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ESTABLISHMENT AND CHARACTERIZATION OF A B-CELL LINE FROM A PATIENT WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Abstract—A permanent lymphoblastoid cell line was established from the peripheral blood of a child with acute lymphoblastic leukemia. The cell line, designated SDK, grows in a stationary suspension culture, forming aggregates, in RPMI medium supplemented with 10% FCS, with a doubling time of 50–60 h.

Immunologic markers and cytological features suggested that the SDK cells should be identified as being of B-cell origin. The cells failed to form rosettes with sheep erythrocytes, did not express T-cell antigens as defined by monoclonal antibodies, and exhibited surface and cytoplasmic immunoglobulin determinants. Chromosome analysis revealed the presence of three cell populations with (a) 46XY; (b) *t*(8q−; 14q+) or (2p−; 14q+) and (c) cells with unidentifiable markers. SDK demonstrated susceptibility to TPA-induced differentiation toward plasma cells.

Key words: Acute lymphoblastic leukemia, B-cell line.

INTRODUCTION

CONTINUOUS cell lines from human peripheral blood leukocytes derived from normal individuals, leukemia or lymphoma patients can be established *in vitro* after exposure to Epstein-Barr virus (EBV) [7, 10, 19, 23, 37]. These cell lines have been shown to be of B-cell origin. Establishment of human T-cell lines has been successful the last few years especially by using the T-cell growth factor [16, 17, 28]. The cultivation and maintenance of B-cell lines *in vitro*, without the interference of EBV, has been considered to be an arduous result [34]. Factors, other than EBV, enabling *in vitro* growth of such cells, are not known at present.

Human lymphoma and leukemia cell lines have been cultured for long or short periods *in vitro*, and have shown considerable diversity regarding the maintenance of morphological cytogenetical and/or cytochemical features of the original lymphoma or leukemic cells [6, 8, 20, 21, 23, 34, 38, 39, 43, 44, 46]. This phenomenon possibly concurs with the variety of cell clones observed in the corresponding patients [3, 42, 49]. The majority of B-cell lines established so far have been reported to exhibit EBV antigens [10, 21, 38, 46]. Here we report on the establishment and characterisation of a continuous B-cell line (SDK) derived from a child with ALL.

Abbreviations: FCS, fetal calf serum; *TPA*, 12-*O*-tetradecanoyl phorbol-13-acetate; *EBV*, Epstein-Barr virus; *ALL*, acute lymphoblastic leukemia; *IF*, immunofluorescence; *PNA*, peanut agglutinin; *PBS*, phosphate buffered saline; *TdT*, terminal deoxyribonucleotidyl transferase; *αNAE*, α-naphthyl acetate esterase; *NASDA*, naphthyl acetate esterase; *AcP*, acid phosphatase; *βGLU*, β-glucuronidase; *Alkp*, alkaline phosphatase; *PAS*, periodic acid Schiff; *EBNA*, EBV-associated nuclear antigen; *EA*, early antigen; *VCA*, viral capsid antigen.

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MATERIALS AND METHODS

Case report

A six-year-old boy was admitted to the hospital for a tonsilectomy. His blood picture was normal upon leaving the hospital. Fifteen days later he complained of pains in the left cheek and ankles and developed fever of 38.5°C. Soon, he developed a left tibia mass and otitis and he was given antibiotics. His condition showed no improvement and, upon examination by his paediatrician who felt the spleen, he was re-admitted to the hospital.

Upon admission, his physical examination revealed small cervical lymph nodes bilaterally and hepatosplenomegaly. The laboratory investigation disclosed WBC 80. O × 10^9/1 (most of which were lymphoblast of the L_2 type), Hg 10.5g/dl and platelets 50 × 10^9/1. Based on these findings the diagnosis of acute lymphoblastic leukemia (ALL) was made, and the child was placed on ALL antileukemic therapy. A month later he went into complete remission.

