Identification of a novel adhesion molecule in human leukocytes by monoclonal antibody LB-2

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Monoclonal antibody LB-2 to a surface antigen on human B cells, lymphoblast, monocytes and vascular endothelial cells largely inhibited adhesion among Epstein Barr virus-immortalized normal B cells (EBV-B) and concanavalin A-stimulated blood mononuclear cells (Con A-BMC) before and after phorbol ester treatment. The antibody inhibited to a lesser extent phorbol ester-induced aggregation of monocytes, U937 cells and fresh BMC and had virtually no inhibitory effect on the adhesion among enriched T cells and granulocytes. A surface glycoprotein band of 84 kDa was obtained from EBV-B cells by immunoprecipitation and gel electrophoresis. Immunological and biochemical studies clearly distinguished this molecule from gp90 and associated glycoproteins which also mediate leukocyte adhesion.

LB-2 antibody; Leukocyte antigen; Adhesion molecule

1. INTRODUCTION

Mononuclear leukocytes interact with each other and with vascular endothelium to generate immune and inflammatory responses. Some of these interactions are known to require cell-cell adhesion [1,2].

Phorbol esters, such as tetradecanoyl phorbol acetate (TPA) and phorbol dibutyrate (P(Bu)2), induce and enhance leukocyte adhesion and aggregation [3]. The intercellular binding is an energy- and temperature-dependent process that requires cell surface structures. Some of these adhesion molecules appear to constitute ligands forming adhesive cell-cell bonds referred to as CAMs (cell-adhesion molecules) [4].

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line PSB-1 was used as source of EBV-B cells. Other cell lines used in this study have been described in [11]. The SKW-3 cell line, derived from a T cell leukemia, was kindly provided by Professor Hans Wigzell. Blood mononuclear cells, a T cell-enriched population, and granulocytes were separated as in [6,7]. Monocytes were obtained from blood mononuclear cells by adherence to plastic dishes and this cell preparation contained 99% OKM1 positive cells and less than 2% T cells (T3 positive). Activated blood mononuclear cells were obtained by incubation with Con A (Sigma, St. Louis, MO) at 5 μg/ml for 3 days. Before use, these cells were extensively washed with 100 mM α-methyl-D-mannoside (Sigma) to remove any residual lectin. Vascular endothelial cells were prepared from human umbilical veins and cultured as in [12].

2.2. Monoclonal antibodies
Antibodies LB-2 (previously named BB-2) and 60.3 were produced as previously reported [5,10,11]. Antibody W6/32 to class I transplantation antigen was purchased from Sera-Lab (London, England). The three antibodies are murine IgG2a and were used as ascites (LB-2 and W6/32, Ig content ≥ 2 mg/ml) or purified Ig (60.3). OKM1 and anti-T3 antibodies were obtained from Ortho Diagnostic Systems (Raritan, NJ) and New England Nuclear (Boston, MA), respectively. Indirect immunofluorescence studies were performed as in [8].

2.3. Measurement of cell aggregation
After extensive washing, cells were resuspended at 5 × 10^6 (cell lines) or 10 × 10^6 (fresh blood cells) cells/ml in RPMI 1640 medium (Grand Island, NY) with 0.5% human albumin (KabiVitrum, Stockholm). Cell suspensions were spun at 100 rpm at 37°C and treated with 60 nM P(Bu)₂ (Sigma) for 20 min. Cell aggregation and the effect of antibodies (at approx. 20 μg/ml, unless otherwise stated) were measured microscopically as reported [6–8].

2.4. Cell surface labelling, immunoprecipitation and gel electrophoresis
EBV-B cells were surface-labelled with ³H after periodate oxidation and NaB₃H₄ reduction [13]. Immunoprecipitation was performed using rabbit anti-mouse IgG (Dakopatts, Copenhagen) in the second step [14]. Polyacrylamide slab gel electrophoresis in the presence of SDS was done according to Laemmli [15] using 8% acrylamide gels. The ³H-labelled gels were treated for fluorography according to Bonner and Laskey [16]. ¹⁴C-labelled standard proteins were obtained from the Radiochemical Centre (Amersham, England).

