Detection of α-galactosides in human tear fluid

The proteins of human tear fluid were separated on a gradient of 5–20% SDS polyacryalmide gel (Laemmli, 1971). Following electrophoretic transfer of proteins to nitrocellulose (Amersham, Freiburg, Germany) at 0.9 mA/cm^2 in a semi-dry blotting apparatus in transfer buffer (48 mM Tris, 39 mM glycine, and 20% methanol (Bjerrum and Schafer-Nielsen, 1986)), the binding sites on the membrane were blocked with 0.1% Tween 20 (Sigma, Prague, Czech Republic) in PBS or with low-fat bovine milk. The membrane was then incubated with a solution containing biotinylated human anti-α-Gal antibody (Dong et al., 1997) in a blocking buffer (1:500). After thorough washing with 0.05% Tween-20 in PBS, the membrane was incubated with a solution containing peroxidase-labelled ExtrAvidin (Sigma, Prague, Czech Republic) diluted with blocking buffer (1:500), washed, and detected by chemiluminescence (ECL kit, Amersham, PPG Zlín, Czech Republic).

Competitive inhibition

Melibiose (Galα1,6Glc; ICN, StarLab, Prague, Czech Republic) at a concentration of 10–40 mM added to a diluted antibody as mentioned above was employed as a competitive inhibitor in immunohistochemical as well in Western blot experiments.

Detection of lactoferrin in tears

We performed Western blotting as described above using rabbit polyclonal anti-human lactoferrin (Sigma, Prague, Czech Republic) diluted 1:50 to detect the glycoproteins in the samples.

Results

Immunohistochemical analysis of α-Gal- and β-Gal-containing epitopes

The purified and labelled immunoglobulin G fractions were tested in solid-phase assays for carbohydrate-dependent activity and then tested as glycohistochemical markers. Epithelial and endothelial cell layers were monitored for reactivity to the human natural anti-carbohydrate immunoglobulin G fraction. Under the conditions used, no signals of probe binding against α-Gal to porcine and human epidermal cells and cells of anterior epithelium of cornea were observed (Figs. 1, 2). The antibody against β-Gal recognized nuclei in porcine and in a lesser extent in human epidermis (Figs. 1, 2). A very low signal intensity only for the presence of β-galactosides was observed in the nuclei of porcine anterior epithelium in contrast to the human corneal epithelium, which was negative (Fig. 2). Pretreatment of epithelium with neuraminidase had no effect on the binding of anti-α-Gal antibodies (not shown).

Cultured foetal porcine cells showed no signal for the presence of α-Gal in contrast to murine 3T3 cells with rather a low, but significant positivity of this glycoepitope (Fig. 3). The endothelium of porcine dermal capillaries expressed epitopes definitely recognized by the antibody fraction against α-Gal (Fig. 4). The endothelial layer of porcine veins was reactive with antibodies against β-galactosides after the neuraminidase treatment (not shown). Human capillaries were highly reactive for the antibody against β-Gal (Fig. 4).

Both porcine liver and human lacrimal gland were highly reactive for the human natural antibody fractions, i.e. against α- or β-Gal (Fig. 5). Preincubation of sections from human lacrimal gland with label-free Gal-3 had no influence on the binding of anti-α-Gal to the lacrimal gland cells (Fig. 6).

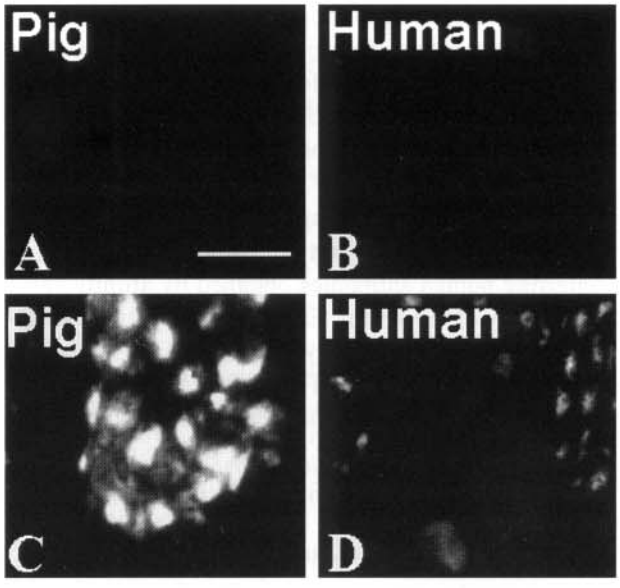

Fig. 1. Detection of glycoepitopes containing probe-reactive α-Gal (A, B) and β-Gal (C, D) moieties in pig (A, C) and human (B, D) epidermis. Scale is 20 μm.

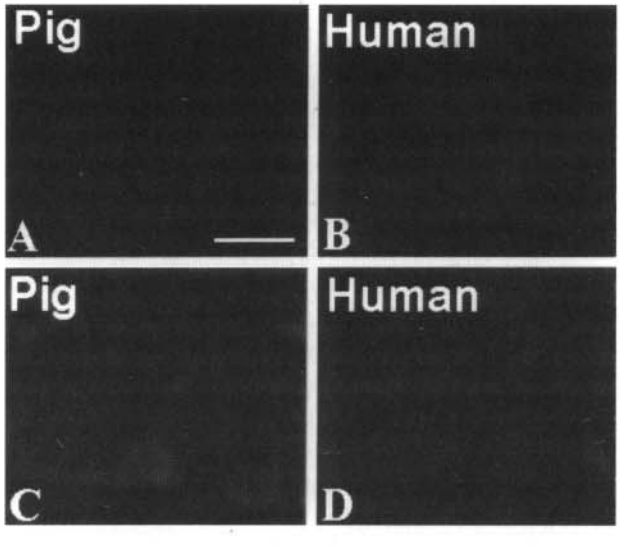

Fig. 2. Detection of glycoepitopes containing probe-reactive α-Gal (A, B) and β-Gal (C, D) moieties in pig (A, C) and human (B, D) anterior corneal epithelium. Scale is 20 μm.