*Acta Medica Okayama*

*Volume* 36, *Issue* 5 1982 *Article* 1

OCTOBER 1982

# Characterization of xenoantiserum produced against B cell acute lymphoblastic leukemia cell line

Tadaatsu Akagi* Hiroshi Sonobe†

Isao Miyoshi‡ Shizuo Yoshimoto**

*Kochi Medical School,

†Kochi Medical School,

‡Kochi Medical School,

**Kochi Medical School,

# Characterization of xenoantiserum produced against B cell acute lymphoblastic leukemia cell line*

Tadaatsu Akagi, Hiroshi Sonobe, Isao Miyoshi, and Shizuo Yoshimoto

## Abstract

Antiserum was produced in white rabbit by intravenously injecting living cells of a B cell acute lymphoblastic leukemia (ALL) line (BALL-1). The reactivity of the antiserum against various lymphoid cell lines was examined by membrane immunofluorescence after appropriate absorption. Serum absorbed with non-T, non-B (NALL-1) and T-ALL (TALL-1) cells recognized B cell antigens distinct from Ia-like antigens on both normal and neoplastic B cells. After further absorption with tonsillar cells or normal B cell line (KO-HL-3), it reacted only with BALL-1 cells and did not react with other leukemia/lymphoma and normal B cell lines. The serum absorbed with tonsillar cells reacted only with BALL-1 and some B cell lines. Thus we were able to obtain antisera with specificity to B cell antigen, B-ALL antigen, and B cell line antigen.

**KEYWORDS:** xenoantiserum, B-cell, acute lymphoblastic leukemia, cell line

---

*PMID: 6184955 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **36**, (5), 325–330 (1982)

# CHARACTERIZATION OF XENOANTISERUM PRODUCED AGAINST B CELL ACUTE LYMPHOBLASTIC LEUKEMIA CELL LINE

Tadaatsu AKAGI, Hiroshi SONOBE*, Isao MIYOSHI**
and Shizuo YOSHIMOTO**

*Department of Pathology, *Department of Clinical Laboratory and **Department of Internal Medicine, Kochi Medical School, Kochi 781-51, Japan*

*Received February 26, 1982*

*Abstract.* Antiserum was produced in white rabbit by intravenously injecting living cells of a B cell acute lymphoblastic leukemia (ALL) line (BALL-1). The reactivity of the antiserum against various lymphoid cell lines was examined by membrane immunofluorescence after appropriate absorption. Serum absorbed with non-T, non-B (NALL-1) and T-ALL (TALL-1) cells recognized B cell antigens distinct from Ia-like antigens on both normal and neoplastic B cells. After further absorption with tonsillar cells or normal B cell line (KO-HL-3), it reacted only with BALL-1 cells and did not react with other leukemia/lymphoma and normal B cell lines. The serum absorbed with tonsillar cells reacted only with BALL-1 and some B cell lines. Thus we were able to obtain antisera with specificity to B cell antigen, B-ALL antigen, and B cell line antigen.

*Key words :* xenoantiserum, B-cell, acute lymphoblastic leukemia, cell line.

Xenoantisera have been produced against fresh acute lymphoblastic leukemia (ALL) cells or ALL cell lines with T or non-T, non-B cell characters, and common ALL (cALL) antigens (1-5), Ia-like antigens (6-8), or human thymus-leukemia antigen(s) (HTLA) (9-11) have been recognized by these antisera. However, B cell ALL is an extremely rare disorder (12-14) and antiserum against it has not yet been produced. We raised antiserum against an unusual B cell ALL line, BALL-1 (15), and examined its reactivity against various lymphoid cell lines after appropriate absorption.

## MATERIALS AND METHODS

*Cell lines.* Nine B cell lines, 6 T cell lines and 4 non-T, non-B cell lines were used. The origins and some marker characteristics of these cell lines are listed in Table 1. Among the B cell lines, five (#1-5) were leukemia-lymphoma cell lines and four (#6-9) were normal B cell lines without malignant characteristics although the CCRF-SB line was derived from ALL. All cell lines were maintained at 37℃ in RPMI-1640 medium supplemented with 10-15% heat-inactivated fetal calf serum (FCS). The BALL-1 cells used for immunization were free from mycoplasma infection.

