Immunological Characterization of Blast Cells in Patients with Acute Lymphoblastic Leukemia. Evaluation of its Clinical Significance.

J. C. Brouet and A. Chevalier

Laboratory of Immunochemistry and Immunopathology, U 108, INSERM, Research Institute on Blood Diseases, Hôpital Saint-Louis, Paris, France.

Summary

A panel of lymphocyte surface markers was used to identify blast cells from 111 patients with acute lymphoblastic leukemia (ALL). Three groups of patients were found. 1) 14 patients with B derived ALL. Only three patients had a common ALL; in the other cases the blastic proliferation was featured by Burkitt's tumor cells or supervened in patients affected with chronic lymphocytic leukemia (CLL). 2) The blast cells from 28 % of the patients with common ALL had T cell properties. 3) The cells from the largest group of patients did not bear B or T cell markers but were featured by the presence of a leukemia-associated antigen revealed by a rabbit antiserum to CLL B cells. Studies with another antiserum to CLL B cells as well as with an antiserum to foetal thymocytes revealed also leukemia-associated antigens but these antigenic determinants were present on all acute leukemia cells which had been tested and were therefore of no help to classify various leukemias. A number of clinical and hematological findings were more frequent in the group of patients with T cell ALL: high white blood cell counts, tumoral disease, thymic enlargement, meningeal involvement, strong acid phosphatase activity in blast cells. However no difference in the survival curve is yet apparent at 30 months.

Introduction

Rwo main immunological approaches were used in the recent years to characterize the blast cells in various leukemias and specially acute lymphoblastic leukemias (ALL). The expression at the surface of ALL blast cells of membrane markers which are considered specific for T or B lymphoid cell lines may help to elucidate the cellular origin of the neoplastic cells. Such studies showed that a minor group of ALL was B cell derived (1, 2) that nearly 30 % of ALL had T cell features (1, 3–8) whereas the majority of ALL blast cells did not possess B or T cell markers. On the other hand the identification at the surface of leukemic cells of some antigenic determinants which are leukemia associated or leukemia specific has been developed (9–14, 18) and there is much evidence now that there is a complexity of serologically detectable antigenic determinants on leukemic cells.

These two approaches to the characterization of leukemic cells are not incon-

sistent with each other. They may both be helpful to classify various leukemias on objective criteria and hopefully will have some therapeutic or prognostic implications. This report deals with the immunological characterization of blast cells in 111 patients with ALL and with a search for possible correlations between the nature of the leukemic cells and clinical, morphological and cytochemical data as well as with the evolution and prognosis of the disease.

Methods and their critical Evaluation

The methods used for the detection of B or T cell membrane markers have been described elsewhere (15, 16).

Three different antisera to B CLL cells and an antiserum to foetal thymocytes were raised in rabbits and tested for their reactivity with various normal and leukemic cells. Rabbits were immunized with 20 to 40. 10^6 cells intravenously, boosted with the same number of cells 14 days later and bled 7 days following each immunization. After heat inactivation, serial absorptions were performed with human liver (x3), human AB erythrocytes (x 3), normal human serum and immunoglobulin (Ig) chains coupled to Sepharose 4B. The anti CLL sera were further absorbed with peripheral T cells (serum 1 and 2) or with peripheral T cells and thymocytes (serum 3). The antiserum to foetal thymocytes was absorbed on CLL B cells. Absorptions were usually carried out with a 3 to 1 serum to packed cells ratio for 60 minutes at + 4 °C. The reactivity of these antisera was assessed by either cytotoxicity or indirect immunofluorescence on 1) normal blood and tonsil lymphocytes. 2) T lymphocytes obtained after nylon filtration of blood or tonsil lymphocytes, 3) B lymphocytes from CLL patients, 4) T lymphocytes from T derived CLL, 5) T or B lymphoid cultured cell lines, 6) thymocytes, 7) phytohemagglutinin transformed lymphocytes, 8) blast cells from various acute leukemias (T derived ALL, non T non B ALL, acute myeloblastic leukemia (AML)).

