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Patterns of Cell Surface Differentiation of cALL Positive Leukemic Blast Cells in Diffusion Chamber Culture

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A. Introduction

The majority of acute lymphoblastic leukemias (ALL) can be characterized by the presence of the common ALL antigen (cALLA) (Greaves et al. 1977). Since this cell marker is only rarely found on bone marrow cells of healthy individuals, the normal cellular equivalent of leukemic cALL positive blasts still remains unknown. However, some observations support the hypothesis that these cells might represent a common stem cell of the T- and B-cell lineage (Janossy et al. 1976). In order to explore a possible relationship between the cALLA and a certain developmental stage during lymphatic ontogeny, peripheral cALL blasts from four children and cells of the Reh line were cultured in diffusion chambers (DC). During the culture period membrane markers were investigated to determine differentiation in either the T- or B-cell axis.

B. Material and Methods

Peripheral blood samples obtained from four untreated ALL patients between 4–11 years of age were separated on an Isopaque-Ficoll gradient. In all cases, leukemic blasts constituted at least 95% of these mononuclear cell fractions, the rest being normal lymphoid cells. The Reh cells were taken from the continuously maintained line which originally had been established from an ALL of the "non-T, non-B" type (Rosenfeld et al. 1977).

Diffusion chambers were filled with 5×10^5 cells of the respective material as already described previously (Lau et al. 1979). Two chambers were inserted into the peritoneal cavity of each CBA mouse preirradiated with 700 rad. At different instances during a 13- or 16-day culture DC were removed and were shaken in an 0.5% pronase solution. In case of the Reh line two experiments of this type were conducted, each lasting 20 days.

Besides total and differential counts the majority of the chamber harvest was processed for cell surface characterization with specific antisera (Rodt et al. 1975). Direct immunofluorescence was performed after the cells had been labeled with polyvalent anti-human immunoglobulin (AIg) or the $F(ab)_2$ fragment of AIg, anti-cALL globulin (AcALLG), and anti-T-cell globulin (ATCG). For double labeling two different antisera were mixed simultaneously with the cells. Cytoplasmic Ig (cIg) was investigated after sedimentation of the cells onto glass slides and fixation in ice cold methanol followed by incubation with antibodies in a moist chamber. In addition, we tested the ability of the cells to form E-rosettes (Bentwich and Kunkel 1973) as well as rosettes with mouse erythrocytes (Forbes and Zalewski 1976).

C. Results and Conclusions

After 6 days of ALL culture, the chamber content had dropped considerably. No further increase of the total cell number was observed throughout the culture period. Cells of the Reh line, on the other hand, grew exponentially from day 3 until the end of both experiments. The types of cells observed during ALL cultivation were mainly leukemic blasts and, to a smaller extent, normal appearing lymphoid cells, macrophages, and granulocytic cells. The morphology of Reh cells resembling that of undifferentiated blast-like cells remained unchanged.

Cell marker analysis revealed remarkable surface changes (Fig. 1). In two patients, T.H. and V.M., the cells had the typical appearance of a cALL on day 0 (Jäger et al. 1979). In both cases the cells developed surface Ig (sIg), mostly in combination with the cALLA

