# Characterization of Antigens on Acute Lymphoblastic Leukemias

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## Introduction

Detectable differences in surface membrane characteristics of normal lymphocyte populations have facilitated the classification of leukemic cells according to cell origin and stage of differentiation. Recent reports show that distinct leukemic cell populations do not only express markers found on normal cell populations. Using specific antisera new antigenic determinants on cells of common acute lymphoblastic leukemia (cALL) and cells of some chronic myeloid leukemias (CML) in blast crisis have been identified [1,11]. The discovery of a cALL-antigen allows the identification of further subgroups of ALL, which has already been described as a heterogenous disease by clinical and morphological criteria [4]. The nature of the cALL-antigen and the question of its occurence during normal hemopoietic development is still speculative. Our studies investigate the distribution of cALL-antigen in correlation with common markers on normal cells, cell lines and leukemic cells of a major group of ALL patients and analyze the effect of anti-cALL antibodies on hemopoietic precursor cell populations.

## Material and Methods

Normal and leukemic blood cells: The preparation of cells from peripheral blood and tissues has been described in detail elsewhere [12].

*Leukemic Cell Lines:* NALM was established from $Ph^+$CML in blastic crisis [6], REH from $E^+$ALL [13], U-698M from a lymphosarkoma using the spongestan grid technique [8] and MOLT-4 [5] and JM [14] from $E^+$ALL. All lines were kindly provided to us by the authors.

*Indicator Systems:* Cytotoxicity, complement fixation, indirect immunofluorescence, rosette formation and colony forming unit (CFU-c) test were used for the analysis of the different cell populations and have been described previously [7,12,15]. *Anti-cALL-globulin (AcALLG):* anti-cALL serum was produced by immunizing rabbits with cALL cells (negative for surface Ig, T-antigen and E-rosettes). The antiserum was absorbed with liver/kidney homogenate, normal spleen cells, peripheral blood lymphocytes, CLL cells,

and selected lymphoblastoid cell lines of the B-cell typem Further details including the purification of the globulin fraction are described elsewhere [10, 12, 17].

*Anti-T-cell globulin (ATCG):* ATCG was prepared from rabbit anti-human thymocyte globulin by absorption with liver/kidney homogenate different CLL-cells of the B-cell type and B-lymphoblastoid cell lines [12]. The globulin fraction (ATCG) was isolated as described for AcALLG. ATCG showed a specific reaction for cells of the T-lymphocyte series and no further crossreactions with other blood cells [9, 12].

## Results and Discussion

Crude antisera against cALL cells (ALL-cells without markers such as E-rosette formation, T-cell antigen, and SMIg) were not able to discriminate between normal blood and leukemic blasts. Subsequent absorption with liver/kidney homogenate, normal lymphocytes, and CLL-cells eliminated the crossreaction with normal lymphocytes and granulocytes. Nevertheless, this antiserum still gave a clear reaction with the immature precursor stages of the myelopoietic and erythropoetic differentiation of normal bone marrow and several lymphoblastoid B-cell lines. These crossreactions of the antiserum were undesirable, since they complicated the determination of specific antigen-positive cells in the bone marrow. Additional absorption of the antiserum with B-lymphoblastoid cell lines, eliminated the crossreactions with bone marrow cells but still left a highly specific reactivity to cALL cells. This was obviously caused by antigens which occured both in a series of normal bone marrow cells and in B-lymphoblastoid cell lines, and which were also found on cALL cells in addition to the cALL antigen itself [11]. All subsequently described investigations were conducted with an antibody preparation isolated from the crude antiserum by absorption with all the cell types specified and followed by a subsequent purification process resulting in the specific AcALLG.

Analysis of normal cell populations with AcALLG revealed that a detectable amount of cALL-antigen was not found in any of the cell sources examined, such as thymocytes, peripheral blood lymphocytes, granulocytes, and bone marrow cells. The reaction of AcALLG with thymus, spleen and bone marrow cells prepared from 3 and 6 month fetuses was also negative. These indicator systems cannot exclude, with absolute certainty, the presence of a very small cALL-positive cell population among a largely negative one. There are indications, that ALL-antigen may be present to a greater extent in early precursor blood cells where it acts as a differentiation antigen [1]. To investigate this question the effect of AcALLG on precursor cells was examined in a colony-forming test (CFU-c) (Fig. 1). Bone marrow was incubated with AcALLG in the presence of complement and then cultivated using in an agar layer technique [7]. The CFU-c-reduction was measured in comparison with a bone marrow preparation incubated with normal rabbit globulin without any activity. While non-absorbed globulin against cALL cells eliminated all the CFU-c up to log 2 titer 7, highly-specific AcALLG did not cause any