Establishment of the 'SDK' cell line

Peripheral blood was obtained when the child was admitted to the hospital and before any therapy. Lymphoid cells were separated by Ficoll-Hypaque gradient centrifugation, washed three times with RPMI 1640 and transferred in a plastic tissue culture bottle (25 cm^2). The number of cells was adjusted to 2 × 10^6 per ml in RPMI 1640 supplemented with 15% fetal bovine serum. The medium was changed every 6 days for the first two months where cell growth was observed, and cell culture was split. Cells showed extensive growth and they have since been maintained in culture (16 months) by replacing one third of cell suspension with medium every 3–4 days. Most of the tests reported here were performed on cells during their 2–4 months in culture.

Surface markers

E rosette assay and receptors for the Fc IgG (FcγR) were detected as previously described [26]. Table 1 lists the murine monoclonal antibodies (MAbs) used in this study with their cellular and/or molecular selectively. All MAbs were used at a final dilution of 1 : 1000 in an indirect immunofluorescence (IF) assay using goat IgG labeled with FITC directed against mouse IgG, at a final dilution of 1 : 20.

The presence of surface immunoglobulins (light and heavy chains) was detected by a direct IF test using FITC labeled specific rabbit antisera to human κ, λ, μ, γ, δ, α chains and total immunoglobulins at a final dilution of 1 : 20. Peanut agglutinin (PNA) binding was detected by a direct IF assay using peanut lectin conjugated with FITC (P-L Biochemicals Inc Milwaukee WL, U.S.A.) at 1 : 200 final dilution of a stock solution of 0.4 mg/ml. For simultaneous PNA and surface IgM labeling, cells reacted first with rabbit anti-human IgM at a final dilution of 1 : 10 and then with goat anti-rabbit IgG-TRIC, (DAKO, Denmark) at a final dilution of 1 : 30 and PNA-FITC.

TABLE 1. MABs USED FOR THE CHARACTERIZATION OF THE HUMAN SDK CELL LINE

MABs	Receptor	References
OKT-3	Pan-T	15
T-28	Pan-T	2
DA	Specific for Ia	5
OKT-11A	Pan-T/anti-SRBC receptor	38
T-5	Specific for cALL	29
OKT-4	Helper/induced T subset	27
OKT-8	Suppressor/cytotoxic T subset	35
OKT-6	Cortical thymocyte	26
WT-1	Pan-T	34

Induction of differentiation

12-*O*-Tetradecanoyl phorbol-13-acetate (TPA) was used as an inducing agent at a final concentration of 16 nM. Exponentially growing SDK cells with an initial cell density of 0.5 × 10^6 cells/ml were treated with agent for three days.

Immunoglobulin production

Ig production was determined by direct haemagglutination of RBC coated with rabbit IgG antibodies against human α, γ, μ and δ heavy chains (DAKO, Denmark) by the chromic chloride method [12]. For the quantitation of the secreted Ig a solid phase RIA was performed with human Ig [the 50% $(NH_4)_2SO_4$ precipitated fraction of

NHS], coupled to CNBr activated-sepharose 4B as a carrier [48]. For the assay standard human IgM concentrations (Bergin) (diluted in growth medium) or supernatant from SDK cultures with known cell density, were mixed in polystyrene tubes with ^{3}H-rabbit IgG directed against heavy and light chains of human Ig at a final volume of 0.5 ml and incubated for 4 h at room temperature. A pretested amount of Sepharose 4B-CNBr/Ig beads was added and the incubation continued at 4°C with constant shaking. Subsequently, the tubes were washed 5 times with cold PBS, the pellet transferred to 5 ml instagel and counted in a β-counter. A standard curve was used for the estimation of the Ig concentration. The per cent inhibition of the binding of the ^{3}H-anti-human Ig on the Sepharose 4B-CNBr/Ig beads caused by the SDK supernatant was compared to the per cent inhibition caused by the known concentrations of human Ig.

Cytochemical assays

Terminal deoxyribonucleotidyl transferase (TdT) content of SDK cells was detected by an indirect IF assay using specific rabbit anti-TdT antibody and stained with FITC goat F(ab)$_2$ anti-rabbit Ig (Bethesda Research Lab., Bethesda, MD, U.S.A.). Cytoplasmic Ig was detected on cytocentrifuged smears by a direct immunofluorescent method.