TABLE 1. ORIGIN AND MARKER CHARACTERISTICS OF TESTED CELL LINES

| Cell line no. | Cell line | Origin* | Cell type | Markers | | | | | Reference no. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | SmIg** | EAC | EA ($\gamma$) | E | EBNA*** | |
| 1 | BALL-1 | ALL | B | + | − | + | − | − | 15 |
| 2 | JBL-2 | Burkitt's | B | + | + | + | − | + | 16 |
| 3 | JBL-3 | Burkitt's | B | + | + | + | − | + | 17 |
| 4 | TK | BL | B | + | + | + | − | − | 18 |
| 5 | ZK-H | HCL | B | + | + | + | − | + | 19 |
| 6 | CCRF-SB | ALL | B | + | + | − | − | + | 20 |
| 7 | KO-HL-2 | Normal | B | + | + | − | − | + | 21 |
| 8 | KO-HL-3 | Normal | B | + | − | + | − | + | 21 |
| 9 | KO-HL-4 | Normal | B | + | + | − | − | + | 21 |
| 10 | TALL-1 | ALL | T | − | − | − | + | − | 15 |
| 11 | CCRF-HSB-2 | ALL | T | − | − | − | + | − | 22 |
| 12 | MOLT-3 | ALL | T | − | − | − | + | − | 23 |
| 13 | MOLT-4 | ALL | T | − | − | − | + | − | 23 |
| 14 | HPB-ALL | ALL | T | − | − | − | + | − | 24 |
| 15 | MT-1 | ATL | T | − | − | − | + | − | 25 |
| 16 | NALL-1 | ALL | non-T, non-B | − | − | − | − | − | 15 |
| 17 | KOP-N-1 | ALL | non-T, non-B | − | − | − | − | − | 26 |
| 18 | NALM-1 | CML-BC | non-T, non-B | − | − | − | − | − | 27 |
| 19 | NALM-18 | ALL | non-T, non-B | − | − | − | − | − | 28 |

* ALL: acute lymphoblastic leukemia; Burkitt's: Burkitt's lymphoma; BL: brain lymphoma; Normal: non-leukemia or lymphoma subject; HCL: hairy cell leukemia; ATL: adult T-cell leukemia; CML-BC: CML in blast crisis.

** Surface membrane immunoglobulin.

*** EB virus nuclear-associated antigen.

*Antiserum.* Anti-BALL-1 serum was raised by injecting white rabbits intravenously with $10^8$ intact BALL-1 cells 4 times at 10 day intervals. The rabbit was bled 7 days after the last injection. The antiserum was heat-inactivated at 56℃ for 30 min and then stored at −80℃.

*Absorption of antisera.* NALL-1, TALL-1, TK and KO-HL-3 cells were used for absorption. The pancreatic cancer cell line, HGC-25 (29) and tonsil lymphocytes prepared from patients with chronic tonsillitis or adenoids were also used. Antisera were also absorbed with FCS insolubilized by glutaraldehyde. Antisera were repeatedly absorbed with 1/5 to one volume of the various absorbing materials until showing no reactivity on the absorbing cells by membrane immunofluorescence. The titer of the unabsorbed anti-BALL-1 serum against BALL-1 cells was 1:5000, and those of the absorbed sera ranged from 1:100 to 1:600. The antisera were used at the concentration corresponding to 4 times the end point dilution.

*Immunofluorescent staining for membrane antigens.* Indirect immunofluorescent staining was used. Viable cells ($5 \times 10^5$) suspended in 0.1 ml of phosphate-buffered saline supplemented with 0.02 N $NaN_3$ (PBS-N) were treated with an equal volume of antiserum at room temperature for 30 min and washed twice. The cells were subsequently stained with 0.1 ml of

fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Medical and Biological Laboratories, Nagoya) absorbed with BALL-1 and HGC-25 cells at room temperature for 30 min. The cells were then washed, mounted with a drop of buffered glycerol saline, and observed under a fluorescent microscope (Olympus BHF) with incident illumination. The antiserum was diluted with PBS-N to prevent capping.