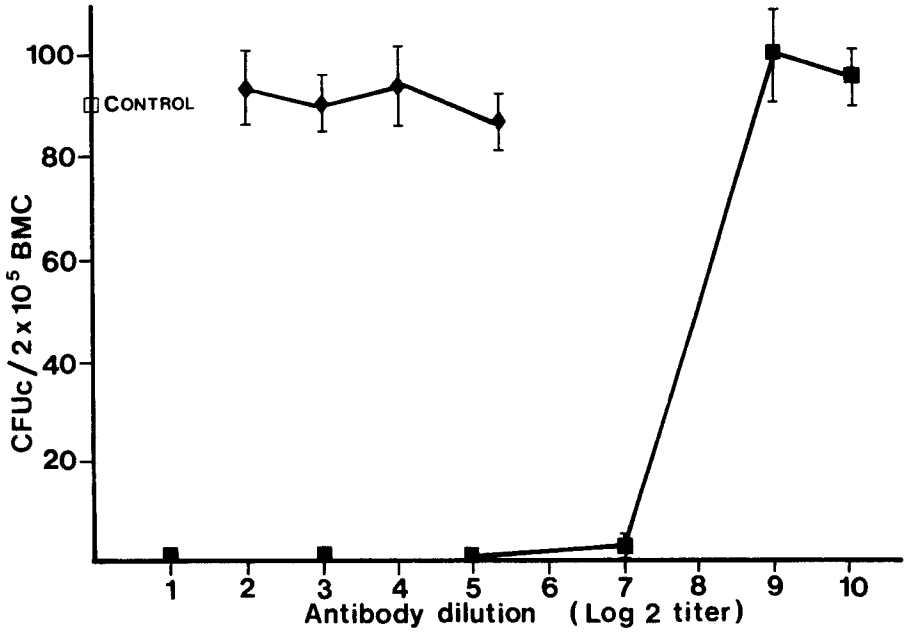


**Fig. 1.** Effect of unabsorbed (■——■) and absorbed (♦——♦) anti-cALL-Globulin on CFU-c of human bone marrow in the presence of complement

detectable CFU-c reduction, even in higher concentrations. Therefore cALL-antigen seems not to be expressed on progenitor cells growing in this test system.

We then analysed the leukemic cells of 174 patients with ALL for the expression cALL-antigen and common markers such as E-rosette formation, T-cell antigen and surface Ig (SIg) (Table 1). 13 leukemias without detectable surface markers remained unclassified (AUL). The leukemias of the remaining 161 patients could be classified in 6 groups: 56 childhood and 8 adult ALL carried only cALL-antigen and were therefore of common ALL-type, 35 childhood and 4 adult ALL expressed both cALL-antigen and T-cell antigen. The latter phenotypic pattern was expressed by the NALM and REH cell line. We found simultaneous expression of cALL-antigen and SIg in 1 childhood ALL. A similar expression of markers was found in the lymphosarcoma cell line U698M. 15 childhood and 9 adult ALL expressed only the T-cell antigen, 23 childhood and 6 adult ALL showed additional E rosette formation. The latter pattern was also found in cell lines like MOLT of JM. 2 leukemias expressed SIg and were identified as B-ALL. Some of the leukemias classified as cALL may possibly belong to a pre-B form described by Vogler et al. [18] showing cytoplasmatic Ig expression, which was only detected in fixed cell preparations. These data show that at least 6 clearly definable subgroups of acute lymphoblastic malignancies can now be identified. The pattern of differentiation markers of each subtype may allow the stage of development of the leukemias to be deduced. It is still not completely clear, whether cALL antigen is a genuine differentiation antigen reflecting a regular cell development, or whether it is the reflection of pathologic changes taking place in this phase due to an agent able to transform the cells. There is no experimental evidence to support the hypothesis that cALL-antigen is an

**Table 1.** Expression of cell surface markers (cALL-antigen, T-cell antigen, spontaneous rosette formation and membrane immunoglobulin) in patients (132 children and 29 adults) with ALL and lymphoid cell lines. Analysis of the populations was performed in several test systems like complement fixation, immunofluorescence, cytotoxicity, rosette formation and immunoautoradiography

| ALL Classification[a] | Children No. (%) | Adults No. (%) | Phenotypic pattern cALL antigen | T-cell antigen | SRBC rosettes | Surface Ig |
|---|---|---|---|---|---|---|
| c-ALL | 56 (42.4) | 8 (27.6) | ⊕ | — | — | — |
| c/T-ALL | 35 (26.5) | 4 (13.8) | ⊕ | ⊕ | — | — |
| Pre-T-ALL | 15 (11.4) | 9 (31.0) | — | ⊕ | — | — |
| T-ALL | 23 (17.4) | 6 (20.7) | — | ⊕ | ⊕ | — |
| c/B-ALL | 1 (0.8) | – | ⊕ | — | — | ⊕ |
| B-ALL | 2 (1.5) | 2 (6.9) | — | — | — | ⊕ |
| Lymphoid cell lines | Origin | | | | | |
| NALM | CML blast crisis | | ⊕ | ⊕ | — | — |
| REH | c-ALL | | ⊕ | ⊕ | — | — |
| U698-M | Lymphosarcoma | | ⊕ | — | — | ⊕ |
| MOLT-4 | T-ALL | | — | ⊕ | ⊕ | — |
| JM | T-ALL | | — | ⊕ | ⊕ | — |
| IFH-16 | PBL[b] | | — | — | — | ⊕ |
| IFH-20 | PBL | | — | — | — | ⊕ |

