monoclonal antibodies against surface antigens. Dot plots represent the isotype control or expression of CD34 and CD80

of cells undergoing apoptosis (data not shown). The abnormal profile of these is a further indication of the malignant origin of the cells remaining in culture.

Morphological changes

Following incubation in cytokines, leukaemic blasts enlarged to 20–30 μm in diameter, with abundant blue-grey cytoplasm containing occasional fine granulation. Their nuclei were large and round, with a single nucleolus. Aggregates of cells became apparent after one week in cytokines and became more numerous with time in culture. These changes were independent of the original FAB subtype.

Table 3 Cell cycle analysis of cells freshly isolated and after 10 days in culture with IL-3, IL-6 and GM-CSF

Cell type	Percentage of cells in growth phase (mean ± SE)		
	G0/G1	G2/M	S
AML blasts, day 1	74 ± 13	4 ± 1	9 ± 5
AML blasts, day 10	71 ± 18	3 ± 1	10 ± 5
Y79	35 ± 5	0	64 ± 5
PBMNC, day 1	98 ± 3	0	0
PBMNC, day 10	64 ± 5	0	0

Cytogenetic results

Two patients were identified with an abnormal karyotype from bone marrow metaphases at diagnosis, including 46,XX,der 14 (t13:14)(q10; q10) and 46,XX-10, + mar (where mar indicates chromosome material of unknown origin). The same cytogenetic abnormalities were identified on flow-sorted CD34/80-positive cells cultured for 20 days in medium containing IL-3, IL-6 and GM-CSF, further indicating the clonal origin of the cultured B7-1^{+} blasts.

Mixed lymphocyte reaction (MLR) to modified leukaemic cells

Autologous or allogeneic PBMNC were incubated under limited serum conditions alone, or with either unmodified leukaemic blasts or leukaemic blasts that had been cultured in cytokines for 20 days. Figure 3 reports the change in cell numbers per well in allogeneic (a) or

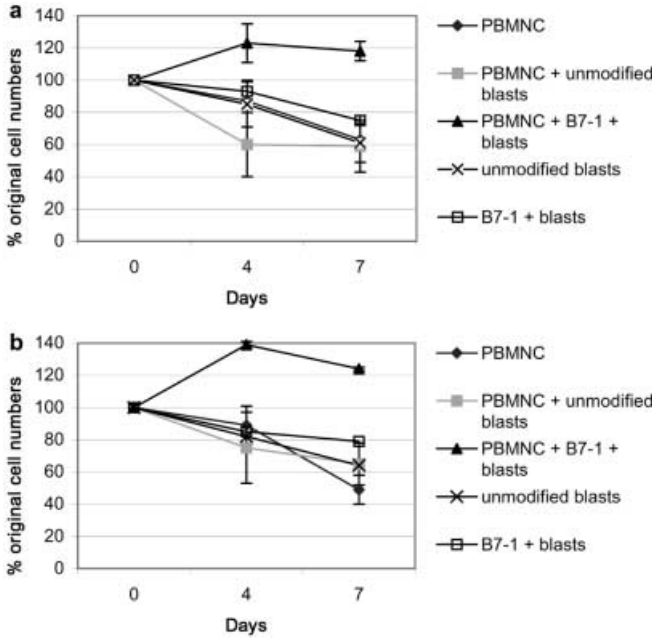

Fig. 3a, b IL-3, IL-6 and GM-CSF stimulated B7-1$^+$ blasts are the most effective stimulatory cells in a MLR. Allogeneic (**a**) or autologous (**b**) MLR were performed with either PBMNC alone (◆), or in co-culture with unmodified leukaemic blasts (■), or IL-3, IL-6, GM-CSF stimulated B7-1$^+$ blasts (▲) in round-bottomed 96-well plates over 7 days. MLRs were performed under limiting serum conditions of RPMI–1640/2% FCS. Other controls included unmodified (x) and B7-1$^+$ (□) blasts cultured alone. Each point represents the mean ± SD of triplicate wells for 3 patients

