Monoclonal Antibody Register

Monoclonal Antibody Produced against Bovine MHC Class I Antigens

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Background

The major histocompatibility complex (MHC) genes encode cell glycoproteins that bind and present antigenic peptides to T cells. The analysis of MHC gene expression, as the key molecule of the immune system, is thus an essential component of studies of immune responses and susceptibility to diseases. The MHC class I molecules present endogenously synthetized peptides to $CD8^+$ cytotoxic T cells (York and Rock, 1996) and these molecules (MHC I) are also recognized by inhibitory receptors of natural killer cells (Lanier, 1998). The „classical" MHC class I molecules of man and other studied species are expressed on most nucleated cells (York and Rock, 1996). They consist of heterodimers of highly polymorphic α chains (Mr 44 kDa) non-covalently associated with the invariant β_2-microglobulin subunit (Mr 12 kDa) (Ploegh et al., 1981).

The bovine MHC is denoted as BoLA. One class I locus, BoLA-A, has been confirmed serologically (Spooner et al., 1979; Davies et al., 1994) and the second locus has been suggested by biochemical evidence (Bensaid et al.,1988, 1991). Cytotoxic T lymphocyte recognition of parasitic antigens associated with MHC class I has also been demonstrated (Goddeeris et al., 1986), confirming the immunological importance of class I MHC molecules in cattle.

Knowledge about MHC class I structure and their biological function has been advanced by the extensive development of monoclonal antibody (mAb) reagents. In cattle, most of the MHC class I molecule analyses were performed by cross-reactive mAb W6/32 (Parham et al. 1979; Kahn-Perles et al., 1987) commonly used for human MHC study. Only a few mouse mAbs generated against bovine cells were reported (Bensaid et al., 1989). This paper describes the antibody IVA-26 formed against bovine cells detecting MHC class I molecule of several species and showing analogical features with mAb MEM-147 (Tran et al., 2001) that recognizes MHC class I on human cells.

Description of the monoclonal antibody IVA-26

Production

Hybridoma cell lines producing mAbs were obtained after intrasplenic immunization of BALB/c mice with bovine lymphocytes using standard procedures for fusion of splenocytes with Sp2/0 myeloma cells, selection and cloning of hybridomas (Dušinský et al., 1988).

Specificity

The IVA-26 was analysed by the indirect immunofluorescent, complement-dependent cytotoxic (CTT) and ELISA test on bovine, pig, ovine rabbit, mouse, Japanese quail and human cells. The indirect immunofluorescent analysis with FITC-conjugated swine anti-mouse immunoglobulin (FITC-SwAM, SEVAC, Czech Republic) revealed that mAb IVA-26 gave positive reaction with the whole population of bovine, ovine, pig and human lymphocytes. There was no reaction in ELISA and CTT. The IVA-26 was also bound to some population of bovine, pig and human granulocytes and weak fluorescence was found on thrombocytes of the same species. No reaction was detected on erythrocytes. The antigen was not expressed in rodents and birds.

IVA-26 immunoprecipitated proteins with molecular weight of 45 kDa (α chain), 14 kDa (β_2m) and 58 kDa (α + β_2m) from lysates of detergent-solubilized, surface-sulphobiotinylated bovine lymphocytes. The same proteins were precipitated by MEM-147, an antibody against human MHC-I class molecules with epitope specificity very similar to classical antibody W6/32 (Tran et al., 2001). IVA-26 also reacted with β_2m molecules of human leukocyte lysates (Fig. 1).

We also analysed the „cross-reactivity" of IVA-26 and MEM-147 mAbs. The proteins immunoprecipitated by IVA-26 and MEM-147 were analysed by Western blot. The precipitate of IVA-26 as well as the precipitate

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Abbreviation: MHC – major histocompatibility complex.

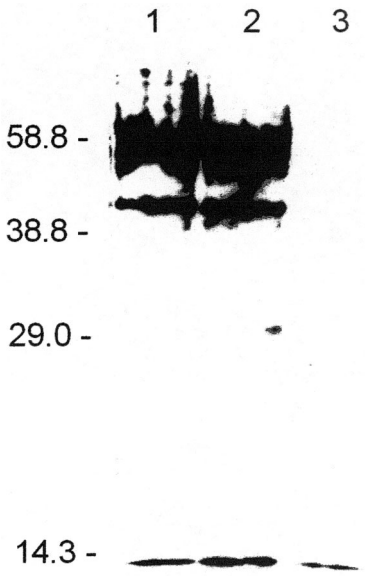

Fig. 1. Specificity of IVA-26 mAb for bovine and human MHC class I molecules was detected by immunoprecipitation (Kessler, 1981). The cells were biotinylated with sulpho-NHS-biotin (Sigma, St. Louis, MO) and solubilized with detergent (1% (v/v) Nonidet P-40). The immunoprecipitated material was analysed by SDS-PAGE (12% gel) under reducing conditions followed by Western blotting with streptavidin-HRP. Recognized proteins were visualized with the chemiluminescent procedure using ECL Western blotting reagents (Amersham, UK). In lanes 1, 2, the bovine lysates were precipitated by IVA-26 or MEM-147, respectively. Proteins of 45 kDa (α chain), 14 kDa (β_2m) or 58 kDa ($\alpha + \beta_2$m), respectively, were detected by both mAbs. Human lymphocyte lysates precipitated with IVA-26 are shown in lane 3.
Molecular weight markers are indicated on the left (in kDa).

