

## Expression of a novel isoform of BEN-like antibody produced against guinea fowl's bursal cells

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A panel of monoclonal antibodies (mAb) was raised against non-fractionated bursal cells to produce anti B cell specific marker. Cell suspension was prepared from bursa of Fabricius of guinea fowl, which contained more than 90% of lymphocytes and few percents of epithelial cells. The supernatants of hybridomas were tested immunocytochemically on adult and embryonic tissues and cloned to select monoclonals.

In recent work we present one of the mAbs, which recognizes highly different tissues during embryonic development suggesting that the expression of this antigen is developmentally regulated and cross-reacts with chicken tissues. In adult bursa of Fabricius of the chicken this mAb designated NAKO recognizes every epithelial components except follicle-associated epithelium. The gut epithelium after closing entoderm is transiently positive. The transient antigen expression emerges first in the notochord of embryonic day (ED) 2 and by day five ceases, when the cells of the sclerotom, which appeared around the notochord begin to express it. DE3 and 4 the dermomyotome also expresses the NAKO positive antigen. Soon after the notochord at 3 DE the floor plate of the neural tube becomes positive, which is followed by the expression of basal plate where the motoneurons differentiate. Outgrowing axons of the motoneurons also express the antigen. Strong NAKO positivity characterizes the spinal sensory ganglions and their central and peripheral processes. By day 5DE the developing intestinal plexus is highly positive. The expression of this antigen continues after hatching on all parts of the peripheral nervous system. In the splanchnic mesenchyme of the ventral part of the embryo strong transient NAKO reaction appears which ceases around 10 DE. On the extraembryonic membranes (amnion) the antigen also appears unlike the ectoderm.

Summarizing the antigen expression we can conclude: In the peripheral nervous system the antigen expression is maintained after hatching, while in the mesoderm- and endoderm- derived structures is transient. The molecular weight and the major immunohistochemical features of the NAKO highly similar to the BEN/SCI/DM-GRASP antibodies (Pourquie et al. 1990; Pourquie et al. 1992; Corbel et al. 1996). Thus the NAKO identified molecule could be a homologue molecule to the BEN. Because the NAKO mAb was produced against guinea fowl cells, not chicken cells and it works in both species, suggests that this molecule is highly conservative. This was confirmed, that homologue molecules were found in fish, rat and human. The NAKO might be a novel isoform molecule of the BEN because its expression differs from it in several tissues *i.e.* cardiac septum and ventral and splanchnic mesenchyme where the cell adhesion function of the NAKO is questionable.

Corbel C, Pourquie O, Cormier F, Vaigot P, Le Douarin NM (1996) BEN/SCI/DM-GRASP, a homophilic adhesion molecule, is required for in vitro myeloid colony formation by avian hemopoietic progenitors. *Proc Natl Acad Sci USA* 93:2844-2849.

Pourquie O, Coltey M, Thomas JL, Le Douarin NM (1990) A widely distributed antigen developmentally regulated in the nervous system. *Development* 109:743-752.

Pourquie O, Corbel C, Le Caer JP, Rossier J, Le Douarin NM (1992) BEN, a surface glycoprotein of the immunoglobulin superfamily, is expressed in a variety of developing systems. *Proc Natl Acad Sci USA* 89:5261-5265.

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