Monoclonal Antibody Register

Characterization of LK-1 Monoclonal Antibody against Human Sialophorin (CD43)

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Background

The CD43 molecule (sialophorin, leukosialin, sialoglycoprotein) is a heavily O-glycosylated and sialylated glycoprotein, consisting of 381 amino acid residues (Hořejší and Stockinger, 1997).

CD43 is a type I transmembrane protein of 95 000–115 000 Mr with an extracellular domain of 239 residues carrying an N-glycan chain and about 70–85 O-linked oligosaccharides (Pallant et al., 1989). A soluble form of CD43, called galactoglycoprotein, was detected in human blood plasma (Schmid et al., 1992).

CD43 is expressed on all human leukocytes except for the majority of resting B lymphocytes. Different cell types express CD43 forms differing in the degree of sialylation of the O-linked oligosaccharide chain. CD43 expression is tightly regulated on cells of hematopoietic origin either by increasing or decreasing surface expression, or by post-translational modification, which results in precisely controlled glycosylated isoforms. The structural characteristics of the CD43 extracellular domain indicate that this molecule has both adhesive and anti-adhesive properties (Ostberg et al., 1998; Seveau et al., 2000). The mucin-like 234 amino acid extracellular domain is extensively O-glycosylated, with 70–85 O-linked carbohydrate chains. These carbohydrate structures, such as the sialyl-Lewis (sLe*) epitope, a ligand for both P- and E-selectin, can potentiate binding to lectin-like molecules. However, the adhesion molecule function for CD43 is counteracted by the presence of over 100 negatively charged sialic acid residues on its extracellular domain.

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Abbreviations: FAB – French-American-British classification, FITC – fluorescein isothiocyanate, IHBT – Institute of Hematology and Blood Transfusion, MoAb(s) – monoclonal antibody(-ies), NHL – non-Hodgkin's lymphoma, PE – phycoerythrin.

Description of the monoclonal antibody LK-1 (IHBT)

Production

BALB/c mice were immunized intraperitoneally at two-week intervals with human leukocytes (1.3×10^7 and 1.7×10^7 cells/ml). Four days after the second immunization, a single-cell suspension was prepared from the spleen for hybridization and 2.3×10^8 cells were fused with 1.2×10^7 NSO/1 myeloma cells using polyethylene glycol (PEG 1500) in RPMI 1640 medium, according to the previously described method (Galfre and Milstein, 1981).

Specificity

The characterization of monoclonal antibody (MoAb) LK-1 (Institute of Hematology and Blood Transfusion (IHBT)) produced by hybridoma was performed by different methods (Koubek, 1989; Koubek et al., 1999).

The results indicate that LK-1 MoAb detected CD43 antigen expressed on various morphological types of human blood cells (monocytes, granulocytes, lymphocytes) (see Table 1). Furthermore, the LK-1 MoAb reacted with the molecules occurring on human leukemic cells of patients with acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute myelomonocytoid leukemia (AMMoL) and with cells of some human cell lines (Table 1). LK-1 MoAb was compared with other standard MoAbs (MEM-59 and BRA-7G) against CD43 antigen (Hořejší and Stockinger, 1997; Shaw 1997). The results showed that LK-1 MoAb had the same reactivity as the standard anti-CD43 MoAb (MEM-59) on the normal peripheral blood lymphocytes and granulocytes detected by indirect immunofluorescence. The reactivity of LK-1 MoAb with CD43 antigen can be well documented by testing the cells of two cell lines (CEM cells and CD43-deficient CEM line) (Manjunath et al., 1993).

Figure 1 demonstrates the comparison of the reactivity of LK-1 and MEM-59 MoAbs in double staining on cells of the CEM cell line and on CD43-deficient cells of the CEM line detected by flow cytometry. The results indicate that LK-1 MoAb did not react with CD43-deficient cells whereas the reactivity of LK-1 MoAb with normal

Table 1. Reactivity of LK-1 monoclonal antibody to different human normal and pathological cells

Cell types	Reactivity (in %)	Number of copies /cell*
granulocytes (peripheral blood) (6**)	>90	two peaks of SABC
monocytes (peripheral blood) (8)	>95	
lymphocytes (peripheral blood) (8)	>90	
erythrocytes (peripheral blood) (8)	<1	
myeloblasts (bone marrow) (5)	>95	
promyelocytes (bone marrow) (5)	>95	
myelocytes (bone marrow) (5)	>95	
metamyelocytes (bone marrow) (5)	>95	
bands (bone marrow) (5)	>95	
acute lymphatic leukemia of T type (4)	>90	72000 ± 10000
acute lymphatic leukemia of B type (2)	>90	32000 ± 10000
acute myeloid leukemia (6)	>90	variable expression
acute myelomonocytoid leukemia (4)	>90	variable expression
chronic myeloid leukemia (BC) (2)	>95	65000 ± 10000
T peripheral lymphoma (2)	>85	47000 ± 5000
B lymphoma (NHL) (2)	<30	16000 ± 5000
T cell lines MOLT-4 (3)	>90	24000 ± 5000
B cell lines ARH-77	>80	15000 ± 5000
myeloid cell lines (ML-1)	>85	48000 ± 10000
fibroblasts (2)	<1	

* calculation of antigen density (number of copies/cell), mean equivalent of standard fluorescence intensity as determined using the microbead standard calibration (DAKO QIFIKIT test, Code No K0078, Glostrup, Denmark), (Koubek et al., 1999).
** number of tests

cells of the CEM line was positive as well as the standard anti-CD43 MoAb.

In summary, the data indicate that LK-1 and MEM-59 MoAbs have the same reactivity and may identify the same CD43 antigen.

Properties

LK-1 MoAb is of the IgG_{2a} isotype. LK-1 antibody imunoprecipitates a single-chain 95 000-Mr glycoprotein from normal lymphocytes (Koubek, 1989). This monoclonal antibody to the CD43 marker can be a useful reagent for research on the signaling function, for adhesive and anti-adhesive cellular functions, for apoptosis in hematopoietic progenitors as well as for immunophenotyping of blood diseases (leukemias and lymphomas, Wiskott-Aldrich syndrome). The different quantitative expression of the CD43 molecule (detected by LK-1 MoAb) was observed on leukemic cells of patients with French-American-British classification (FAB) (M2 – M4) subtypes of acute myeloid leukemia (Koubek, unpublished preliminary results). LK-1 MoAb was licensed No. 273656 by the Industrial Property Office, Prague, Czech Republic.

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