## RESULTS

The antisera to the B cell ALL line, BALL-1 was tested against various human lymphoid cell lines after appropriate absorption. The results of the membrane immunofluorescent tests are presented in Table 2.

Unabsorbed anti-BALL-1 serum reacted with all B, T, and non-T, non-B cell lines (data not shown). The serum absorbed with NALL-1, TALL-1 and HGC-25 cells, and FCS (B-1) reacted with all B cell lines, but did not react with T and non-T, non-B cell lines. Thus B-1 antiserum seems to recognize B cell antigens on both normal and neoplastic B cells. The serum absorbed with NALL-1 and TALL-1 cells, and further with tonsil lymphocytes (B-2) or KO-HL-3 cells (B-3) reacted only with BALL-1 cells and did not react with other leukemic or normal B cell lines. The serum absorbed with tonsil lymphocytes, HGC-25 cells and FCS (B-4) reacted only with BALL-1 cells and some other B cell lines of normal or neoplastic cell types, so it seems to recognize the antigen(s) common to some B cell lines, but not existing on normal tissue B lymphocytes. Thus we were able to obtain antisera with specificity to B cell antigen,

TABLE 2. REACTIVITY* OF THE ABSORBED ANTI-BALL-1 SERA WITH THE VARIOUS CELL LINES BY MEMBRANE IMMUNOFLUORESCENCE

| Antisera | Absorption | B cell | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | BALL-1 | JBL-2 | JBL-3 | TK | ZK-H | CCRF-SB | KO-HL-2 | KO-HL-3 | KO-HL-4 |
| B-1 | NALL-1, TALL-1, HGC-25, FCS | ++ | + | ND** | + | + | ++ | ++ | + | + |
| B-2 | NALL-1, TALL-1, Tonsil | ++ | − | − | − | − | ND | − | ND | − |
| B-3 | NALL-1, TALL-1, KO-HL-3 | ++ | − | − | − | − | − | − | − | − |
| B-4 | Tonsil, HGC-25, FCS | ++ | ± | ± | + | − | + | ± | + | − |

TABLE 2. continued

| T cell | | | | | | non-T, non-B cell | | | |
|---|---|---|---|---|---|---|---|---|---|
| TALL-1 | CCRF-HSB-2 | MOLT-3 | MOLT-4 | HPB-ALL | MT-1 | NALL-1 | KOP-N-1 | NALM-1 | NALM-18 |
| − | ± | ± | − | ± | − | − | ± | ND | ± |
| − | − | − | − | − | − | − | − | − | ND |
| − | − | − | − | − | − | − | − | − | − |
| − | − | − | − | − | − | − | − | − | ± |

* −: All cells were negative; ±: less than 50% of the cells were stained very weakly; +: more than 50% of the cells were moderately positive; ++: all cells were strongly positive.
** Not done.

B-ALL antigen and B cell line antigen.

## DISCUSSION

Antisera have been raised against normal fresh B lymphocytes (30) or B cell lines (9, 11), but the production of antisera against B-ALL has not yet been reported. We used B-ALL line, BALL-1, as immunizing antigens and various lymphoid cell lines as the target cells, respectively. Because of homogeneity in their antigenicity, cell lines have some advantages over fresh cells although they sometimes loose antigens or gain alien antigens in the course of long-term culture. B-1 antiserum may have specificity apparently similar to antiserum prepared to normal B cell lines (9, 11), but B-1 antiserum does not detect Ia-like antigens since it did not react with the non-T, non-B cell lines positive for Ia-like antigens. Thus, it may be useful as a probe for B cell marker antigen although there is a posibility that it may detect the antigen(s) of some B cell subpopulations. B-2 or B-3 serum reacted only with BALL-1 cells. Although the possibility cannot be completely excluded that these antisera detect alloantigen(s) of BALL-1 cells, such is unlikely since there are no HLA antigens existing only in BALL-1 cells alone (data not shown). BALL-1 cells may have unique B-ALL antigen(s) different from cALL antigen or HTLA. Recently,

cALL antigen has been suggested to be a kind of differentiation antigen expressed on pluripotential stem cells (14). It is doubtful that B-ALL antigen be a differentiation or a stem cell antigen of the B cell series because other B cell lines lack the B-ALL antigen, and furthermore BALL-1 cells have several characteristics specific to mature B cells. The possibility that they recognize a subpopulation of B cells also seems excludable. B-4 serum reacted with some normal and leukemic B cell lines including BALL-1 and did not react with adult normal B cells. It may be that B-4 serum can detect the B cell-associated antigen expressed on some B cells under cell culture conditions, but there remains the possibility that it might just as well react with some fresh neoplastic B cells.