Staining for Sudan black B, peroxidase, α-naphthyl acetate esterase (αNAE), naphthyl acetate esterase (NASDA), NASDA with sodium fluoride inhibition (NASDA + NaF), acid phosphotase (AcP), AcP with tartaric acid inhibition (AcPT), β-glucuronidase (β-GLU), alkaline phosphatase (Alkp) and periodic acid Schiff (PAS) were carried out using known cytochemical methods [25].

Cytogenetic studies

Cells obtained 24 h after refeeding were treated with colcemid (0.2 μg/ml) subjected to hypotonic treatment (0.21% KCl, 0.17% NaCl) for 40 min at 37°C and fixed in methanol-glacial acetic acid (3 : 1). Air dried metaphase preparations were stained with Giemsa after trypsin banding [36].

Presence of the EBV-antigens

Detection of EBV-associated nuclear antigen (EBNA) was carried out according to the method previously described [29]. The expression of early antigen (EA) and viral capsid antigen (VCA) of EBV was tested by the immunofluorescence method [14].

RESULTS

After two months *in vitro* lymphoblast-like cells started to grow well in suspension and formed loose clumps. Cells seeded in culture flasks at 6×10^4 cells/ml reached a density of 3×10^5 cells/ml within 7 to 8 days. The doubling time of the cells was 50 to 60 h. SDK cells were round ranging in diameter from 16 to 30 μm, with large usually round, nuclei with fine chromatin, containing 1 to 3 large round eosinophilic nucleoli, with abundant and deeply basophilic cytoplasm when stained with May-Grunwald Giemsa (Fig. 1.). These morphological features preliminarily indicated that the cells were of B-cell origin according to Parker *et al.* [27].

Table 2 summarizes the results of surface and intracellular marker tests. SDK cells lack common surface receptors and other immunological markers such as E-rossettes, OKT 3, 4, 6, 8 and 11 and WT-1 specific for T-cells and T-cell leukemias.

Cells were positive for surface and cytoplasmic κ light and μ heavy chains. Presence of α, γ and δ heavy chains was not detected. Cells secreted IgM as identified by direct haemagglutination and the amount of the secreted IgM by 1×10^6 cells in 24 hr was about 100 ng/ml. Ia/Da antigen was expressed in a high percentage of SDK cells. They also bound PNA and in double labelling with surface IgM it was found that a small percentage of IgM positive cells bound also PNA.

Table 3 lists the effects of 16 nm TPA on SDK cells, treated with the inducing agent for 3 days. The percentage of cells with surface IgM decreased considerably, whereas the percentage of cells with cytoplasmic IgM increased. The FITC staining of the cytoplasmic IgM was more intense in the treated cells than in the controls, indicating an increase in IgM content. Treated cells secreted more IgM than control cells. Ia/DA and PNA binding did not change. Most of the TPA treated cells adhered to the surfaces of the plastic culture flasks and the proliferation rate decreased.

The results of the cytochemical reactions are listed in Table 4. Nearly 100% of the cells were αNAE and AcP positive. Both reactions revealed an intense focal end product; mainly localized in a paranuclear cytoplasmic area. Cells were also positive with the PAS reaction in a granular form. TdT, as well as other enzymes tested, was uniformly negative.

TABLE 2. CELL SURFACE AND CYTOPLASMIC MARKERS OF SDK CELL LINE

Tests	Results
J-5	—
OKT-3	—
T-28	—
OKT-11A	—
OKT-4	—
OKT-8	—
WTI	—
OKT-6	—
E Rossettes	—
DA (Ia)	88% cells
Fc (OXIgG)	—
Fc (AggIgG)	—
IgM (cytoplasmic)	39% cells
κ (cytoplasmic)	30% cells
λ (cytoplasmic)	10% cells
IgM (surface)	67% cells
κ (surface)	75% cells
λ (surface)	—
PNA	40% cells
PNA and IgM	10% cells
IgG (surface and cytoplasmic)	—
IgA (surface and cytoplasmic)	—
IgD (surface and cytoplasmic)	—

—, Negative.

