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Studies on Normal B-Cells and Common Acute Lymphoblastic Leukemia Blast Cells Using a Colony Assay

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Human B-cell populations contain a subset of cells that are capable of forming colonies in culture [1]. This in vitro assay permits the study of growth and differentiation of the clonogenic cells both in normal and various malignant diseases of the B-cell lineage. It is also applicable to common acute lymphoblastic leukaemia (cALL) [2] and B-cell chronic lymphocytic leukaemia [3]. The specificity of the assay depends on exhaustive depletion of T-cells from B-cell containing populations because the conditioned medium used in the assay (PHA-TCM) also contains factors that promote T-cell colony formation. PHA-TCM is prepared from purified T-cells cultured in the presence of 1% phytohaemaglutinin for 3 days. Finally, normal T cells must be included as feeder cells in the culture mixture, after treatment with mitomycin-C or radiation; myeloma cells are an exception as they do not require feeder cells [1, 4]. Methyl cellulose is used as viscous support medium; alpha-MEM or Iscove's medium (GIBCO) and fetal calf serum complete the culture mixture. Colonies are scored after 7 days of culture.

In this paper we present preliminary data on some of the characteristics of the cells that give rise to B-cell and common ALL colonies.

Blood cells were obtained from normal volunteers and separated using density gradients, sheep red cell rosetting and adherent cell depletion techniques as previously described [1]. A T-cell rich fraction (E+ cells) and a B-cell rich fraction (E^- cells) depleted of T cells (<2%) and adherent cells were obtained.

The E+ cells were used as source of feeder T cells and to prepare PHA-TCM. The B-cell rich fraction contained 20%–50% B cells. This fraction was further separated using the fluorescent-activated cell sorter (FACS 1) and two markers specific for B-lymphocytes, a polyvalent goat anti-human immunoglobulin conjugated with fluorescein to label surface immunoglobulin-(SIg) positive B cells and a monoclonal antibody (B1) that reacts with all blood B-lymphocytes [5] (Coulter Electronics Ltd, Hialeah, Florida). The monoclonal antibody was developed with a goat anti-mouse immunoglobulin fluorescein-conjugated.

Cells from the unsorted fraction, the purified B-lymphocytes (sIg+ or B1+ cells) and a B-cell depleted (sIg- or B1- cells) fraction were cultured using the B-cell colony assay. After 7 days in culture, the colonies were counted and then analysed for cell markers. First, the cytoplasm of cells from individual colonies from the sIg+ cell fraction were simultaneously stained with a mixture of a goat anti-human kappa rhodamine-conjugated and goat anti-human lambda fluorescein-conjugated. Each colony was scored twice for red or green fluorescence using fluorescence microscopy. In a first experiment 17 of 22 colonies were kappa (+) only and 2 of 22 colonies were lambda (+) only. In a second experiment 14 of 24 colonies were kappa (+) only and 8 of 24 were lambda (+) only. These results suggest that each colony derives from a single cell B-cell precursor, therefore fulfilling an important requisite for a clonogenic assay.

Table 1. Monoclonal antibodies

Antibody	Specifity	Source	Reference
J5	Common ALL antigen	Coulter	[12]
B1	B-cell specific	Coulter	[5]
UCHT-2	Pan-T cell	P Beverley	[7]
UCHT-1	Mature T cell	P Beverley	[8]
OKT11	Sheep Red Cell Receptor	Ortho	[6]
Leu 2a	Suppressor Cycotoxic T cells	Beckton-Dickinson	[11]
Leu 3a	Helper-Inducer T cells	Beckton-Dickinson	[11]
Leu 7	NK cells	Beckton-Dickinson	[9]
OKM1	NK cells, monocytes	Ortho	[10]

Table 2. Fluorescent-activated cell sorter analysis of B-cell colony forming cells

Antibody	Experiment no.	Cell sorter Fraction	No. of colonies per 2×10^4 cells	Colonial cell phenotype
Surface				
Ig	1	Control	237	n.t.[a]
(polyvalent)		Negative	102	n.t.
		Positive	332	*B cells only*[b]
	2	Control	130	n.t.
		Negative	51	n.t.
		Positive	215	*B cells only*[b]
B1	1	Control	107	B+T cells
(monoclonal)		Negative	157	T cells only
		Positive	99	B cells only
	2	Control	192	B+T cells
		Negative	162	T cells only
		Positive	115	*B cells*

[a] n.t., not tested
[b] Used to determine kappa and lambda distribution in single colonies

Secondly, colonies from each fraction were collected, pooled, and a single cell suspension was prepared and stained with B- and T-cell markers (Table 1). The results shown in Table 2 reveal that pure B-cell colonies are obtained only in positively purified B-cell fractions (sIg+, B1+), indicating that the B-cell colony forming cell in blood has a mature B-cell phenotype with sIg and the B1 antigen on its surface. The table also shows that T-cell growth occurs in unsorted E^- cells, suggesting that this level of cell separation in normal blood is not sufficient to obtain pure B-cell colonies. The colonies obtained from the B-cell depleted B1(–) cells do not react with B-cell markers but react with some pan-T cell markers, OKT11 (94%) and UCHT-2 (70%) [6, 7], but not with a mature T-cell marker, UCHT-1 (3%) [8], suggesting that these colonies contain cells belonging to a different T-cell subset. Markers of NK cells (natural killers) are found amongst these cells, Leu 7 (8%) [9], and OKM1 (24%) [10] as well as markers of helper T cells [11], Leu 3a (48%). No Leu 2a (suppressor) [12] positive cells were detected. Further analysis is required to confirm these findings and to determine if these T-cell colonies derived from contaminating T-cells or from T-cell marker negative cells.

Blood cells from patients with common ALL were depleted of T cells as described above [2] and then further separated in the cell sorter using a monoclonal antibody (J5) against the cALL antigen. cALL-positive (J5+) and cALL-negative (J5–) fractions were collected. In two experiments

the unsorted, the J5– and the J5+ fractions all gave rise to cALL colonies (Experiment 1: 295, 201 and 235 colonies per 4×10^4 cells; Experiment 2: 105, 96 and 126 colonies per 4×10^4 cells).

In summary, (a) B-cell colonies from normal blood cells arise from single cells that have a mature B-cell phenotype: surface Ig+, B1 positive; (b) the common ALL antigen is not a marker of all common ALL clonogenic cells, and (c) T-cell colonies arise from B- and T-cell depleted fractions; they may belong to a subset of T-cells that includes NK cells.

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