The present data are restricted to the reactivities of the serum against cell lines. Reactivities against normal and neoplastic lympho-hematopoietic tissues are now under study.

## REFERENCES


1. Billing, R., Minowada, J., Cline, M., Clark, B. and Lee, K. : Acute lymphocytic leukemia-associated cell membrane antigen. *J. Natl. Cancer Inst.* **61**, 423-429, 1978.
2. Brown, G., Hogg, N. and Greaves, M.: Candidate leukaemia-specific antigen in man. *Nature* **258**, 454-456, 1975.
3. Greaves, M.F., Brown, G., Rapson, N.T. and Lister, T.A.: Antisera to acute lymphoblastic leukemia cells. *Clin. Immunol. Immunopathol.* **4**, 67-84, 1975.
4. LeBien, T.W., Hurwitz, R.L. and Kersey, J.H.: Characterization of a xenoantiserum produced against three molar KCl-solubilized antigens obtained from a non-T, non-B (pre-B) acute lymphoblastic leukemia cell line. *J. Immunol.* **122**, 82-88, 1979.
5. Pesando, J.M., Ritz, J., Levine, H., Terhorst, C., Lazarus, H. and Schlossman, S.F.: Human leukemia-associated antigen: Relation to a family of surface glycoproteins. *J. Immunol.* **124**, 2794-2799, 1980.
6. Billing, R., Rafizadeh, B., Drew, I., Hartman, G., Gale, R. and Terasaki, P.: Human B-lymphocyte antigens expressed by lymphocytic and myelocytic leukemia cells. I. Detection by rabbit antisera. *J. Exp. Med.* **144**, 167-178, 1976.
7. Janossy, G., Goldstein, A.H., Capellaro, D., Greaves, M.F., Kulenkampff, J., Pippard, M. and Welsh, K.: Differentiation linked expression of p28, 33 (Ia-like) structures on human leukaemic cells. *Br. J. Haematol.* **37**, 391-402, 1977.
8. Schlossman, S.F., Chess, L., Humphreys, R.E. and Strominger, J.L.: Distribution of Ia-like molecules on the surface of normal and leukemic human cells. *Proc. Natl. Acad. Sci.* **73**, 1288-1292, 1976.
9. Anderson, J.K. and Metzgar, R.S.: Detection and partial characterization of human T and B lymphocyte membrane antigens with antisera to HSB and SB cell lines. *J. Immunol.* **120**, 262-271, 1978.
10. Borella, L., Sen, L. and Casper, J.T.: Acute lymphoblastic leukemia (ALL) antigens detected with antisera to E rosette-forming and non-E rosette-forming ALL blasts. *J. Immunol.* **118**, 309-315, 1977.
11. Tsubota, T., Minowada, J. and Pressman, D.: Reaction of T-lymphoid and B-lymphoid cell lines with rabbit antisera against these lines. *J. Natl. Cancer Inst.* **59**, 399-404, 1977.

