ACQUISITION OF CELL SURFACE IgD
AFTER IN VITRO CULTURE OF NEOPLASTIC
B CELLS FROM THE MURINE TUMOR BCL1*

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The differentiation of bone marrow-derived lymphocytes in mice is characterized
by the sequential appearance of different surface markers. IgM is the first isotype to
appear, and IgM+ cells subsequently acquire IgD. The acquisition of IgD appears to
be antigen and T cell independent (1). The roles of these two surface isotypes in the
triggering of B cells is the subject of intensive study. When mature B cells bearing
both isotypes are triggered by mitogens, IgD is lost and the cells differentiate into
IgM secreting plasma cells (1-3).

Recent evidence indicates that several nonsecreting B cell tumors from humans (4,
5) and mice (6) have the capacity to secrete IgM under the influence of mitogens (5),
T cells (4), or after fusion to myeloma cells (7). One of the murine tumors, BCL1, has
been used in the present studies to investigate the pathway of B cell differentiation
that is characterized by the acquisition of IgD. The uncultured tumor cells bear large
quantities of surface IgM and trace amounts of IgD (8-10). Thus, both the phenotypic
characteristics (6) and, in addition, the functional properties (9, 10) of BCL1 cells
suggest that they are analogous to immature B cells. In the present studies we have
demonstrated that cultivation of BCL1 cells results in markedly increased expression
of surface IgD in the absence of IgM secretion.

Materials and Methods

Mice. BALB/c mice were obtained from Cumberland View Farms, Clinton, Tenn. The
BCL1 tumor was maintained in vivo by intravenous passage of 10⁵ spleen cells obtained from
a tumor-bearing mouse.

Culture Conditions. Peripheral blood and spleen cells were obtained from mice that had
carried the tumor for 8-12 wk and were prepared and cultured as previously described (11)¹
without the addition of either 2-mercaptoethanol or lipopolysaccharide (LPS). Greater than
90% of the cell populations employed are tumor cells as judged by morphology and expression
of idiotype (9, 12). Cells were incubated in 250-ml culture flasks (Falcon Labware, Div. of
Becton, Dickinson & Co., Oxnard, Calif.) at 2 × 10⁶/ml (30-50 ml/flask), in RPMI-1640 with
10% fetal calf serum (FCS) (Grand Island Biological Co., Grand Island, N. Y.). Under these
conditions, BCL1 cells do not secrete IgM.¹

Immunofluorescence. Indirect immunofluorescence analysis of tumor cells on the fluorescence-
activated cell sorter (FACS III, Becton, Dickinson & Co., Rutherford, N. J.) was performed as

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FIG. 1. Immunofluorescence analysis of BCL₁ cells stained with anti-δ. Blood cells from tumor-bearing animals were cultured at 2 × 10⁶/ml for 4 d and, along with uncultured blood cells, were stained with hybridoma anti-δ or the control mouse IgG₂A (RPC-5) and F1-RAMy F(ab')₂. Cells were analyzed on the FACS, and the results were plotted as cell number vs. fluorescence intensity.

Results

Immunofluorescence Analysis. Prior studies of BCL₁ tumor cells with both immunofluorescence and biochemical techniques demonstrated that these cells bear large quantities of surface IgM but only trace amounts of IgD (8–10). Thus, Fig. 1 shows that freshly prepared BCL₁ cells stained minimally with anti-δ. In contrast, 86% of BCL₁ cells cultured for 4 d without added mitogens stained brightly with hybridoma anti-δ but not with a control myeloma protein of the same subclass (RPC-5).
cultured cells that stained with anti-8 were very heterogeneous with respect to fluorescence intensity, which suggests a variable density of surface IgD. This finding indicates that the vast majority of BCL1 tumor cells differentiate in vitro and acquire the surface immunoglobulin phenotype of a more mature B cell.

Biochemical Analysis of Surface Ig. To obtain biochemical evidence that cultured BCL1 cells acquire IgD, cells were iodinated, either before or after culture, and the lysates were analyzed by immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Immunoprecipitation with rabbit anti-mouse Ig (RAMIg) of lysates from uncultured cells demonstrated substantial quantities of #-chain but little or no &chain (Fig. 2). Lysates from radioiodinated cultured cells, however, contained readily detectable IgD as determined by the presence of radioactive &-chain on SDS-PAGE. Estimation of the areas under the #- and &-peaks in several experiments showed at least a three- to fourfold decrease in the #:&-ratio of cultured cells. Analogous results with regard to &-chain were obtained with affinity-purified goat anti-8 (data not shown).