TABLE 3. INDUCTION OF DIFFERENTIATION IN SDK CELL LINE

	Control cells	TPA cells treated
Cytoplasmic*		
Ig	39%	61%
κ	30%	58%
λ	10%	10%
Surface*		
Ig	67%	5%
κ	70%	10%
λ	0	0
Ia	77%	79%
PNA	40%	46%
Secreted†		
IgM	105	148

*Percent positive cells determined by IF.
†ng/ml secreted IgM by 1×10^4 cell.

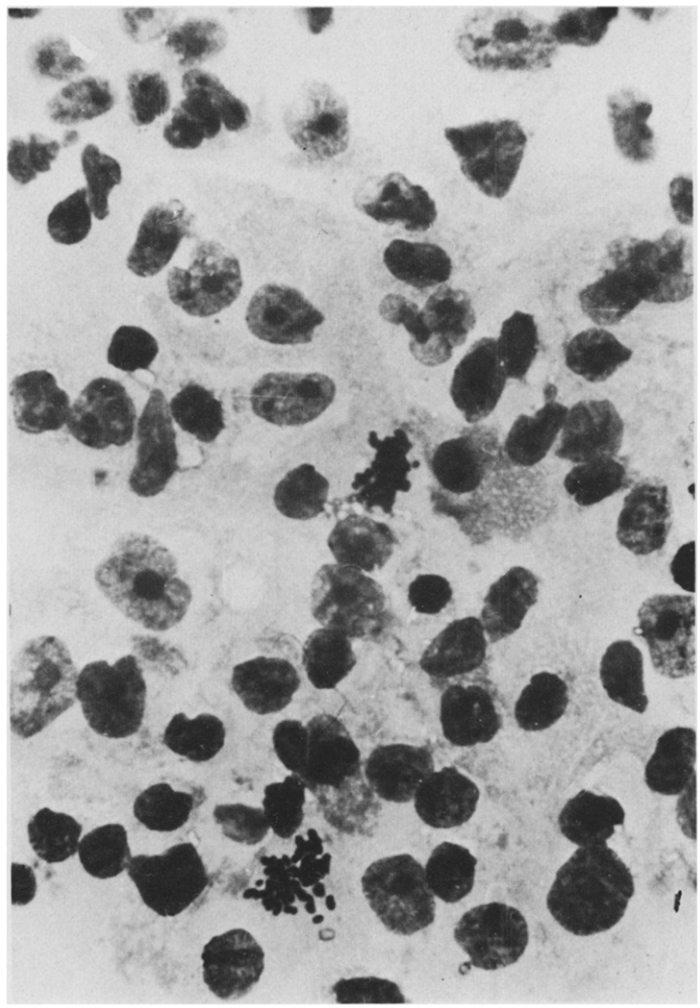

FIG. 1. Morphological characteristics of SDK cells in culture. Cells are round or polygonal with large nuclei and large round nucleoli. Giemsa, × 390.

TABLE 4. CYTOCHEMICAL FINDINGS IN SDK CELL LINE

Reaction	Result	Comments
Peroxidase	–	
SBB	–	
αNAE	+	strong focal positivity
NASDA	–	
NASDA + NaF	–	
AcP	+	strong focal positivity
AcPT	–	
βGLU	–	
Alkp	–	
PAS	+	granular cytoplasmic positivity
TdT	–	

SBB, Sudan black B; αNAE, α-naphthyl acetate esterase; NASDA, naphthol AS-D acetate-esterase; NaF, sodium fluoride; AcP, acid phosphatase; AcPT, acid phosphatase + tartrate; βGLU, β-glucuronidase; Alkp, alkaline phosphatase; PAS, periodic acid-Schiff; TdT, terminal deoxyribonucleotidyl transferase. –, Negative; +, positive.

The SDK cells after 4 months in continuous culture consisted of 3 cell type populations; (a) cells with a normal 46XY karyotype represented about 60% of the total cell population; (b) cells with a total of 46 chromosomes exhibiting a *t*(8q–; 14q+) or a *t*(2p–; 14q+) translocation represented about 14% of the total cell population and (c) cells with numerous chromosomal translocations producing unidentifiable markers represented about 25% of the total cell population. Extra long markers were observed frequently in these cells. Chromosome loss was pronounced in all three groups of cells (Fig. 2). Detailed cytogenetic analysis of SDK cells is in progress.

