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DETERMINATION OF A SHARED EPITOPE ON CELLS FROM ACUTE MYELOGENIC LEUKEMIA (AML) AND T-ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL)

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ABSTRACT

Two IgM monoclonal antibodies (MAb) with strong reactivity for granulocytes and to a lesser extent for Jurkat cell lines were established by immunizing BALB/c mice with a histiocytic cell line (U937). These two MAbs (designated as 6C9 and 4C4) reacted with blast cells of T-acute lymphoblastic leukemia (T-ALL) and acute myelogenous leukemia (AML) patients as well as leukemic cells from patients known as unclassified leukemia (UL). According to their pattern of reactivity, it is most probable that these two IgM MAbs react with some highly glycosylated membrane determinants such as 3-fucosyl-N-acetyllactosamine (3-FAL) which are exclusively expressed on some subsets of granulocytes and AML cells.

Keywords: Granulocyte, Leukemia, CD15

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INTRODUCTION

Myelo-monocytic cell series with their heterogenous morphology originate from the bone marrow. The differentiation and maturation of these cells are characterized mostly according to their morphology and cytochemical features. Phagocytosis, antigen processing and presentation are the most important immunological functions of myelo-monocytic cell series. The cell-cell interaction, migration and adhesion that are essential and prerequisites for phagocytosis and antigen recognition are mediated via their membrane-associated molecules.[1] Various cell surface markers exist on the membrane of myeloid cells, some of which are shared by other leukocytes. There are also molecules known as lineage and stage specific markers on these cells.[2,3] The role and function of some of these molecules have been studied, but the immunological function of the majority has remained obscure.[2] In the present study, we describe two IgM monoclonal antibodies exclusively reactive with normal peripheral granulocytes and myeloid leukemic blast cells. Comparing the tissue distribution of these two antibodies with those of known myeloid specific antibodies suggest that our MAbs may react with certain membrane-associated molecules similar to CD15.

MATERIAL AND METHODS

Media and reagents

RPMI-1640 supplemented with 10% fetal calf serum (FCS) and penicillin and streptomycin were purchased from Gibco, Scotland. HAT medium, polyethylene glycol (1350), and FITC-conjugated goat anti-mouse Ig was obtained from Sigma (St. Louis).

Cells and cell lines

Purified granulocytes, platelets and red cells were prepared

from healthy laboratory personnel. Leukemic blast cells were provided from patients referring to hospitals affiliated to the Shiraz University of Medical Sciences. Normal and leukemic cells were purified by Ficoll-Hypaque density gradient centrifugation as described elsewhere.[5,6] Granulocytes were purified using 3% dextran solution.

Production of 6C9 and 4C4 hybridoma clones

Details on the production of monoclonal antibodies and procedures for their screening have been described previously.[5]

RESULTS AND DISCUSSION

The pattern of various cell lines with these two monoclonal antibodies is shown in Table I. As indicated, 6C9 and4C4 reacted with 90% of the U937 cell line. B-cell lines were found to be less than 10% reactive with these two antibodies. On the other hand Jurkat cell line (a T-ALL phenotype) was found to be 20% and 50% reactive with 4C4 and 6C9 monoclonal antibodies respectively (Table I).

Reactivities of these two antibodies for normal blood mononuclear cells were less than 10% (Table II). 6C9 and 4C4 MAbs reacted with 72% and 67% of purified granulocytes respectively (Table II), while only 5% of blood monocytes reacted with these antibodies. Moreover,no reactivity was found with red cells and platelets (Table II).

As indicated in Table III, all 7 cases of AML and one case of chronic myelogenous leukemia (CML) were found to react with these two antibodies. The mean percentage of fluorescent activity for 7 cases of AML was found to be 46.14% with 4C4 and 54.14% with 6C9. Similarly, one case of T-ALL was also found to carry a reactive epitope for 4C4 and 6C9 monoclonal antibodies (Table III). Leukemic cells from two cases diagnosed as unclassified leukemia also reacted with these two antibodies.

As shown in Table III, 4C4 and 6C9 MAbs reacted with most leukemic cells of these two patients. The tissue distribution of epitopes recognized by these two IgM monoclonal antibodies indicated a line of identity similar to that reported for certain monoclonal antibodies clustered as CD15.[7] The comparison of data obtained with 4C4 and 6C9 monoclonal antibodies indicate that these two antibodies probably recognize structurally identical or highly related epitopes. The data presented in this study indicate that monoclonal antibodies of IgM isotype secreted by two hybridoma clones 6C9 and 4C4 react with 90% of U937, a monocytic cell line. Apart from reactivities seen with Jurkat cell line, no significant reactivities were found with B-cell lines. Despite lack of reactivity of normal mononuclear cells with 6C9 and 4C4 MAbs, the majority of normal granulocytes were found to carry a reactive epitope for these two antibodies. This pattern of reactivity again confirms the similarity between the tissue distribution of CD15 molecules and what we have seen for6C9 and4C4 reactive epitopes. Monoclonal antibodies clustered in the CD15 antigen recognize an epitope known as 3-fucosyl-N- acetyllactosamine (3-FAL).[4]

Table I. Percentage of reactivity of 4C4 and 6C9 antibodies with various cell lines.