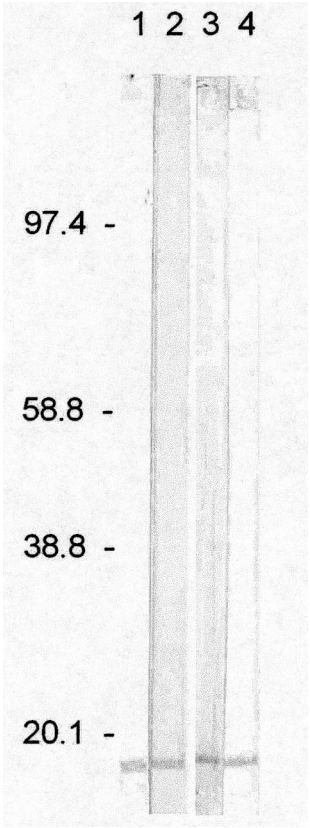

Fig. 2. The bovine lymphocytes solubilized with detergent (1% (v/v) Nonidet P-40) were precipitated with IVA-26 (lanes 1, 2) or MEM-147 (lanes 3, 4). The precipitates were analysed after separation on SDS-PAGE (10% gel) under reducing conditions by Western blot with IVA-26 (lanes 2, 3) or MEM-147 (lanes 1, 4). Molecular weight markers in kDa are indicated on the left.

of MEM-147 reacted in Western blot with both mAbs (MEM-147 and IVA-26). They both bound the same 14 kDa protein (β_2m-precipitate) under reducing conditions (Fig. 2).

IVA-26 failed in standard Western blot analysis under reducing as well as non-reducing conditions.

The immunoperoxidase staining of cryostat tissue sections with mAb IVA-26 has shown weak reactions in many body tissues of cattle and pig. Stronger staining was found mainly in the lymph node (cortical region), kidney (glomerules) and small intestinal epithelium (crypts). The IVA-26 distinguished the medullar and the cortical zone of the thymus similarly as the mAb IL-A19 described by Bensaid et al. (1989). Intensive reaction was found with medullar and no reaction with cortical thymocytes (Fig. 3).

Properties

IVA-26 is of the IgG_1 isotype. It is suitable for detection of MHC class I antigens by immunofluorescence (on bovine, ovine, pig and human intact lymphocytes), immunoprecipitation (on bovine cells) and by immunohistochemistry (on bovine and pig cells). It failed to react in Western blot analysis under reducing as well as non-reducing conditions.

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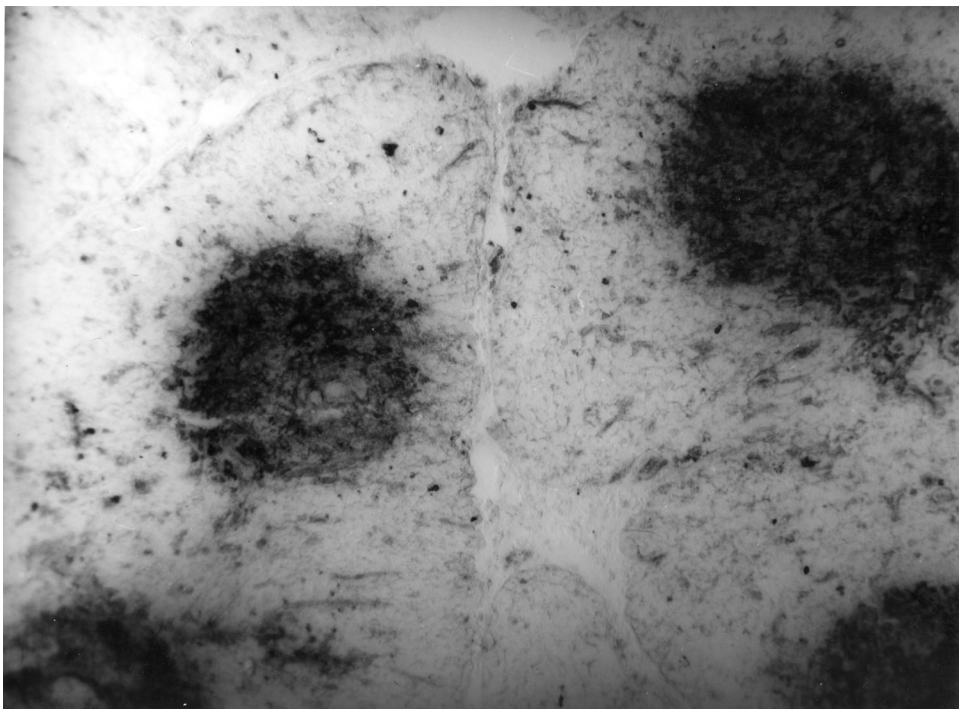

Fig. 3. Immunoperoxidase-revealed reactivity of mAb IVA-26 on a cryostat section of the pig thymus. The section was fixed with a mixture of acetone-methanol (1 : 1). The indirect immunoperoxidase method using swine anti-mouse IgG conjugated with peroxidase (Swam/Px, SEVAC, Prague, Czech Republic) and staining with DAB (3,3' diaminobenzidine tetrahydrochloride, Sigma) was applied. Intensive staining in the zone of medulla (black part of the picture) and only scattered cells in the part of cortex were observed. Magnification 40x.

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