The interpretation of the results of such investigations requires much caution since they are exposed to a number of pitfalls. Among lymphocyte surface markers, membrane bound Ig detected by immunofluorescence constitute the most reliable marker of B cells and may represent a clonal marker of B cell proliferations when monospecific antisera to the various Ig chains are used. But the mere presence of immunoglobulins at the surface of a cell does not necessarily mean that they are produced by that cell. Erroneous interpretations may result from the attachment of circulating immune complexes or IgG aggregates to any cell carrying receptors for C3 or Fc, from an anti-IgG activity of membrane bound IgM (17) or from the presence of antibodies directed to lymphocyte surface determinants (16). In vitro experiments may therefore be required to ensure that surface immunoglobulins are synthesized by the cells under study (17). Most heteroantisera to B or T cells are only relatively specific. When they can be used only in cytotoxicity tests and not in immunofluorescence, they do not allow direct checking for other markers and direct examination of the positive cells.

Current studies have shown that the delineation between B and T lymphocyte markers is less clear cut than previously appraised and that cell subpopulations characterized by different surface markers exist within these two broad categories. Is should be stressed that a classification of cells in terms of B or T

origin which may be relatively safe for normal lymphocytes may not be valid when extrapolated to undifferentiated or neoplastic cells. Such cells may express membrane antigens only at certain stages of the cell cycle or experience surface changes which prevent their identification.

Reactive antigenic determinants on leukemic cells may represent viral coded determinants, foetal, stem cell or even derepressed normal antigens. The precise nature and significance of leukemic associated antigens remains largely unknown. Some antisera appear truly leukemia specific since they react only with leukemic cells, usually of various cellular types (10–14); other antisera reveal leukemic associated antigens which may also be found on a population of normal cells (14, 18, 19).

Results

Lymphocyte membrane markers in 111 patients with ALL

B derived ALL

A monoclonal B cell proliferation was found in 14 patients. The cells bore a homogeneous surface IgM in all cases but one where IgG $\varkappa$ molecules were detected. These blast cells also bound IgG aggregates but lacked T cell markers in all cases studied.

Most of these patients were not affected with a common ALL. In 3 cases the blastic proleferation supervened in patients previously affected with common CLL. In one of these patients the finding of an identical anti-IgG antibody activity of the surface Ig on both small lymphocytes and blast cells ascertained that the two cell populations derived from the same clone (20). In 8 cases the blast cells possessed all the cytological features of Burkitt's tumor cells (2). Only 3 patients with a monoclonal B cell process belonged to the group of unselected patients with common ALL. In 2 of these cases the blast cells showed some unusual cytological features similar to that of cells from lymphosarcoma.

T derived ALL

Blast cells from 28 patients out of 100 cases of common ALL (i.e. patients with Burkitt's tumor cells or with ALL supervening on CLL excluded) had T cell surface characteristics (Table I). In 26 of these 28 patients the blast cells formed spontaneous rosettes. In 10 cases only the percentage of rosetting blasts was higher than 60 %; in the remaining 16 patients the percentage of rosette forming blast cells was lower (10 to 60 %). The cells from 18 patients were studied with an antiserum to peripheral T cells. In 17 cases including the 2 patients whose blast cells did not form rosettes, the cells were killed by this antiserum.

Non T non B ALL (Table I)

In 59 patients, all blast cells were devoid of surface Ig and did not bind IgG aggregates. In the remaining 10 patients a small percentage (less than 15 %) of cells were stained by antisera to γ, $\varkappa$, λ and sometimes μ chains. The staining pattern was unusual with an irregular or grossly spotted aspect. These cells did not bind IgG aggregates. In the 2 cases where trypsinization experiments were per-

Table I: B and T membrane markers on blast cells in common ALL.

	N ° studied	N ° positive
Surface Ig	100	3 $\mu\kappa$ $\mu\lambda$ $\mu\lambda$
Fc receptor	70	0
Spontaneous rosette formation	100	26 (only 8 with > 80 % of RFC)
Killing by anti-T serum	50	17

formed, no evidence in favor of an actual Ig synthesis by the cells in vitro was obtained.