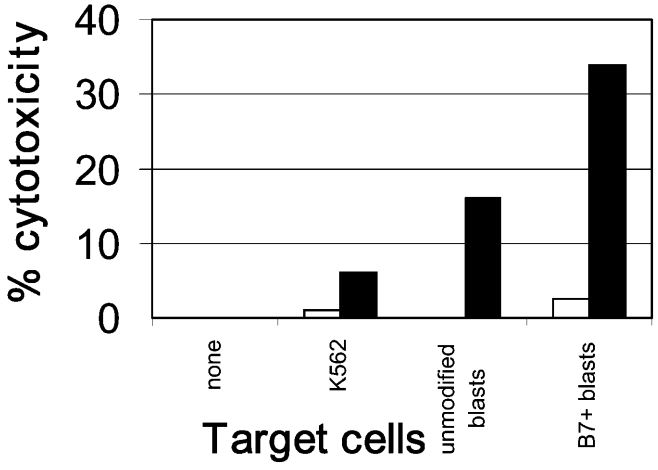

Fig. 4 Pre-exposure of T-lymphocytes to autologous B7-1$^+$ AML blasts prior to CTL assays increases the anti-tumour response against both modified and unmodified AML cells. Peripheral blood T-lymphocytes (>93% CD3$^+$) primed with cytokine-modified (B7-1$^+$) AML cells (■) were compared to T-cells with no priming (□) for their ability to lyse target cells in CTL assays. Primed T-cells were exposed for 3 days at a 3:1 ratio with B7-1$^+$ blasts. CTL assays were performed at an effector to target ratio of 10:1 in 200 µl RPMI–1640 containing 2% FCS for 6 h in phenol-red free medium. Spontaneous release by effector and target cells was controlled by separate incubation of the respective populations. The harvested cell-free supernatants were incubated with lactate dehydrogenase (LDH) substrate and absorbance measured as an indicator of cell lysis

autologous (b) experiments as compared with original cell numbers on day 0 (expressed as 100%). The minimal culture conditions of the MLR were unable to support the proliferation of PBMNC, unmanipulated blasts or B7-1$^+$ blasts cultured alone. The B7-1$^+$ blasts had been cultured prior to the MLR for 20 days, as described, at which time normal mononuclear cells were unable to survive (Figure 1). PBMNC numbers also gradually decreased over 7 days when they were co-cultured with unmodified leukaemic cells. In contrast, co-culture of B7-1$^+$ leukaemic blasts with PBMNC resulted in an increase in cell numbers between days 4 and 7 in both the allogeneic and autologous experiments (126% and 138% of original cell numbers, respectively). Immunophenotyping of these mixed cell populations before and at days 4 and 7 of co-culture, indicated that the expansion of cell numbers resulted from a 40% (+/− 21%) increase in T-lymphocytes (CD3$^+$, data not shown). These results show that the cytokine-modified blasts were enhanced stimulators of both allogeneic and autologous T-lymphocytes.

CTL assays

We examined the effect of priming T-lymphocytes with cytokine-modified B7-1$^+$ leukaemic cells prior to CTL assays. T-cells from peripheral blood (>95% CD3$^+$, data not shown) with no pre-treatment, exhibited minor killing of B7-1$^+$ leukaemic cells (2.5%) with no detectable lysis of unmanipulated blasts or K562 cells (Fig. 4). However, pre-exposure of the T-cells for 3 days prior to the CTL with B7-1$^+$ blasts, generated augmented cytotoxicity against both the target B7-1$^+$ blasts (34%) and the unmanipulated blasts (16%). Non-specific killing accounted for 6% of cytotoxicity in the latter experiments (Fig. 4).

Discussion

Leukaemic cells possess two of the three requirements for T-cell activation. They usually express MHC and appropriate cell adhesion molecules, but not co-stimulatory molecules. B7-1 stimulation of CD28 on T-cells by antigen-presenting cells is thought to be essential in the activation, proliferation and cytotoxic function of T-cells [1]. Failure of expression of such co-stimulatory molecules may be responsible for the failure to activate tumour-specific CTL.

Leukaemic cells have been successfully modified to express CD80 by transduction with the retroviral, and recently the lentiviral, vector for B7-1. Numerous models have illustrated the efficacy of using transduced B7-1 blasts in murine leukaemia models, resulting in protective and therapeutic immunity against established leukaemia [3, 16, 22, 33, 47], and more recently in murine

Philadelphia chromosome-positive acute lymphoblastic leukaemia (ALL) [44]. Further, transfection of CD80 cDNA into a human erythroleukaemic cell line has been demonstrated to stimulate an allogeneic response [31]. Similarly, Yang and co-workers [48] have shown that modification of melanoma tumour cells to express B7-1 resulted in a dramatic increase in autologous and HLA-matched allogeneic CTL responses.

This study has shown that significant upregulation of B7-1 expression by AML cells can be achieved with subsequently enhanced immunogenicity. Partial differentiation of leukaemic blasts has been induced by culture in GM-CSF, IL-3 and IL-6 and has resulted in upregulated co-stimulatory molecule expression in all patients examined. This finding is supported by a report of Hirst et al. [23], in which B7-1 expression was induced by culture of blasts from two patients with acute monoblastic leukaemia in a combination of GM-CSF, SCF, and IL-3. The differentiation of AML cells to a dendritic cell phenotype following culture in GM-CSF, IL-4 and TNF-α has been reported [4, 38, 40], confirming that the AML blast phenotype can be modified by in vitro exposure to cytokines and this may result in upregulation of their antigen presenting function. Mutis et al., [34] reported an increase in MHC class I and II on AML blasts cultured with GM-CSF, IL-4 and TNF-α, although in their hands there was no increase in CD80 expression.

