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# Differentiation Capacity of Null-AL(L) Cells in Culture *

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## A. Introduction

The cellular phenotype of the blast cells has remained unclassified in a small percentage of patients with acute leukemia (AL) (Greaves et al. 1983). Although markers are expressed on the cells, these are not sufficiently lineage specific to allow an unequivocal allocation of these null-AL cells to one of the defined myeloid and lymphoid cell lineages. We therefore investigated the differentiation potential of leukemic blast cells from patients with null-AL cells under in vivo and in vitro culture conditions to find out whether lineage-specific markers can be induced and whether differentiation is restricted to a single lineage or can occur along several lineages.

## B. Patients and Methods

Five patients with newly diagnosed acute leukemia were selected because of the absence of morphological and immunological features characteristic of a particular cell lineage. Peripheral blast cells were separated on a Ficoll-Isopaque (1.077 g/cm$^3$) gradient. For differentiation induction the blast cells were cultured in vivo within diffusion chambers (DC) implanted into the peritoneal cavity of host mice preirradiated with 7.5 Gy (Hoelzer et al. 1977). Chamber contents were harvested after 1, 7, and 14 days of DC culture and investigated for total and differential cell counts and for the expression of immunological cell markers.

In three patients the blast cells were cultured in vitro for 1–3 days in BM-86 Wissler medium (Boehringer, Mannheim, West Germany) supplemented with 50 μM 2-mercaptoethanol and 10% heat-inactivated fetal calf serum and incubated at 37 °C in a 5% $CO_2$ humidified atmosphere. During suspension culture the cells were exposed continuously to 5 n*M* 12-O-tetradecanoylphorbol-13-acetate (TPA) (Sigma, Munich), or 1 unit/ml porcine platelet-derived growth factor (PDGF) (Speywood Laboratories, Nottingham, England), or 10% medium conditioned by the Burkitt cell line X-308 (X308-CM) (Heit et al. 1983), 1 μ*M* retinoic acid (RA) (Sigma), or 1 m*M* butyric acid (BA) (Sigma). Appropriate control cultures were set up in parallel.

Cells attached to poly-l-lysine coated glass slides (Morich et al. 1983) were analyzed with a panel of antibodies using an immunoalkaline phosphatase technique. The monoclonal antibodies used are listed in Table 1. Surface $\mu$ was determined by a polyclonal antibody (Sigma). Cytoplasmic $\mu$ was detected by a peroxidase-antiperoxidase method.

## C. Results and Discussion

The objective of this study was to determine whether leukemic blast cells of a null-AL phenotype can differentiate in cul-

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**Table 1.** Monoclonal antibodies used in this study

| Selectivity | Designation | Reactive structure | Source |
|---|---|---|---|
| Hematopoietic progenitor cells | RFB-1 | | Bodger, Christchurch |
| Common ALL/lymphocyte progenitor associated | J 5 | p100 | Coulter |
| | BA-3 | p100 | Hybritech |
| T lineage associated | | | |
| Intrathymic subset | OKT 6 | | Ortho |
| Mature T | OKT 3 | p19–29 | Ortho |
| Helper T | OKT 4 | p62 | Ortho |
| Suppressor T | OKT 8 | p76 | Ortho |
| B lineage associated | BA-1 | p30 | Hybritech |
| | BA-2 | p24 | Hybritech |
| Granulocytic-monocytic lineage associated | 82 H 5 | | Janowska-Wieczorek, Edmonton |
| | B 4.3 | | Lansdorp, Amsterdam |
| | B 13.9 | | Lansdorp, Amsterdam |
| | VIM-D5 | | Knapp, Vienna |
| | MO-2 | | Coulter |
| Erythroid lineage | VIE-G4 | Glycophorin | Knapp, Vienna |
| Megakaryocytic lineage | C 17.28 | gp IIIa | Lansdorp, Amsterdam |
| Pan-leukocyte | T 29/33 | p200 | Hybritech |
| Anti-transferrin receptor | B 3/25 | p90 | Hybritech |
| HLA-DR "framework" | OKIa 1 | | Ortho |

ture and thereby reveal the cell lineage to which they are affiliated.

The results of differentiation induction by in vivo DC culture and in vitro suspension culture are given in Tables 2 and 3. No lineage-specific marker expression was found on the cells prior to culture.

The DC culture system, which has previously been shown to support proliferation and differentiation along the various myeloid (Hoelzer et al. 1977, 1981) and lymphoid (Lau et al. 1979) cell lineages, promoted lymphoid differentiation in two of five patients with null-AL (patients 1 and 2), leading to the expression of cALLA and of cytμ, respectively. Differentiation along the B cell lineage was probably already determined in the original blast cells of both patients, a supposition supported by the lack of expression of T cell markers during culture and recent findings by Korsmeyer et al. (1983) of rearranged $\mu$ heavy chain genes in the blast cells of most patients with cALLA-negative non-T, non-B ALL. In patient 1, the expression of markers in the in vitro suspension culture paralleled the results of DC culture, whereas myeloid (B 4.3) (Tetteroo et al. 1984) and stem cell markers (RFB-1) (Bodger et al. 1982) in addition to the lymphoid markers were induced in the cells of patient 2 only during suspension culture. In addition, the expression of 82H5, reported as a myeloid but also as a pluripotent stem cell marker (Janowska-Wieczorek et al. 1984), was further enhanced, indicating that a common lymphoid-myeloid progenitor might have been involved in the leukemic process in this particular case.