In 63 cases the blast cells did not form rosettes with sheep erythrocytes. Small cells (presumably the residual T lymphocytes present in the suspension) accounted for most of all of the few rosette-forming cells. However in the last 6 patients, it was difficult to exclude the possibility that a small percentage of the blasts were rosetting cells. These 6 patients could not be studied with other T cells markers. In the 32 patients who were studied with an antiserum to peripheral T cells, the blast cells were unreactive. By contrast in most cases the cells were killed by an anti-CLL serum which recognized leukemia associated antigens (see infra).

Correlations between lymphocyte membrane markers on blast cells and clinical and hematological data

All patients with a B derived ALL experienced a very poor prognosis. Patients with Burkitt's tumor cells did not respond to chemotherapy or relapsed after a short remission. Among the patients with B derived "common" ALL one 5-year old child died from hematological relapse after five years survival whereas two male adults had no remission under chemotherapy.

Among patients with T ALL or non T non B ALL, there was no difference in the sex and age distribution. The cytological subclassification of ALL did not differentiate the two groups. Cytochemical studies showed that a strong positivity for acid phosphatase was more often found in blast cells from T derived ALL ($p = 0.01$). PAS positivity or beta-glucuronidase activity were not different in the two groups of patients.

Initial anemia or thrombocytopenia were equally found in both groups of patients. An initial high white blood cell count was more often associated with T ALL ($p = 0.05$). A tumoral presentation occurred in 36 patients; 17 of these patients were affected with a T ALL ($p = 0.05$). 15 patients had a mediastinal involvement. Seven of those 8 patients who had a predominantly antero-superior mediastinal enlargement and hence presumably a thymic enlargement were affected with T ALL.

The actuarial survival curve shows no difference between the group of patients with T ALL and non T non B ALL at 30 months. Hematological relapses were found equally in both groups of patients. 5 patients experienced a meningeal in-

volvement in the group of T ALL whereas a single patient had a meningeal relapse in the non T non B group ($p = 0.02$).

Reactivity of ALLblast cells with antisera to CLL B cell or foestal-thymoctes

The reactivity of anti-B CLL cells sera 1 and 2 on normal and leukemic cells is shown on Table II. These antisera reacted with B cells from normal or leukemic individuals and from various B cell lines. They gave negative reactions with peripheral T cells, on T derived cultured lines, T derived CLL and PHA transformed cells. However they did react with thymocytes. Among patients with leukemias they did not react with AML cells or T derived ALL blasts (with a single exception) but were positive on blast cells from non T non B ALL cases. The reactivity with the latter cells was abolished by absorption with thymocytes and vice versa. The results of this cross-absorption experiments and the presence of at least one determinant on thymocytes showed that these leukemia associated antigens shared by CLL, non T non B ALL and thymocytes are distinct from B cell antigens.

Table II: Reactivity of leukemic and normal cells with rabbit antisera to CLL B cells (sera 1 and 2)

Absorption	B cells	Peripheral T cells	Thymocytes	T ALL	Non T non B ALL	AML
A						
RBC						
Liver	+	–	+	–	+	–
Ig						
Peripheral T cells						
B						
Abs. A						
+						
Thymocytes	+	–	–	–	–	–
C						
Abs. A +						
Non T non B						
All cells	+	–	–	–	–	–

Another serum raised in rabbits to CLL B cells had a different pattern of reactivity (Table III). It was absorbed on peripheral T cells and thymocytes. It contained antibodies to B cells and gave negative results on peripheral T cells or thymocytes. However it was reactive with PHA transformed T lymphocytes. This reactivity was only transient and disappeared in late cultures. This serum stained AML and ALL blast cells; the latter reactivity disappeared after absorption with AML cells and it is most likely that this antiserum contained antibodies to cell cycle antigens.

Table III: Reactivity of leukemic and normal cells with a rabbit antiserum to CLL B cells (serum 3)

Absorption	B cells	Periph-eral T cells	Thymo-cytes	Non T non B ALL	AML	PHA transformed T cells	
						3rd day	8th day
RBC							
Liver							
Ig	+	–	–	+	+	+	–
Peripheral T cells							
Thymocytes							
Idem + AML cells	+	–	–	–	–	–	–

A rabbit antiserum to foetal thymocytes, reacted after suitable absorptions with T cells and blast cells from ALL or AML (Table IV). Absorption of this antiserum with AML cells abolished the reactivity with non T non B ALL blast cells but not with T ALL and T cell antibodies were not removed by this absorption.