3. RESULTS
Antibody LB-2 inhibited phorbol ester-enhanced aggregation of EBV-B cell (72%) in a concentration-dependent manner (fig. 1). At 20 μg/ml, the antibody blocked the cell aggregation in more than 70%. Half inhibition was obtained with approx. 50 ng/ml. The spontaneous cell aggregation (22%) was also largely blocked by both antibodies while the characteristic phorbol ester-induced morphological changes [8] were not affected (not shown). Antibody LB-2 inhibited intercellular adhesion in EBV-B cells from four different lines which had been passaged in vitro for a few months up to several years (not shown). In contrast, antibody W6/32 as well as many other antibodies to cell-surface antigens did not inhibit cell-cell binding [4,8]. Antibody LB-2 also inhibited to a large extent phorbol ester-enhanced adhesion among lectin-activated blood mononuclear cells while a minor inhibitory effect was obtained when monocytes, U937 cells or fresh...
### Table 1

Effect of monoclonal antibodies LB-2, 60.3 and W6/32 on phorbol ester-induced adhesion among different leukocytes

<table>
<thead>
<tr>
<th>Leukocytea</th>
<th>Per cent cell aggregationb</th>
<th>Per cent inhibition of cell aggregation by monoclonal antibodiesc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LB-2</td>
<td>60.3</td>
</tr>
<tr>
<td>EBV-B cells</td>
<td>67</td>
<td>72(^d^)</td>
</tr>
<tr>
<td>Con A-blood mononuclear cells</td>
<td>60</td>
<td>73</td>
</tr>
<tr>
<td>Monocytes</td>
<td>72</td>
<td>32</td>
</tr>
<tr>
<td>U937 cells</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>Blood mononuclear cells</td>
<td>58</td>
<td>13</td>
</tr>
<tr>
<td>Enriched T cells</td>
<td>31</td>
<td>3</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>90</td>
<td>0</td>
</tr>
</tbody>
</table>

- All cells were resuspended in RPMI 1640 medium with 0.5% human albumin at 5 x 10^6 (cell lines) or 10 x 10^6 (fresh blood cells) per ml
- Leukocyte aggregation was measured by counting aggregated or single cells after 20 min treatment with 60 nM P(Bu)\(_2\) and rotation at 100 rpm at 37°C
- Approx. 20 μg/ml of antibodies was added to the cells before phorbol ester treatment
- Mean of at least two experiments

### Table 2

Reactivity of monoclonal antibodies LB-2 and 60.3 with different cell types

<table>
<thead>
<tr>
<th>Cells (type)</th>
<th>Immunofluorescence reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LB-2</td>
</tr>
<tr>
<td>Blood mononuclear cells</td>
<td>42 ±/+</td>
</tr>
<tr>
<td>Enriched T cells</td>
<td>23 ±</td>
</tr>
<tr>
<td>Monocytes</td>
<td>90 +</td>
</tr>
<tr>
<td>Con A-blood mononuclear cells</td>
<td>64 ±/+ + +</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>13 ±</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>0</td>
</tr>
<tr>
<td>Vascular endothelial cells</td>
<td>99 ++/+ + +</td>
</tr>
<tr>
<td>PSB-1 (EBV-B)</td>
<td>80 ++/+ + +</td>
</tr>
<tr>
<td>Raji (Burkitt's lymphoma-B)</td>
<td>96 ++/+ + +</td>
</tr>
<tr>
<td>Molt-4 (T leukemia)</td>
<td>97 ±, 3 ++ +</td>
</tr>
<tr>
<td>SKW-3 (T leukemia)</td>
<td>82 ±, 18 ++</td>
</tr>
<tr>
<td>U937 (histiocytic lymphoma)</td>
<td>98 ++/+ +</td>
</tr>
<tr>
<td>K562 (erythroleukemia)</td>
<td>85 ++</td>
</tr>
</tbody>
</table>

Percentage of positive cells and labelling intensity were measured in a fluorescence microscope: (±) very weak, (+) weak, (+++) intermediate and (++++) strong
blood mononuclear cells were used (table 1). In contrast to antibody 60.3, no significant effect was observed with the antibody on enriched T cells and granulocytes.

As reported in [10,11], antibody LB-2 reacted weakly with some blood cells, particularly monocytes, while T cells, granulocytes and erythrocytes were virtually negative (table 2). After a 3 day stimulation of blood mononuclear cells with Con A, labelling with the antibody was stronger in a larger number of cells. The reactivity of the antibody with cells from T, B, myelomonocytic and erythroid lineages was confirmed and in addition, the antibody was found to react strongly with cultured vascular endothelial cells and to have a different specificity from antibody 60.3 (table 2).