Radioimmunoassay for Surface Ig. To further quantify the relative amounts of IgM and IgD on cultured BCL1 cells, cells were treated with radioiodinated, affinity-purified antibodies directed against #- or &-chains. As shown in Table I, BCL1 cells bound substantially more 125I-anti-# than normal spleen cells (assuming 40% of normal spleen cells and 100% of BCL1 cells are IgM positive) (9). In the experiment shown, binding of 125I-anti-# was unchanged on cells cultured for 6 d; in other experiments, increased binding of 125I-anti-# (up to a twofold increase) was often observed. In contrast, binding of 125I-anti-8 to uncultured BCL1 cells, although

![Figure 2](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3204745/bin/7a.png)
Table I

Radioimmunoassay for Cell Surface IgM and IgD on BCL₁ Cells

<table>
<thead>
<tr>
<th>Cell source</th>
<th>Days in culture*</th>
<th>Bound to cells</th>
<th>( \mu : \delta )**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( ^{125}\text{I-anti-( \mu )})</td>
<td>( ^{125}\text{I-anti-( \delta )})</td>
</tr>
<tr>
<td>BALB/c spleen</td>
<td>0</td>
<td>598</td>
<td>2,582</td>
</tr>
<tr>
<td>BCL₁ spleen**</td>
<td>6</td>
<td>2,764</td>
<td>933</td>
</tr>
<tr>
<td>BCL₁ spleen**</td>
<td>0</td>
<td>2,529</td>
<td>3,144</td>
</tr>
<tr>
<td>BCL₁ blood**</td>
<td>0</td>
<td>2,467</td>
<td>855</td>
</tr>
<tr>
<td>BCL₁ blood**</td>
<td>6</td>
<td>2,540</td>
<td>2,603</td>
</tr>
</tbody>
</table>

* Cells were cultured at \( 2 \times 10^6 / \text{ml} \).
\( ^{12} \) \( 1 \times 10^5 \) cpm input (\( 2 \times 10^6 \) cpm/µg).
\( ^{2} \) \( 2 \times 10^6 \) cpm input (\( 2 \times 10^6 \) cpm/µg).
\( ^{3} \) cpm anti-\( \mu \) bound: cpm anti-\( \delta \) bound.
\( ^{4} \) \( 5 \times 10^5 \) cells/tube were assayed.
\( ^{**} \) \( 2 \times 10^5 \) cells/tube were assayed.

Discussion

The present studies indicate that culture of BCL₁ tumor cells can result in a markedly increased expression of cell surface IgD. The IgD was measured by three techniques: immunofluorescence using the FACS, immunoprecipitation of radioiodinated surface immunoglobulin and analysis by SDS-PAGE, and a radioimmunoassay with heavy chain-specific antibodies. The results of the latter assay suggest that the
density of IgD on uncultured BCL₁ cells is ~1/10th of that found on normal adult spleen cells. After 3–5 d of culture, the density of IgD increased three- to fourfold. Earlier studies have shown a loss of surface IgD during differentiation of B cells into plasma cells (1-3) but to our knowledge, this is the first demonstration of in vitro acquisition of surface IgD.

A significant development in B cell immunology was the demonstration by Fu et al. (4, 5) and Kishimoto (13) that neoplastic human B lymphocytes could be stimulated in vitro to differentiate into Ig-secreting plasma cells. Similar observations have been made with the BCL₁ tumor, i.e., cultivation of BCL₁ cells with LPS stimulates them to secrete IgM (11) bearing the same idiotype as the surface Ig of the unstimulated cells (12). The present findings extend the concept that tumor cells can differentiate. Thus, cultivation of BCL₁ cells in the absence of LPS results in differentiation along a different pathway, i.e., acquisition of an Ig phenotype characteristic of a more mature B lymphocyte. It is unclear whether this is a spontaneous maturation event related to removal of host suppressive influences or caused by trace amounts of stimulatory factors (growth factors or mitogens) in the FCS. Regardless, the BCL₁ cells may provide a useful model for analysis of the events involved in expression of IgD and the factors that determine which pathway of differentiation is chosen by B cells.

Summary

Murine BCL₁ tumor cells bear large amounts of surface IgM and trace amounts of surface IgD. In the present studies we have shown that cultivation of these cells, in the absence of lipopolysaccharide, results in the acquisition of IgD by virtually all the cells. These results suggest that BCL₁ cells can differentiate in vitro into more mature B cells and offer an attractive model for analyzing the factors controlling appearance of IgD on a monoclonal cell line.

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References