Table IV: Reactivity of leukemic and normal cells with a rabbit antiserum to foetal thymocytes

Absorption	B cells	Peripheral T cells	Thymocytes	T ALL	non T non B ALL	AML
RBC						
Liver						
Ig	–	+	+	+	+	+
B CLL cells						
Idem + AML cells	–	+	+	+	–	–

Discussion

The study of the expression of B or T membrane markers on blast cells in ALL allowed the distinction between three groups of patients. A minor group is characterized by B derived blast cells. In our experience these B ALL cells bore homogeneous surface Ig and thus are presumably of monoclonal origin. Patients with B ALL belonged mostly to two specific antities namely ALL supervening on CLL and ALL featured by Burkitt's tumor cells. It is striking that in 2 out of 3 patients with a "common" B ALL, the cells had unusual cytologic features suggestive of poorly differentiated lymphocytic lymphoma, a disease which is usually of B cell origin in adults. These findings suggest that most if not all patients with B type ALL may in fact be affected with a lymphoma with leukemic presentation.

Less than a third of common ALL are featured by T derived blast cells. This study and other reports (4–8) demonstrated that such patients often had leukemias with high risk factors, such as high WBC, tumoral presentation and often a thymic mass. It is thus likely that the survival of patients with T ALL will be poorer than that of patients with non T non B ALL, although at present no difference is apparent at 30 months. It will be important to look for the survival of patients with T ALL or non T non B ALL which will be matched for initial clinical parameters in order to determine whether the T nature of the blast cells is by itself of poor prognostic value.

In the largest group of patients with ALL the blast cells did not possess any of the current B or T lymphocyte membrane markers. The question of the true cellular origin of non T non B ALL remains presently unsettled. The involvement of lymphoid stem cells devoid of mature B or T lymphocyte markers or the occurrence of surface changes due to the malignant process may explain the lack of detectable T cell markers. The finding of a specific thymic enzyme in blast cells from 11 out of 13 ALL patients (21) would support this hypothesis and it seems unlikely that these non T non B cells do not involve the lymphoid differentiation pathway at all. In this context, it is of great interest that blast cells from non T non B ALL were characterized in this study by a positive finding, i.e. a leukemia ssociated antigens as discussed below.

The proposed immunologic classifcation of ALL in three main groups appears to be valid. Hopefully the classification will not remain purely academic but will prove to be of some practical value. If confirmed by the study of a large number of patients, the results obtained in this report give some hope that a battery of less sophisticated methods such as for instance myeloperoxidase and acid phosphatase staining, E rosette formation and binding of IgG aggregates will improve in any case the accuracy and reproducibility of the classification of acute leukemias.

The study of leukemic cells with rabbit antisera to feotal thymocytes or CLL B cells pointed to the presence of distinct leukemia associated antigens. The former antiserum reacted with blast cells from different cellular origin (i.e. AML or ALL cells). A wide pattern of reactivity among leukemic cells was also found by Mohnakumar and Metzgar using a primate antiserum to thymocytes (19). The results obtained with antisera to thymic cells must therefore be carefully interpreted when evaluating the possible T origin of leukemic cells.

Another approach to leukemic surface antigens was evaluated in this study using antisera to CLL B cells. Two antisera reacted, after suitable absorptions, with thymocytes (and not peripheral T cells), CLL and non T non B ALL cells whereas negative results were obtained with T ALL or AML blasts. It is of interest that such a distinction between non T non B ALL cells and T derived blasts was also achieved by Greaves et al. using a rabbit antiserum to non T ALL cells (14). Since their antiserum did not react with CLL cells it is likely that at least two different sets of leukemia related antigenic determinants are expressed on non T non B ALL cells.

