

Monoclonal Antibody Register

Monoclonal Antibodies Specific for Bovine CD18

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Background

The molecule CD18 is a subunit of leukocyte adhesion molecules belonging to the $\beta 2$ integrins (CD11/CD18). Recently, free forms of intracytoplasmic CD18 molecules have been described (Drbal et al., 2000). Integrins are polypeptide heterodimers consisting of a common 95-kD β chain (CD18) non-covalently linked to a unique α subunit: 180-kD αL (CD11a), 170-kD αM (CD11b), or 150-kD αX (CD11c). The molecule CD11a/CD18 (LFA-1) is primarily expressed on lymphocytes, CD11b/CD18 (MAC-1) on neutrophils and monocytes, CD11c/CD18 on monocytes and macrophages (Gahmberg et al., 1997). The integrins are type I membrane proteins containing an N-glycosidic carbohydrate (Asada et al., 1991). The extracellular domains of integrins mediate cell-matrix and cell-cell contacts, while their cytoplasmic tails associate with the cytoskeleton. Integrins are not active in resting cells, but need activation to become adhesive. The CD18 molecule has been shown to play a central role in regulating activity (Green et al., 1998). The $\beta 2$ integrins bind to intercellular adhesion molecules ICAM and to several soluble proteins, many of which are involved in inflammation (Gahmberg et al., 1998). Their pivotal importance is best evident in individuals lacking functional CD11/CD18 due to mutation in the CD18 gene with LAD (leukocyte adhesion deficiency) syndrome in man (Arnaut, 1990) or BLAD (bovine leukocyte adhesion deficiency) syndrome in cattle (Kehrli et al. 1990; Shuster et al., 1992). This syndrome is characterized by repeated infections.

Description of the monoclonal antibodies IVA35 and IVA218

Production

Hybridoma cell lines producing monoclonal antibodies (mAbs) were obtained after two different immuniza-

tions of BALB/c mice with bovine lymphocytes using standard procedures for fusion of splenocytes with Sp2/0 myeloma cells, selections and cloning.

Specificity

Cytofluorometric analysis of bovine peripheral blood cells revealed that antibodies IVA35 and IVA218 gave positive reactions with 98-100% lymphocytes, monocytes and granulocytes. However, neither of these mAbs reacted with leukocytes from BLAD-homozygous cattle, lacking the CD18 molecules. The antibody IVA35 was tested at the 3th Workshop on Ruminant Leukocyte Antigens and recognized the CD11a/CD18 antigen [mAb code: 3W-340 (IVA35)] (Naessens and Hopkins, 1996).

The reactivity of both mAbs was also characterized by indirect immunoperoxidase staining of serial sections from healthy cattle tissues such as spleen, lymph node,

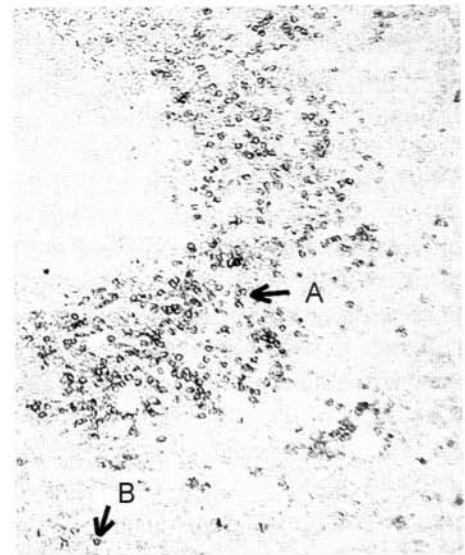


Fig. 1. Indirect immunoperoxidase staining of macrophages in bovine spleen. A - marginal zone area, B - red pulp. Tissue sections 6-8 μ m thick were fixed with acetone-methanol, treated with 0.6% hydrogen peroxide in phosphate-buffered saline and then incubated with foetal calf serum. The sections were incubated with mAbs and then with swine anti-mouse peroxidase conjugate (SEVAC a.s., Prague, Czech Republic). Peroxidase activity was visualized with 3,3'-diaminobenzidine (Sigma, St. Louis, MO) and the sections were tinged faintly by Harris's haematoxylin.

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