Cell line	Antibody 4C4	Antibody 6C9
U-937	90	90
NALM-6	0	0
BJAB	6	10
DAUDI	10	0
RAMOS	8	5
JURKAT	20	50

Table II. Percentage of reactivity of 4C4 and 6C9 antibodies with hematopoietic cells.

Blood cell	Antibody 4C4	Antibody 6C9
PBG	67	72
PBM	6	8
PBMC	5	6
RBC	0	0
PLT	0	0

PBG: peripheral blood granulocyte
PBM: peripheral blood monocyte
PBMC: peripheral blood mononuclear cell
RBC: red blood cell
PLT: platelet

Table III. Percentage of reactivity of 4C4 and 6C9 antibodies with leukemic cells

Case no.	Antibody 4C4	Antibody 6C9
I	40	55
II	53	51
III	44	45
IV	74	71
V	50	90
VI	50	53
VII	12	14
VIII	20	20
IX	45	31
X	0	0
XI	0	0
XII	56	69
XIII	47	53

I-VII: AML VIII: CML
IX: T-ALL X: B-ALL
XI: CLL XII, XIII: UCL

Although the 3-FAL epitope is restricted to neutrophils, it is widely expressed on non-hemopoietic cells such as neural tissues.[4] Of particular interest is the expression of the 3-FAL epitope on leukemic blast cells from AML patients. From among the different CD15 monoclonal antibodies which have been tested by other investigators, all have been shown to react strongly with AML cells.[8] We have tested 7 different AML cells for their reactivity for 6C9 and 4C4 MAbs, and all 7 cases were shown to react with these two antibodies. The present data and the results of another investigation[9] have shown no correlation between the expression of CD15 on AML cells and the FAB classification, although both antibodies expressed a weak response on AML-M_4 cells. The relative reactivity of a group of leukemic cells phenotypically characterized as unclassified leukemia with these two antibodies support their use as an important tool in diagnosing the type of leukemia.

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REFERENCES

1. Zimmerman GA, Prescott SM, McIntyreTM: Endothelial cell interactions with granulocytes: tethering and signalling molecules. Immunol Today 13: 93-100, 1992.
2. Bordessoule D, Jones M, Gatter KC, Mason DY: Immunohistological pattern of myeloid antigens: tissue distribution of CD13, CD14, CD16, CD31, CD36, CD65, CD66 and CD67. British J Hematology. 83: 370-383, 1993.
3. Favaloro EJ, Bradstock KF, Grimsley P, Zowtyi H, Zola H: Further characterization of myeloid antigen (gp: 160, gp: 150, gp: 67): investigation of epitopic heterogeneity and non-hematopoietic distribution using a panel of monoclonal antibodies belonging to CD11b and CD13. British J Hematology 69: 163-171, 1988.
4. Tetteroo P, Geurts Van Kessel AD: Expression of CD15 (FAL) on myeloid cells and chromosomal localization of the gene. Histochem J 24: 777-782, 1992.
5. Amirghofran Z, Ghaderi AA: Determination of a 45 KDa leukocyte common antigen and its unique expression on B-cell development. Iran J Med Sci 17: 109-115, 1992.
6. Westerwoudt RJ: Improved fusion methods. IV. Technical aspects. J Immunol Methods 77: 181-194, 1986.
7. Gooi HC, Feizi T, Kapadia A, Knowles BB, Solter D, Evans MJ: Stage specific embryonic antigen involved alpha-1 goes to 3 fucosylated type 2 blood group chains. Nature 292: 156-158, 1981.
8. Ball ED, Schwarz L: Reactivity of CD15 MAb with normal and malignant myeloid cells and with hemopoietic progenitor cells. In: Knapp W, Dorken B, Giks WR, et al (eds). Leukocyte Typing IV. White cell differentiation antigens. Oxford University Press, pp. 805-806, 1989.
9. San Miguel JF, Gonsalez M, Canizo MC, Anta JP, Zola H, Lopez BA: Surface marker analysis in acute myeloid leukemia and correlation with FAB classification. British J Hematology 64: 547-560, 1986.