[a] 13 cases of acute leukemias (6 children, 7 adults) remained unclassified (AUL) and were not listed

[b] Established by infection of normal peripheral lymphocytes with EBV

exogenous virus-coded antigen of any of the known oncogenic viruses ([2], unpublished observations). The lack of cALL-positive cells in normal human bone marrow and fetal liver in our experiments may only reflect the scarcity of cells carrying this antigen during a transitory stage of hemopoietic development. Our findings show that in a defined number of ALL-cases as well as in several lymphoid cell lines the cALL-antigen can be detected together with T-cell antigen or SIg, which means that the cALL-antigen is expressed by cells committed to differentiate along the T or B cell axis. Leukemic cells, carrying cALL-antigen only, may represent a differentiation stage of a common lymphoid precursor cell (LyP) not yet committed to one of the two pathways of differentiation. On the other hand our data do not support the postulate of Janossy and coworkers that cALL antigen is expressed on pluripotent stem cells (PSC) [3]. The proliferative activity of colony-forming and diffusion chamber stem cells was not affected by an incubation with AcALLG. Both test systems analyze the growth of early precursor cells of human bone marrow. The diffusion-chamber provides a suitable milieu for the growth of certain pluripotent stem cells [15] and in the CFU-c assay mainly myeloid precursors give rise to colonies. Additional investigations in the CFU-c test have been performed with bone marrow cells cultured in diffusion chambers. The growth pattern of these cells indicate an influx from pluripotent stem cell

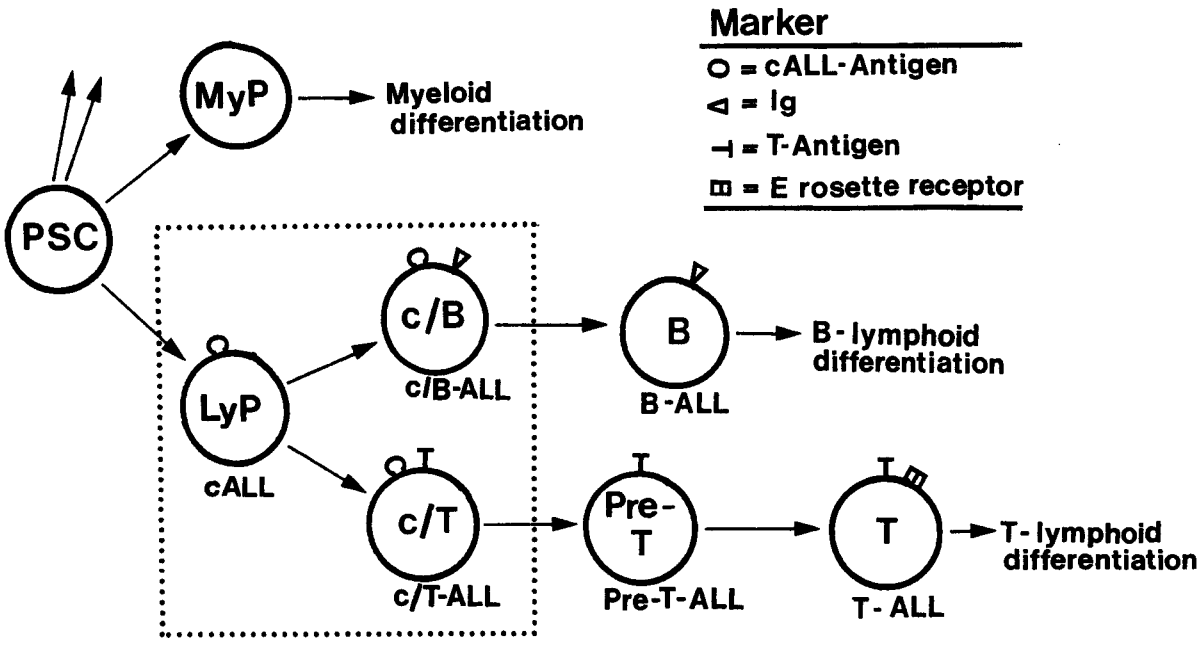


**Fig. 2.** Different ALL phenotypes as counterparts of differentiation steps in a possible scheme of normal hemopoietic development (abbrevations explained in the text)

pool in addition to the limited self replication of the committed myeloid precursor cells. Colony development under these conditions is not influenced by incubation treatment with AcALLG prior to the chamber implantation as reported by Netzel et al. [7] which may exclude the elimination of stem cells by cALL-specific antibodies. On the basis of the reported findings and comparative investigations of normal fetal cells (Thiel et al., submitted) the following differentiation scheme can be proposed (Fig. 2). If ALL cells and cell lines represent arrested stages of normal hemopoiesis, cALL antigen is expressed transitorily on cells of early lymphopoietic development (LyP, c/B, c/T).

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