Studies in various animal and in vitro human models have shown the induction of B7-1 expression on tumour cells is an efficient mechanism to increase their immunogenicity and subsequently confer protection against tumour rechallenge [2–4, 8, 15, 25, 32, 35, 43, 47]. In this study, the cytokine modified B7-1^+ blasts stimulated augmented proliferation of T-lymphocytes in both allogeneic and autologous MLR. Non-manipulated leukaemic cells did not activate T-lymphocytes in these assays. Furthermore, priming T-lymphocytes with B7-1^+ blasts greatly increased the CTL activity of autologous T-cells against both B7-1^+ blasts and, to a lesser degree, unmodified blasts. It is interesting that cytokine modified blasts produced an amplified cytotoxic response. This may have been due to the demonstrated increase in MHC class I expression, which could facilitate an increase in MHC/T-cell receptor interaction, or may be due to a direct amplification of effector response to increased B7-1 expression, although it is not currently reported to have this effect. The amplified cytotoxicity may also be due to unknown variables, such as a possible increase in tumour antigen expression as a result of culture in cytokines. These results indicate the increased antigen presenting function of the cytokine-modified leukaemic cells. A number of groups [4, 10] have reported that dendritic cells derived from AML cells by culture in GM-CSF, IL-4 and TNF-α or CD40 ligand have also stimulated autologous, anti-leukaemic T-cell responses. In our study, the blasts cultured in the GM-CSF, IL-3 and IL-6 combination were stimulated to express B7-1 and MHC classes I and II; they may also act as antigen-presenting cells with immune stimulatory ability, similar to the AML-derived dendritic cells.

In vitro, most blast cells from patients with AML have a low proliferation rate, since they are arrested in G1 [18] and are considered to have reached the end of their maturation [11, 41]. Only a small subset of leukaemic cells (the clonogenic cells) can be induced to proliferate and are less differentiated than their progeny [5, 6, 21, 50]. They have the capacity to undergo a limited, although abnormal, differentiation to non-proliferative cells [5, 21, 50]. We report here that approximately 13% of the peripheral blood AML blasts were in G2/M and S phase of the cell cycle, both when freshly isolated and after 10 days in culture with cytokines. Whilst AML cells depend on GM-CSF in vitro for proliferation [7], IL-6 has been shown to inhibit growth as well as inducing differentiation of myeloid cell lines and blasts [20, 42]. In this study, the proliferative effect of GM-CSF may have been counteracted by the inhibitory effect of IL-6, to produce malignant cells that were slowly cycling and differentiating. The change in blast phenotype from CD34 to CD33 expression, as well as the increase in cell size, indicates a process of maturation with culture in cytokines.

A number of lines of evidence highlight that the cultured B7-1^+ cells were derived directly from AML cells. First, normal PBMNC were demonstrated not to remain viable in culture for 20 days with this cytokine combination and after 10 days were not in the proliferative phase of the cell cycle, compared with 13% of AML cells in G2/M or S phase. Second, in both cases with an identical cytogenetic abnormality at diagnosis, the cultured AML cells retained that karyotypic abnormality. Third, there was a small proportion of B7-1^+ cells, which co-expressed the early differentiation marker CD34 at the end of the period in culture, that was not present in the normal populations. Similar observations have also been reported by Boyer et al. [4].

Myeloid regulatory proteins have been previously reported to induce in vivo terminal differentiation of leukaemic blasts [14]. GM-CSF and IL-3 are known to stimulate the most immature cells [30, 46] and induce rapid cell multiplication. IL-6, in combination with IL-3 and GM-CSF, has been shown to have a synergistic effect on peripheral blood AML blasts in the stimulation of blast colony formation [24]. The colony-forming cells have the capacity to undergo limited, although abnormal, differentiation to non-proliferative cells [5, 21, 28]. Interleukin-6 inhibits the growth of mouse myeloid leukaemic cells and induces their differentiation into macrophages or more mature myeloid cells [20, 42]. We have used this particular cytokine combination with the objective of stimulating differentiation of early-stage arrested myeloid blasts to a phenotype associated with antigen-presenting potential.

Due to the increased immunogenicity of the cytokine-modified AML cells described in this study, these cells may be useful in the context of immunotherapy. Despite a number of studies exploring the potential for gene

therapy vaccines (reviewed by Dunussi-Joannopoulous [15]), the need for a safe vector system remains. This study has highlighted the possibility of using modified tumour cells as antigen-presenting cells in a vaccine without the need for performing gene modification.

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