Under reducing conditions, a surface glycoprotein band of 84 kDa was obtained from EBV-B cells by immunoprecipitation with antibody LB-2 and gel electrophoresis (fig.2C), while an apparent molecular mass of 77 kDa was observed under non-reducing conditions (fig.2F). Some labelled material remained on the top of the gel indicating aggregation. This may occur between the gp84 molecules or between these and other molecules like the monoclonal antibody present in the immune precipitate. Antibody 60.3 precipitated two major surface glycoproteins with apparent molecular masses of 90 and 160 kDa from the same cells (fig.2D), as previously reported [8].

4. DISCUSSION

The inhibitory effect of antibody LB-2 on cell aggregation induced or enhanced by phorbol esters indicates the participation of gp84 in the intercellular adhesion. We have previously tested several hundred different monoclonal antibodies directed against human leukocyte surface glycoproteins, and with one exception (antibody 60.3), no antibody inhibited cell adhesion. Fab fragments from 60.3 inhibited adhesion as well. Since the LB-2 antibody does not affect induction of morphological changes, this surface molecule is apparently not involved in stimulation of the cells by these compounds. Thus, it seems probable that gp84 is directly involved in the intercellular binding and it may constitute an adhesive ligand or CAM. It is also likely that gp84 interacts with a previously described adhesion molecule [6–8]. Interestingly, inhibition of cell aggregation by antibody LB-2 was less than that shown by antibody 60.3 and never complete, suggesting the participation of additional adhesion molecules. When tables 1 and 2 are compared, the inhibitory effect of antibody LB-2 on cell aggregation correlates well with the expression of its antigen on the cells. Since the antigen expression increases after stimulation of the cells with lectins or EBV, gp84 seems to mediate adhesion mainly in ‘activated’ mononuclear leukocytes. Moreover, the strong reactivity of the antibody with vascular endothelial cells suggests that the antigen constitutes an adhesion molecule also in these cells.

In contrast to antibody LB-2, antibody 60.3 inhibits adhesion among all types of leukocytes tested thus far. The latter antibody recognizes gp90, which is separately and non-covalently associated to gp160, gp155 and gp130 identified by monoclonal antibodies anti TA-1/LFA-1, OKM1

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**Fig. 2.** Polyacrylamide slab gel electrophoresis patterns of 3H-labelled EBV-B cells. (A) 14C-labelled standard proteins: M, myosin; PHb, phosphorylase b; BSA, bovine serum albumin; OA, ovalbumin; CA, carbonic anhydrase; (B) pattern of surface-labelled EBV-B cells: CLA, common leukocyte antigen; GP100, major sialoglycoprotein; HLA-A, class I transplantation antigen heavy chain; HLA-D, class II transplantation antigen; (C) pattern obtained by precipitation with antibody LB-2 under reducing conditions: GP84, glycoprotein with apparent molecular mass of 84 kDa; (D) pattern obtained by precipitation with antibody 60.3 under reducing conditions; (E) pattern with no monoclonal antibody; (F) pattern obtained by precipitation with antibody LB-2 and run under nonreducing conditions.
and anti-LeuM5, respectively [4,17]. All members of this protein complex are distinct from gp84 in both structure and cell distribution. In addition, the genes coding for gp90 and gp84 have been assigned to chromosome 21 and 19, respectively [9,18].

Slight changes in the electrophoretic mobility of the sialoglycoprotein recognized by antibody LB-2 suggest the presence of intra-chain disulfide bonds. The small difference in molecular mass from that described in the original publications, p76 [10,11], may be due to technical reasons.

Interestingly, cells which react with antibody LB-2, namely monocytes, B lymphoblast, U937, K562 and endothelial cells, are known to exert an 'accessory function' in T cell proliferation induced by lectins [19–21]. gp84 may mediate adhesion in this cell–cell interaction as well as in antigen presentation by the former cells. Moreover, adhesion of endothelial cells to lymphocytes may allow recirculation and localization of the latter cells in different lymphoid organs [2].

Recently, monoclonal antibody RR1/1 was reported to define a novel cell surface molecule involved in phorbol ester-induced aggregation of EBV-B cells [22]. Although the structure and cell distribution of this molecule are similar to those of gp84, only an extensive and parallel comparison of the two antigens will determine their relationship.

Adhesion of mononuclear leukocytes to each other and to vascular endothelial cells may allow proliferation and differentiation of the former cells and control of EBV-B cells in vivo [6,8]. On the other hand, abnormal adhesion may contribute to the development of leukemia and lymphoma.

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