DETERMINATION OF A SHARED EPITOPE ON 
CELLS FROM ACUTE MYELOGENIC LEUKEMIA 
(AML) AND T-ACUTE LYMPHOBLASTIC LEUKEMIA 
(T-ALL) 
ABBAS A. GHADERI, M.H. AGAHI, Z. AMIRGHOFRAN AND 
S. ARDEHALI 
From the Dept. of Microbiology and Immunology, Medical School, Shiraz University of Medical 
Sciences, Shiraz, Islamic Republic of Iran. 

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 


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. 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. The role and function of some of these molecules 
have been studied, but the immunological function of the 
majority has remained obscure. 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 (1351), 
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 

Downloaded from mjiri.iums.ac.ir at 16:34 IRDT on Friday August 23rd 2019
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.\textsuperscript{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.\textsuperscript{5}

RESULTS AND DISCUSSION

The pattern of various cell lines with these two monoclonal antibodies is shown in Table I. As indicated, 6C9 and 4C4 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.\textsuperscript{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 for 6C9 and 4C4 reactive epitopes. Monoclonal antibodies clustered in the CD15 antigen recognize an epitope known as 3-fucosyl-N-acetyllactosamine (3-FAL).\textsuperscript{4}

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

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Antibody 4C4</th>
<th>Antibody 6C9</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-937</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>NALM-6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BJAB</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>DAUDI</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>RAMOS</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>JURKAT</td>
<td>20</td>
<td>50</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Blood cell</th>
<th>Antibody 4C4</th>
<th>Antibody 6C9</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBG</td>
<td>67</td>
<td>72</td>
</tr>
<tr>
<td>PBM</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>PBMC</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>RBC</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PLT</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Antibody 4C4</th>
<th>Antibody 6C9</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>40</td>
<td>55</td>
</tr>
<tr>
<td>II</td>
<td>53</td>
<td>51</td>
</tr>
<tr>
<td>III</td>
<td>44</td>
<td>45</td>
</tr>
<tr>
<td>IV</td>
<td>74</td>
<td>71</td>
</tr>
<tr>
<td>V</td>
<td>50</td>
<td>90</td>
</tr>
<tr>
<td>VI</td>
<td>50</td>
<td>53</td>
</tr>
<tr>
<td>VII</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>VIII</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>IX</td>
<td>45</td>
<td>31</td>
</tr>
<tr>
<td>X</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>XI</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>XII</td>
<td>56</td>
<td>69</td>
</tr>
<tr>
<td>XII</td>
<td>47</td>
<td>53</td>
</tr>
</tbody>
</table>

I-VII: AML
IX: T-ALL
XI: B-ALL
XII: CLL
XIII: UCL
Although the 3-FAL epitope is restricted to neutrophils, it is widely expressed on non-hemopoietic cells such as neural tissues. 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. 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 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, 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.

ACKNOWLEDGEMENT

This work was supported by a grant from the Shiraz University of Medical Sciences (Grant No. 7048).

REFERENCES