Expression of CD15 (FAL) on myeloid cells and chromosomal localization of the gene

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Summary

Different CD15 murine monoclonal antibodies were studied. These antibodies appeared to react specifically with the human myeloid-lineage-derived cell types in both peripheral blood and bone marrow.

The antigens recognized by these antibodies were immunoprecipitated from lysates of ¹²⁵I-labelled neutrophilic PMNs of healthy donors and subsequently analysed by electrophoresis on SDS-polyacrylamide gel and autoradiography. All antibodies precipitated the same membrane polypeptides from the membrane-iodinated PMN lysates: 105 and 150-kDa as most prominent, together with 260-, 230-, 67- and 52-kDa polypeptides.

Absorption studies were performed with synthesized carbohydrate molecules. Antibody B4.3 appears to be directed against 3-α-fucosyl-N-acetyl-lactosamine (FAL). Competition experiments with ¹²⁵I-labelled B4.3 demonstrated complete inhibition of binding by B4.3 and three other CD15 antibodies (VIM D5, UJ308, MI/N1), and partial inhibition by three additional antibodies (FMC10, FMC12, FMC13), indicating binding to the same antigenic structure. None of the antibodies reacted with monocytes using the immunofluorescence technique, but after neuraminidase digestion of these cells, positive reactions were obtained with all antibodies. Immunoprecipitation with lysates of both native and neuraminidase-digested monocytes showed no polypeptide bands. Monocytic differentiation of the myeloid cell line HL60 by 12-O-tetradecanoylphorbol-13-acetate (TPA) was accompanied by a decrease in reactivity with the antibodies, which could be reversed by neuraminidase digestion. This indicates that 3-α-fucosyl-N-acetyl-lactosamine is masked for the detection with antibodies upon monocytic differentiation by sialylation.

Human × mouse myeloid cell hybrids were obtained after fusion of human myeloid cells and the HPRT-deficient murine myeloid cell line WEHI-TG. These hybrids were tested for reactivity with the anti-CD15 antibodies. The CD15 panel exhibited very similar reactivity patterns with the hybrid clones. Chromosomal analysis of hybrid cell metaphases revealed that the gene(s) involved in the expression of FAL must be located on human chromosome 11 in the region q12-qter.

Introduction

Molecular changes in the cell membrane occurring during myeloid and monocytic differentiation can be studied with monoclonal antibodies. Changes in structures related to specific functions of the mature cells were initially identified with cell-lineage-specific antibodies. The specificity of such antibodies was defined by immunofluorescence assays on normal peripheral blood cells or bone marrow, and on cells of established human leukaemic cell lines. Such antibodies have been used in studies of myeloid and monocytic differentiation as well as for the diagnosis of leukaemia.

Human × mouse myeloid cell hybrids segregating human chromosomes appeared to be suitable for studying genetic control over the expression of human myeloid differentiation characteristics (Geurts van Kessel et al., 1983).

In the present study the molecular nature of the PMN-associated antigen CD15 and the chromosomal localization of the gene(s) involved in the expression of this structure is presented.

Materials and methods

Cells

Polymorphonuclear cells (PMN) were obtained from EDTA-anticoagulated peripheral blood of healthy donors. The red cells were sedimented with dextran. Subsequently, the leucocyte-rich plasma was centrifuged on Ficoll-Isopaque. The remaining red cells were removed from the PMN-containing pellet by lysis with ammonium chloride. Suspensions of platelets, red cells, lymphocytes and monocytes were prepared as described by Brutel de la Rivière et al. (1976).
Somatic cell hybridization

Peripheral leucocytes from various normal and leukaemic donors were used for cell fusion experiments. They included chronic myeloid leukaemia, acute myeloid, and acute lymphocytic leukaemia patients. In all cases the human leucocytes were fused with a hypoxanthine phosphoribosyltransferase-deficient (HPRT-) mutant of the murine myeloid cell line WEHI-3B (Geurts van Kessel et al., 1983). Hybrid clones were isolated and grown in RPMI 1640 medium supplemented with 10–15% fetal calf serum, 2 mM glutamine, 100 U ml⁻¹ penicillin, and 100 μg ml⁻¹ streptomycin.

Antibodies and immunoassays

A panel of seven PMN-specific monoclonal antibodies (all IgM) were selected because they behaved similarly in their reaction with various cell types and were thought to react with the same antigen: B4.3 (Van der Reijden et al., 1983), MI/N1, UJd08 (Kemshead et al., 1981), VIM D5 (Majdic et al., 1981) and FMC 10, 12, 13 (Zola et al., 1981).

Binding of the antibodies to normal or enzyme-treated cells was tested by indirect immunofluorescence as described by Tetteroo et al. (1984a).

