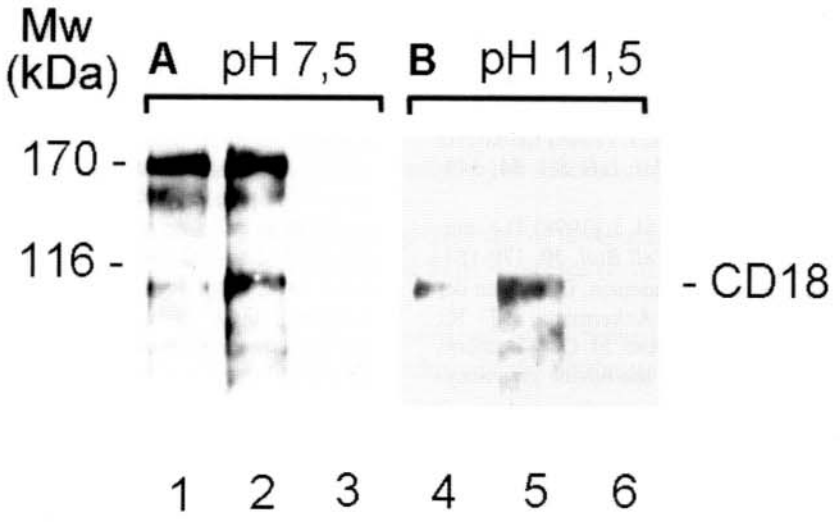

Fig. 2. Immunoprecipitation of a native CD11/CD18 molecule or its dissociated α and β subunits by the mAbs IVA35 and IVA218. Sulpho-NHS-biotin (Sigma, St. Louis, MO)-labelled, detergent (1% (v/v) Nonidet P-40)-solubilized extract from bovine granulocytes was treated at pH 7.5 (*panel A)* or pH 11.5 (*panel B)* for 30 min, and then neutralized (Sanchez-Madrid et al., 1983) and assayed for immunoprecipitation with the mAb IVA35 in *lines 1 and 4*, IVA218 in *lines 2 and 5*, Dulbecco's modified Eagle's medium (negative control) in *lines 3 and 6*. Immunoprecipitated material was analysed by SDS-PAGE (8% gel) under reducing conditions followed by Western blotting with streptavidin-horseradish peroxidase (Amersham Pharmacia Biotech, Little Chalfont, England). Recognized proteins were visualized with a chemiluminescent procedure using ECL Western blotting detection reagents (Amersham Pharmacia Biotech, Little Chalfont, England). Molecular weight markers, in kDa, are shown on the left.

thymus, liver, lung, brain, skin and small intestine. Similarly to mAbs detecting CD18 molecules on human cells, the reaction of macrophages was dominant in all reactive tissues under study. The macrophages were stained in the marginal zone and red pulp in the spleen (Fig. 1) and in paracortex and medulla in the lymph node. Tissue macrophages were also reactive in thymus, skin and small intestine. The liver exhibited strong staining of a portion of Kupffer cells. In the brain some microglial cells were positive. Furthermore, reactions with some non-macrophage cell populations and tissues such as hepatocytes, pulmonary mucosa and vascular endothelium were observed. Both mAbs immunoprecipitated proteins with apparent molecular weights of 170, 150 and 95 kDa, corresponding to CD11b, CD11c and CD18 molecules, from lysates of detergent-solubilized, surface sulphobiotin-labelled granulocytes (Fig. 2A). The same values were also obtained with lymphocytes. The standard CD18 monoclonal antibody produced against bovine cells is not commercially available at present; therefore, our two mAbs were compared with a standard antibody to bovine CD11b on bovine granulocytes. The mAbs IVA35, IVA218 and the anti-CD11b antibody brought down molecules having the identical molecular weights 170 and 95 kDa, besides a 150 kDa molecule (data not shown). To determine a target subunit, both mAbs were further assayed for their capacity to immunoprecipitate α or β subunits from the lysate that had been treated at pH 11.5 for 30 min, a procedure known to dissociate the α/β heterodimer. As shown in Fig. 2B, epitopes for both mAbs were found to be present on the β subunit (CD18). Our results clearly demonstrate that IVA35 as well as IVA218 specifically recognize the CD18 molecule.

Properties

IVA35 and IVA218 are of the IgG1 isotope. They can be used for detection of CD18 in bovine lymphocytes, granulocytes and monocytes by ELISA, immunofluorescence, immunohistochemistry and immunoprecipitation. They failed in Western blot analysis under reduced as well as non-reduced conditions.

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