Haematology and Blood Transfusion Vol. 26
Modern Trends in Human Leukemia IV
Edited by Neth, Gallo, Graf, Mannweiler, Winkler
© Springer-Verlag Berlin Heidelberg 1981

Serologic Subtyping of cALL*

W.-M. Becker, W.-H. Schmiegel, H. Kabisch, R. Arndt, and H.-G. Thiele

Recently we have found that sera of patients with common ALL contain a glycoprotein reacting with antibodies directed to common ALL associated antigen (Ag cALL) (Kabisch et al. 1979). The molecular properties of the serum Ag cALL, i.e., the apparent molecular weight of 125,000 and the binding specificity to lens culinaris lectin, are in good accordance with those estimated for Ag cALL as solubilized from common ALL cells (Kabisch et al. 1978) or obtained from established leukemic cell lines (Sutherland et al. 1978). These findings indicate an in vivo shedding or secretion of this antigen.

Proceeding from the assumption that cALL cells of different patients or even single established leukemic cell lines represent distinct stages of cellular development characterized by particular patterns of cell surface structures and that shedding of Ag cALL from cALL cells may not be restricted to this antigen but may also occur with respect to other differentiation antigens, it seemed to be promising to use cell culture supernatants of such cell lines as starting material for antigen preparation and antiserum production. In comparison to intact tumor cells or membranes, secreted or shed cell material has the advantage for the purpose of immunization that the antibodies obtained will be of restricted diversity. The specificity of such antisera can be improved when before immunization the secreted or shed material is further purified and defined. Such antisera may be of value for subclassification of cALL. We here report on our preliminary results with antisera raised against distinct glycolysated structures released into the culture medium by the established non-T, non-B cell leukemia lines Reh and Nalm (Rosenfeld et al. 1977; Minowada et al. 1977).

Purification of such structures was performed by lectin affinity chromatography on agarose immobilized (a) lens culinaris hemagglutinin (LCH), (b) ricinus communis agglutinin 60 (RCA 60), and (c) ricinus communis agglutinin 120 (RCA 120). Since purification of cell surface antigens frequently is associated with reduction of immunogenicity, the antigenic material as eluted from the lectin columns by competing sugars was incorporated into egg lecithin liposomes by use of n-octyl-glucoside following the method described by Helenius et al. (1977). After reconstitution to vesicles a significant increase of the antigenicity of Ag cALL as obtained from lectin-purified supernatants was observed in an antibody-binding inhibition assay.

Antisera were raised in rabbits by repeated injections of lectin purified antigens incorporated into liposomes (0.7–2.5 mg protein/dose). The antisera produced in this way were absorbed with AB erythrocytes, with glutardialdehyde fixed AB serum and human liver. By means of indirect immunofluorescence and by using different target cells the specificities of the three antisera were compared with that of an antiserum which had been raised by immunizing rabbits with cALL cells and which after extensive absorptions had been proved to be specific for Ag cALL (Table 1, column 1). As may be seen, antiserum 319, which was raised against LCH binding antigens derived from Nalm Cells culture supernatants, recognized membrane structures not only on Nalm cells and on cells derived from common ALL patients but also

* This work was supported by the Stiftung Volkswagenwerk

Table 1. Reaction of single antisera with various cell types (indirect immunofluorescence assay)[a]

Target cells	Percentage of cells reacting with different antisera			
	A-cALLS[b]	As 319[c]	As 365[d]	As 317[e]
I. ALL-derived cell lines				
REH	21	N.D.	99	89
NALM	17	55	40	30
KM-3	23	N.D.	3	6
II. Common ALL (patients)				
$cALL_{Er}$	52	51	0	42
$cALL_{Br}$	42	N.D.	31	0
$cALL_{Sc}$	72	N.D.	36	9
III. Other leukemic cells				
Acute myeloctic leukemia (AML)	0	85	0	0
Acute myelocytic-monocytic leukemia (AMML)	0	0	1	0
Acute monocytic leukemia (AMOL)	0	0	0	0
Chronic myelocytic leukemia (CML)	0	N.D.	0	0
Chronic lymphatic leukemia				
T-CLL	0	0	1	0
B-CLL	N.D.	96	12	0
T-ALL	0	0	1	5
IV. Normal cells				
Peripheral blood lymphocytes (PBL)	0	3	0	0
Bone marrow cells (BMC)	0	1	N.D.	N.D.
Tonsil lymphocytes (TOL)	0	3	11	0

[a] Second antibody: tetraethylrhodamine-isothiocyanate conjugated goat anti-rabbit globulin (Kabisch et al. 1978)
[b] Preparation of A-cALLS (Kabisch et al. 1978)
[c] Rabbit antiserum against antigen derived from Nalm cells supernatants purified by inity chromatography on LCH
[d] Rabbit antiserum against antigen derived from Reh cells supernatants purified by affinity chromatography on RCA 60
[e] Rabbit antiserum against antigen derived from Reh cells supernatants purified by inity chromatography on RCA 120

reacted with AML and B- CLL cells (Table 1, column 2).

Antiserum 365 raised to RCA 60 binding antigens derived from Reh cell supernatants revealed a different reacting pattern in that it did not react with AML but recognized a subpopulation of B-CLL as well as of tonsil lymphocytes (Table 1 column 3). When these results are compared with the selectivity of A-cALLS (Table 1, column 1), it becomes evident that antiserum 365 discriminates subtypes of Ag cALL bearing leukemic cells.

The recognition spectrum of antiserum 317 raised against RCA 120 binding antigens derived from Reh cell culture supernatants was found to be restricted to cALL cells, although its ability to discriminate cALL subtypes is evidently distinct from that of antiserum 365. Thus, antiserum 317 stained 42% of cALL cells of patient Er., whereas antiserum 365 had no labeling effect on these cells. On the other hand, antiserum 365 stained 31% and 36% of cALL cells of patients Br. and Sc., respectively. These target cells showed no (patient Br.) or only little (9%, patient Sc.) fluorescence when tested with antiserum 317.

These data confirm the assumption that antisera towards molecular-defined shed or secreted cell surface molecules with different lectin binding specificities are indeed a valuable tool for further differentiating of common ALL cells characterized by expression of Ag cALL.

References

Helenius A, Fries E, Kartenbeck J (1977) Reconstitution of semliki forest virus membrane. J Cell Biol 75:866–880 – Kabisch H, Arndt R, Thiele H-G, Winkler K, Landbeck G (1978) Partial molecular characterization of an antigenic structure associated to cells of common ALL. Clin Exp Immunol 32:399–404 – Kabisch H, Arndt R, Becker W-M, Thiele H-G, Landbeck G (1979) Serological detection and partial characterization of the common-All cell associated antigen in the serum of cALL patients. Leuk Res 3:83–91 – Minowada J, Tsubota T, Greaves MF, Walters RT (1977) A non-T, non-B human leukaemia cell line (Nalm 1): Establishment of the cells and presence of leukaemiaassociated antigens. J Natl Cancer Inst 59:83–87 – Rosenfeld C, Goutner A, Choquet C, Venuat AM, Kayibanda B, Pico JL, Greaves MF (1977) Phenotypic characterisation of a unique non-T, non-B acute lymphoblastic leukaemia cell lines. Nature 267:841–843 – Sutherland R, Smart J, Niaudet P, Greaves MF (1978) Acute lymphoblastic leukaemia associated antigen. II. Isolation and partial characterisation. Leuk Res 2:115–126