In contrast previous studies performed with antisera to B cell line extracts or B CLL cells yielded different results. The antigenic systems involved in such studies were found on all ALL and CLL cells tested when monkey antisera were used (10) whereas rabbit antisera reacted with all acute leukemias (12, 13, 22). In the present report one out of three rabbit antisera to CLL cells had such a broad reactivity; it

contained presumably antibodies to cell cycle dependent antigens since normal lymphocytes were transiently positive when stimulated by PHA.

The purification and characterization of leukemia associated antigens (23, 24) are clearly warranted to evaluate the potential use of such antisera in the management of patients with acute leukemia.

We are grateful to Dr. H. R. Toben who provided some antisera used in this study. This work was supported in part by INSERM (grant ATP 1.73.16.17) and DGRST (Grant 75.7.0786).

References

1. Brouet, J. C., Toben, H. R., Chevalier, A., Seligmann, M. *Ann. Immunol.* Paris, *125C*, 691, 1974.
2. Flandrin, G., Brouet, J. C., Daniel, M. T., Preud'homme, J. L. *Blood, 45*, 183, 1975.
3. Belpomme, D., Dautcher, D., Du Rusquec, E., Grandjon, D., Huchet, R., Pouillart, P., Schwarzenberg, L., Amiel, J. L., Mathe, G. *Biomedicine, 20*, 109, 1974.
4. Sen, L., Borella, L. *New Engl. J. Med., 292*, 828, 1975.
5. Brown, G., Greaves, M. F., Lister, T. A., Rapson, N., Papamichael, M. *Lancet, 11*, 753, 1974.
6. Catovsky, D., Goldman, J. M., Okos, A., Frisch, B., Galton, D. A. G. *Brit. Med. J., 2*, 643, 1974.
7. Chin, A. H., Saiki, J. H., Trujillo, J. M., Williams, R. C. *Clin. Immunol. Immunopath., 1*, 499, 1973.
8. Kersey, J. H., Sabad, A., Gajl-Peczalska, K. J., Hallgren, H. M., Yunis, E. J., Nesbit, M. E. *Science, 182*, 1355, 1973.
9. Bias, W. B., Santos, G. W., Burke, P. J., Mullins, G. M., Humphrey, R. L. *Science, 178*, 304, 1972.
10. Metzgar, R. S., Mohanakumar, T., Miller, D. S. *Science, 178*, 986, 1972.
11. Baker, M. A., Ramachandar, C., Taub, R. N. *J. Clin. Invest., 54*, 1273, 1974.
12. Mann, D. L., Rogentine, G. N., Halterman, R., Leventhal, B. *Science, 174*, 1136, 1971.
13. Billing, R., Terasaki, P. I. *J. Nat. Cancer Inst., 53*, 1639, 1974.
14. Greaves, M. F., Brown, G., Rapson, R., Lister, A. *Clin. Immunol. Immunopath., 4*, 67, 1975.
15. Seligmann, M., Preud'homme, J. L., Brouet, J. C. *Transpl. Rev., 16*, 85, 1973.
16. Preud'homme, J. L., Seligmann, M. *Blood, 40*, 777, 1972.
17. Preud'homme, J. L., Seligmann, M. *Proc. Nat. Acad. Sci., 69*, 2132, 1972.
18. Brouet, J. C., Preud'homme, J. L., Seligmann, M. *Blood Cells, 1*, 81, 1975.
19. Mohanakumar, T., Metzgar, R. S. *Cellul. Immunol., 12*, 30, 1974.
20. Brouet, J. C., Preud'homme, J. L., Seligmann, M., Bernard, J. *Brit. Med. J., 4*, 23, 1973.
21. McCaffrey, R., Harrison, T. A., Parkman, R. B. S., Baltimore, D. *New Engl. J. Med., 292*, 775, 1975.
22. Mohanakumar, T., Metzgar, R. S., Miller, D. S. J. *Nat. Cancer Inst., 52*, 1435, 1974.
23. Metzgar, R. S., Mohanakumar, T., Greeen, R. W., Miller, D. S. Bolognesi, D. P. *J. Nat. Cancer Inst., 52*, 1445, 1974.
24. Billing, G., Terasaki, P. I. *J. Nat. Cancer Inst., 53*, 1645, 1974.