In addition to 82H5, during DC culture the cells of patient 3 sequentially expressed both lymphoid and myeloid markers, possibly due to sequential proliferation and maturation of two separate cell populations along two different lineages, whereas the cells of patient 4 mainly expressed 82H5 and lymphoid and myeloid markers were found only on a small number of cells. In patient 4 the growth pattern in DC culture was paralleled in suspension culture, where there was a marked increase in cells reactive with RFB-1 and 82H5 and appear-

**Table 2.** Differentiation induction in diffusion chamber (DC) culture[a]

| Pt | Days in culture | Cells/DC ($\times 10^{-5}$) | % Positive cells | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | OKIa | BA-1 | BA-2 | cALLA | cyt μ | 82H5 | B4.3 | B13.9 |
| 1 | 0 | 8.3 | 98 | 89 | – | – | – | – | – | – |
| | 7 | 2.5 | 99 | 45 | 18 | 58 | 4 | – | – | – |
| | 14 | 2.0 | 79 | – | 29 | 53 | – | – | – | – |
| 2 | 0 | 7.5 | 61 | 44 | – | – | – | 60 | – | – |
| | 7 | 1.0 | 40 | 42 | – | – | 17 | 66 | 5 | – |
| | 14 | 0.8 | 41 | 10 | NT | 4 | 60 | 77 | 4 | 3 |
| 3 | 0 | 5.5 | 45 | 97 | – | – | – | 15 | – | – |
| | 7 | 2.6 | 25 | 47 | 49 | 4[b] | – | 10 | 9 | – |
| | 14 | 8.2 | 24 | – | – | – | – | 82 | 78 | 42 |
| 4 | 0 | 8.0 | 90 | – | 73 | – | – | 9 | – | – |
| | 7 | 6.0 | 98 | 11 | 14 | 11 | 14 | 32 | 11 | 13 |
| | 14 | 5.8 | 98 | 3 | – | 8 | 5 | 68 | 10 | 2 |
| 5 | 0 | 5.1 | – | – | – | – | – | – | – | – |
| | 7 | 2.3 | – | – | – | – | – | 25 | 13 | – |
| | 14 | 2.6 | – | – | – | – | – | 36 | 38 | – |

[a] The percentage of blast cells on day 0 were 98% for patient 1; 95% for patient 2; 80% for patient 3; 97% for patient 4; and 99% for patient 5; cells were negative for RFB 1, OKT 6, OKT 3, MO 2, C 17.28, VIE G4

[b] 50% cALLA-positive on day 1

NT, not tested

**Table 3.** Differentiation induction of null-AL(L) cells in suspension culture

| Pt | Days in culture | Agent | Cells/ml ($\times 10^{-5}$) | % Positive cells | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | OKIa | BA-1 | BA-2 | cALLA | cyt μ | RFB 1 | 82H5 | B4.3 |
| 1 | 0 | – | 10.0 | 98 | 89 | – | – | – | – | – | – |
| | 3 | – | 8.9 | 87 | 50 | – | 59[a] | 28 | – | – | – |
| | 3 | TPA | 3.6 | 91 | 35 | – | 33[a] | – | 3 | – | – |
| 2 | 0 | – | 10.0 | 61 | 44 | – | – | – | – | 60 | – |
| | 3 | – | 11.2 | 97 | 8 | 48 | 42[a] | – | 44 | 100 | 26 |
| | 3 | TPA | 6.6 | 72 | 18 | 90 | 7[a] | NT | 65 | 100 | 52 |
| 4 | 0 | – | 10.0 | 90 | 4 | 73 | – | – | – | 9 | – |
| | 3 | – | 11.0 | 74 | – | – | 8[a] | – | 31 | 39 | 8 |
| | 3 | TPA | 3.0 | 93 | – | – | 18[a] | – | 38 | 55 | 26 |

[a] Weak but positive reaction on mononuclear cells

NT, not tested

ance of small but significant percentages of cells reactive with anti-cALLA and B 4.3, consistent with a differentiation arrest at the level of the lymphoid-myeloid progenitor cell. Finally, in patient 5 only myeloid markers, of doubtful significance, were found after DC culture.

The exposure of the cells to TPA in patients 1, 2, and 4 increased the percentage although not the absolute number of cells carrying myeloid markers (B4.3); however, the expression of cALLA was not influenced. Only in patient 2 did TPA induce the de novo expression of RFBI. While

PDGF, X308-CM, and RA did not significantly alter cell growth or marker expression, BA had a profound cytotoxic effect.

Incubation of the cells in glutaraldehyde prior to cell surface analysis, as used here, has been shown to increase the sensitivity of cALLA detection substantially (Kranz et al. 1984), which could explain the demonstration of cALLA in our studies as opposed to earlier ones. In vitro experiments have not previously succeeded in inducing expression of cALLA or cytoplasmic immunoglobulins in null-AL cells (Cossmann et al. 1982; LeBien et al. 1982; Nadler et al. 1982). The reason for successful induction of cALLA and cytoplasmic immunoglobulins reported here might be related to the conditions of the in vivo DC culture system used and to the differences in the in vitro suspension cultures, e.g., the different culture media, as well as to differences in the nature of the cells under investigation.

The induction experiments reported here reveal the heterogeneity within this group of unclassified leukemias, with some cases expressing only lymphoid or myeloid markers and other cases which, upon culture, simultaneously develop markers of lymphoid-myeloid progenitor cells as well as of both lymphoid and myeloid cells. Since we do not have selective markers for pluripotent stem cells and their malignant counterparts, and therefore have to rely on the behavior of the cells in culture (Greaves et al. 1983), expression of both lymphoid and myeloid markers could well imply that in these cases common lymphoid-myeloid progenitor cells were involved in the malignant transformation event with subsequent block of differentiation.

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