12. Brouet, J-C., Valensi, F., Daniel, M-T., Flandrin, G., Preudhomme, J-L. and Seligmann, M. : Immunological classification of acute lymphoblastic leukaemias: Evaluation of its clinical significance in a hundred patients. *Br. J. Haematol.* **33**, 319-328, 1976.
13. Brown, G., Greaves, M.F., Lister, T.A., Rapson, N. and Papamichael, M.: Expression of human T and B lymphocyte cell-surface markers on leukaemic cells. *Lancet* **2**, 753-755, 1974.
14. Greaves, M.F. and Janossy, G.: Patterns of gene expression and the cellular origins of human leukaemias. *Biochim. Biophys. Acta* **516**, 193-230, 1978.
15. Miyoshi, I., Hiraki, S., Tsubota, T., Kubonishi, I., Matsuda, Y., Nakayama, T., Kishimoto, H. and Kimura, I.: Human B cell, T cell and null cell leukaemic cell lines derived from acute lymphoblastic leukaemias. *Nature* **267**, 843-844, 1977.
16. Miyoshi, I., Hiraki, S., Kimura, I., Miyamoto, K. and Sato, J.: 2/8 translocation in a Japanese Burkitt's lymphoma. *Experientia* **35**, 742-743, 1979.
17. Miyoshi, I.: Lymphoid cell lines and EB virus. *Soshikibaiyo* **6**, 137-141, 1980 (in Japanese).
18. Miyoshi, I., Kubonishi, I., Yoshimoto, S., Hikita, T., Dabasaki, H., Tanaka, T., Kimura, I., Tabuchi, K. and Nishimoto, A.: Characteristics of a brain lymphoma cell line derived from primary intracranial lymphoma (in press).
19. Miyoshi, I., Hiraki, S., Tsubota, T., Hikita, T., Masuji, H., Nishi, Y. and Kimura, I.: Hairy cell leukemia: Establishment of a cell line and its characteristics. *Cancer* **47**, 60-65, 1981.
20. Adams, R.A., Pothier, A., Flowers, A., Lazarus, H., Farber, S. and Foley, G.E.: Comparison of cells isolated *in vitro* and *in vivo* from a patient with acute lymphoblastic leukemia. *Exp. Cell Res.* **62**, 5-10, 1970.
21. Akagi, T., Matsuda, Y. and Taniuchi, K.: Establishment of three lymphoblastoid cell lines from the primary lesion and the metastatic lymph nodes of gastric cancer. *Kochi J. Med.* **2**, 47-51, 1975 (in Japanese).
22. Kaplan, J., Shope, T.C. and Peterson, W.D.: Epstein-Barr virus-negative human malignant T-cell lines. *J. Exp. Med.* **139**, 1070-1076, 1974.
23. Minowada, J., Ohnuma, T. and Moore, G.E.: Rosette-forming human lymphoid cell lines. I. Establishment and evidence for origin of thymus-derived lymphocytes. *J. Natl. Cancer Inst.* **49**, 891-895, 1972.
24. Morikawa, S., Tatsumi, E., Baka, M., Harada, T. and Yasuhira, K.: Two E-rosette-forming lymphoid cell lines. *Int. J. Cancer* **21**, 166-170, 1978.
25. Miyoshi, I., Kubonishi, I., Sumida, M., Yoshimoto, S., Hiraki, S., Tsubota, T., Kobayashi, H., Lai, M., Tanaka, T., Kimura, I., Miyamoto, K. and Sato, J.: Characteristics of a leukemic T-cell line derived from adult T-cell leukemia. *Jpn. J. Clin. Oncol.* **9** (Suppl.), 485-494, 1979.
26. Nakazawa, S., Kaneko, K., Koide, R. and Watanabe, S.: Establishment of null cell leukemic cell lines (KOPN) and its characteristics. *Rinshoketsueki* **20**, 189, 1978 (in Japanese).
27. Minowada, J., Tsubota, T., Greaves, M.F. and Walters, T.R.: A non-T, non-B human leukemia cell line (NALM-1): Establishment of the cell line and presence of leukemia-associated antigens. *J. Natl. Cancer Inst.* **59**, 83-87, 1977.
28. Kubonishi, I., Miyoshi, I., Kimura, I. and Minowada, J.: Establishment and characterization of a pre-B cell human acute lymphoblastic leukemia (ALL) line. *Proc. Jpn. Cancer Assoc.* **38**, 123, 1979 (in Japanese).
29. Akagi, T. and Kimoto, T.: Establishment and characteristics of a human pancreatic cancer cell line (HGC-25). *Acta Pathol. Jpn.* **27**, 51-58, 1977.
30. Ishii, Y., Koshiba, H., Ueno, H., Maeyama, I., Takami, T., Ishibashi, F. and Kikuchi, K.: Characterization of human B lymphocyte specific antigens. *J. Immunol.* **114**, 466-469, 1975.