No EBNA staining was detected and the cells did not express EA or VCA.

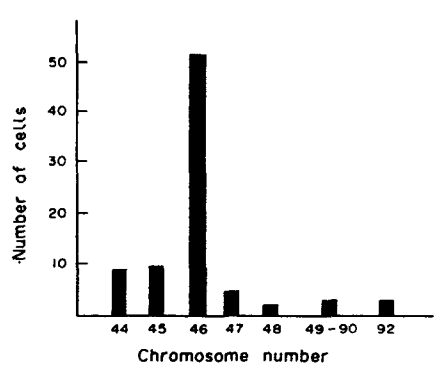

FIG 2. Histogram of chromosome number distribution in SDK cells, at 25th passage.

DISCUSSION

Classification of lymphoblastoid cell lines based on lymphocyte surface markers [13, 15] used in leukemias was also employed in this study. Most of the markers characterizing T cells such as E-rosetting, OKT-3, T-28, OKT-IIA, WT-1, OKT-4, OKT-8, were absent from the SDK cell line. Furthermore, these cells were not stained with a monoclonal antibody (J-5) specific for non-T non-B nule lymphocytes. In contrast the cells exhibited positivity with anti-Ig antisera and anti-Ia indicating that they belong to the B cell lineage. The presence of cIgM, $\varkappa$ light chain as well as the secretion of IgM, showed that a fraction of the cell population had already matured to plasma cells [2]. Cytoplasmic IgM without the light chains has been shown to be present in pre-B cells [47]. It has been reported that cell lines derived from human leucocytes and leukemic blasts bear $\varkappa$ light chain and δ and μ heavy chains but these cell lines were EBV positive and they were not positive for cytoplasmic immunoglobulin [21].

TPA-induced differentiation of the SDK cells towards plasma cell with an increase of cIgM staining and secretion and a decrease of the proportion of cells with sIgM positive was shown. Fc receptors, which are usually present on B cells were lacking from our cell line. This is perhaps due to the loss of Fc receptors during the differentiation of B cells into plasma cells.

PNA which interacts specifically with D-galactosyl residues has been shown to bind with cortical thymocytes but not with mature T cells [32]. Based on this finding it has been suggested that PNA can be used as a differentiation marker of T cells. However, a subsequent paper reported that PNA also binds to leukemic cell lines of B cell origin [11]. Our results agree with the later observation.

The study of marker chromosomes in leukemic cells in culture aim to establish a correlation between chromosomal alterations and the neoplastic process [3, 42]. Such studies are complicated due to the rapid karyotypic changes occurring during prolonged cultivation *in vitro*. The presence of three cell populations in the SDK cells with different chromosomal constitutions suggests an active process of re-arrangements and genetic instability towards adaptation in the *in vitro* environment. The presence of 14q+ marker in a fraction of the cell population studied and its connection with leukemic cells [3, 34, 42] suggests that at least this fraction of the SDK cells should be considered as a leukemic determinant, apart from the immunological properties. Abnormalities involving chromosome 14 have often been connected with a variety of lymphoid cancers. *t*(8q−; 14q+) has been found in Burkitt lymphoma [1, 49] in ALL with B cell markers and in L_3 type leukemia [3, 9, 34].

Involvement of chromosome 2 has also been found in an ALL patient with the L_3 type [35]. Our results agree with these reports and further suggest a relationship between chromosome 14 abnormalities and lymphoid leukemia with B cell characteristics.

The cytochemical findings of a strong and focal AcP and αNAE positivity of our lymphoblastic cell line is of considerable interest in view of the fact that this pattern of positivity has been frequently seen in T-lymphoblastic proliferations. Nevertheless, AcP positive cases with a similar pattern have also been observed in a few cases of common ALL, null-ALL, pre-T-ALL and pre-B-ALL [25]. The latter observations and our present findings suggest that more information would be needed in order to determine the precise validity of the AcP and αNAE reactivity in the various subpopulations of proliferating lymphoblastic cells. The PAS positivity observed cannot be of significant value in the further characterization of these cells since previous studies have indicated that this reaction may vary from negative to strongly positive in acute lymphoblastic leukemias [3, 4, 25].

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