Immunoadsorption with synthetic carbohydrates

The specificity of B4.3 was studied with absorption with synthetic immunoabsorbents (SYNSORB), kindly provided by Chembiomed, Edmonton, Canada. Table 1 shows the various carbohydrate structures attached to silica (0.5 μmol sugars g⁻¹ silica). Three hundred microlitres of appropriately diluted ascites fluid were incubated with 30 mg immunosorbent for 3 h at room temperature with continuous shaking. The supernatants were collected and were used in the indirect immunofluorescence assay with cells to determine the percentage absorption as described by Tetteroo et al. (1984a).

Competition experiments

Antibody B4.3 (IgM) was purified from ascites fluid by gel filtration on Sephacryl S-300 (Pharmacia, Uppsala, Sweden). The purity was tested by immunoelectrophoresis. The antibody was labelled with ¹²⁵I by the iodogen method. Competition experiments with ¹²⁵I-labelled B4.3 were performed as described by Perussia et al. (1982), on PFA-fixed PMN. Granulocytes (3 × 10⁸ cells) were added to a mixture of 20 μl unlabelled and ¹²⁵I-labelled B4.3 (100 000 c.p.m.) or the granulocytes were incubated first with unlabelled antibody, then washed and subsequently incubated with ¹²⁵I-labelled B4.3. The unlabelled antibody was added as a 1-in-10 dilution of ascites. Cells were incubated at room temperature for 30 min and then washed in a discontinuous gradient of 20% sucrose in phosphate-buffered saline (PBS). Bound radioactivity in the pellet was counted in a Philips (Eindhoven, The Netherlands) gamma-counter.

Neuraminidase digestion

Cells were treated with neuraminidase (EC 3.2.1.18, Vibrio cholerae) as described by Tetteroo et al. (1984a, b).

Immunoprecipitation

Before radiolabelling with ¹²⁵I, the PMNs were incubated with 2 mM of protease inhibitor disopropyl fluorophosphate in PBS for 5 min at 4°C. Cell-surface proteins of the cells were iodinated with Na¹²⁵I (Amersham) with 1,3,4,6-tetrachloro-3,6-diphenylglycoluril (Iodogen, Pierce Chemical Co., Rotterdam, The Netherlands) as catalyst. Cells, 20 × 10⁶, were labelled using 1 mCi = 37 MBq ¹²⁵I. The labelling efficiency was variable (1–15%).

Immunoprecipitation was performed by using the immune complex method with rabbit-anti-mouse Ig (Nordic), the antibody and radiolabelled lysates being as previously described (Tetteroo et al., 1983). After labelling, the PNM were lysed at 4°C for 1 h in a 0.01 M Tris–HCl buffer, pH 7.8, containing 1% NP40, 0.15 M NaCl and the following protease inhibitors: 1 mM 3% 6% diphensylglycoluril (Iodogen, Pierce Chemical Co., Rotterdam, The Netherlands) as catalyst. Cells, 20 × 10⁶, were labelled using 1 mCi = 37 MBq ¹²⁵I. The labelling efficiency was variable (1–15%).

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### Table 1. Synthetic immunosorbents for absorption experiments

<table>
<thead>
<tr>
<th>Structure Code</th>
<th>Structure</th>
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<tbody>
<tr>
<td>SYNSORB-H₁</td>
<td>β-D-Gal(1→3)β-D-GlcNAc... 2 † 1 α-L-Fuc</td>
</tr>
<tr>
<td>SYNSORB-H₂</td>
<td>β-D-Gal(1→4)β-D-GlcNAc... 2 † 1 α-L-Fuc</td>
</tr>
<tr>
<td>SYNSORB-Leₐ</td>
<td>β-D-Gal(1→3)β-D-GlcNAc... 4 † 1 α-L-Fuc</td>
</tr>
<tr>
<td>SYNSORB-Leₐᵇ</td>
<td>β-D-Gal(1→3)β-D-GlcNAc... 2 4 1 1 α-L-Fuc α-L-Fuc</td>
</tr>
<tr>
<td>SYNSORB-X</td>
<td>β-D-Gal(1→4)β-D-GlcNAc... 3 † 1 α-L-Fuc</td>
</tr>
<tr>
<td>SYNSORB-Y</td>
<td>β-D-Gal(1→4)β-D-GlcNAc... 2 3 † 1 α-L-Fuc α-L-Fuc</td>
</tr>
<tr>
<td>SYNSORB-B</td>
<td>α-D-Gal(1→3)β-D-Gal... 2 † 1 α-L-Fuc</td>
</tr>
<tr>
<td>SYNSORB-A</td>
<td>α-D-GalNAc(1→3)β-D-Gal... 2 † 1 α-L-Fuc</td>
</tr>
</tbody>
</table>

3% 6% diphensylglycoluril (Iodogen, Pierce Chemical Co., Rotterdam, The Netherlands) as catalyst. Cells, 20 × 10⁶, were labelled using 1 mCi = 37 MBq ¹²⁵I. The labelling efficiency was variable (1–15%).

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