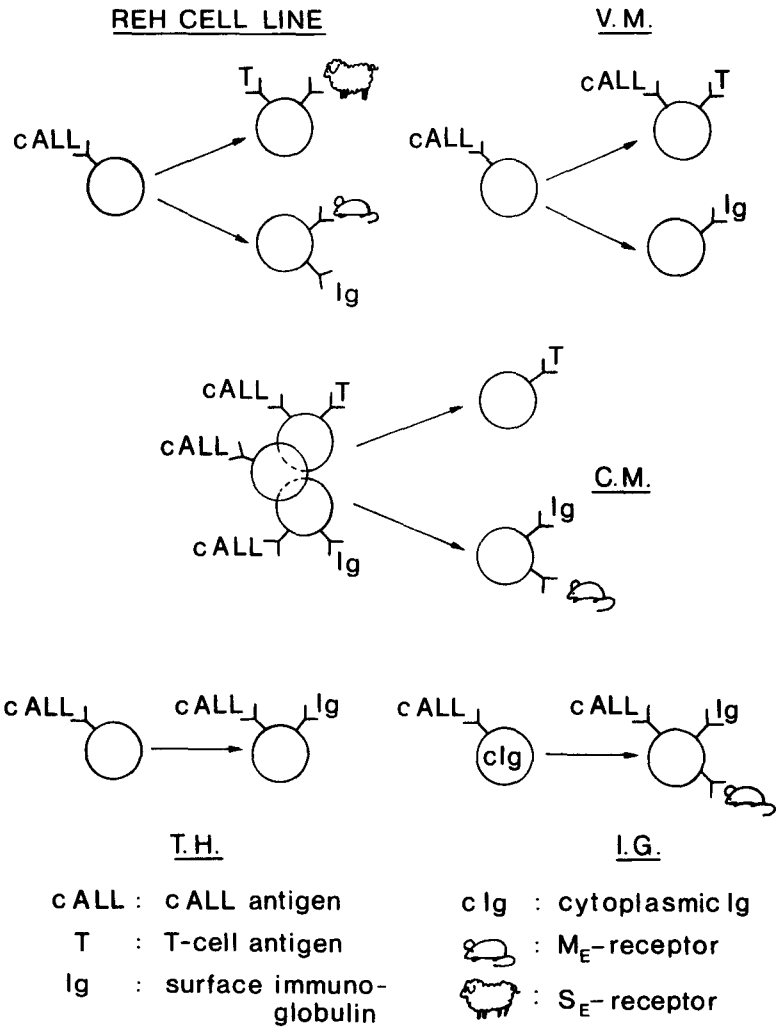

Fig. 1. Changes in the membrane phenotypes of cALL positive blasts from four children and of the Reh cells during culture in DC

(70%/75/%). Furthermore, a fairly constant portion of cells (24%) carrying the cALLA together with the T-cell antigen was seen from day 6 on in V.M.

In addition to 50% of the chamber input showing the cALLA alone, 28% of the cells in patient C.M. could already be characterized by double labelling with AcALLG and AIg(Fab)$_2$ on day O (Jäger et al. 1979). At the same time 23% of the cells expressed the cALLA together with the T-cell antigen. More mature descendants of these cells with only sIg or the T-cell antigen were already seen early in the culture. A receptor for mouse erythrocytes known to be a B-cell characteristic (Forbes and Zalewski 1976) was detected in this case in 6% of the cells on day 0 and increased up to 30% on day 9.

No change in the percentage of cALL positive cells could be registered during the whole culture period in patient I.G. (25%–33%); cIg was demonstrated in more than 90% of the blast cells already on day 0. At the end of the culture, sIg was found together with the cALLA in 30% of the cell harvest. This change was accompanied by the occurance of 33% of mouse rosette forming cells (M-RFC). With regard to the phenotypic characteristics of the Reh line cells (Lau et al. 1979) the cALLA was the only membrane marker found at the beginning of both experiments (10%/70%). Towards the end of the first culture period 70% T-positive cells developed along with 14% E rosetting cells. In the course of the second culture Reh cells could onyl be classified as belonging to the T-cell series according to their expression of the T-cell antigen. In this instance, M-RFC appeared on day 9 in 27% and on day 16 in 7% of the cell yield. During two further experiments performed with thymectomized mice as DC recipients a considerable portion of Reh cells (22% vs. 65%) was found to stain positively with AIg(Fab)$_2$ (unpublished observations).

Although a rise in the number of apparently normal lymphoid cells was evident in all ALL cases, two observations support the view that the cALL positive leukemic blasts were truly

responsible for lymphatic differentiation: (1) At the onset of the culture the leukemic cells of I.G. contained cIg which enabled us to identify these cells as B-lymphocyte precursors. During cultivation the cells expressed sIg and a receptor for mouse erythrocytes. (2) Cells of the Reh line fulfilling all criteria of a leukemic cell line developed membrane markers of T-lymphocytes as well as characteristics of B-cells. The process of differentiation was accompanied by neither a change in morphology nor in the karyotype (Lau et al. 1979). On the basis of these findings we conclude that leukemic cALL blasts represent arrested early lymphatic progenitors. Under the environmental conditions of the DC culture the block in differentiation was at least partially released, and the cells were induced to further mature, mimicking a development into either the B- or T-cell series.

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