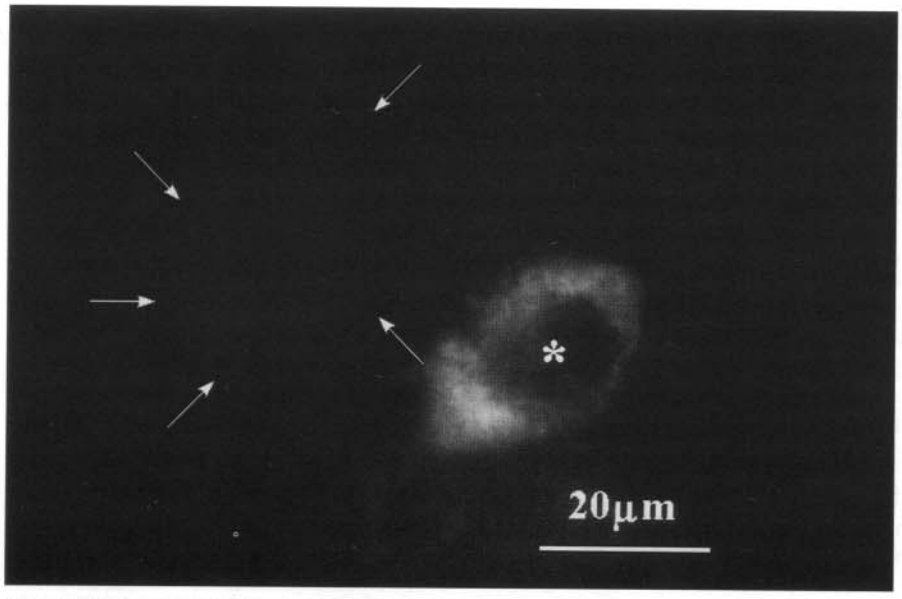

Fig. 3. Detection of glycoepitopes containing probe-reactive α-Gal in cultured pig foetal epidermal cells (position of one cell is indicated by arrows). A murine 3T3 fibroblast is marked with an asterisk. Scale is 20 μm.

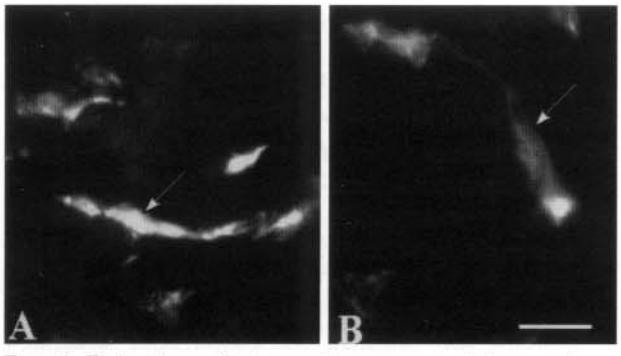

Fig. 4. Detection of glycoepitopes containing probe-reactive α-Gal (A) and β-Gal (B) moieties in pig (A) and human (B) capillaries (arrows). Bar is 20 μm.

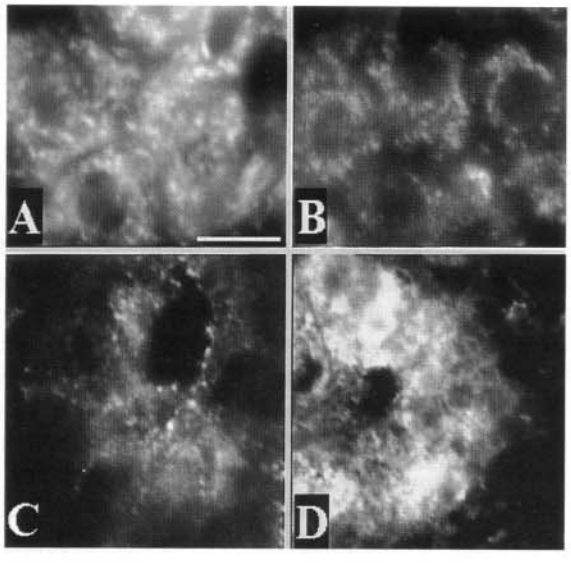

Fig. 5. Detection of glycoepitopes containing probe-reactive α-Gal (A, C) and β-Gal (B, D) moieties in pig liver (A, B) and human lacrimal gland (C, D). Bar is 20 μm.

Detection of α-galactosides by immunoblotting

Distinct bands were detected in blots of the (glyco)protein mixture of tear fluid of healthy persons and the proband with the postherpetic lesion. No bands were detected when bovine milk was used for blocking. No positivity was observed in the tear fluid sample from a patient with idiopathic chronic conjunctivitis. Preincubation of antibody with melibiose had a strong blocking effect on the antibody reactivity, proving the sugar-dependent antibody binding (Fig. 7).

The Western analysis of tear fluid showed that the human lactoferrin bands had identical mobility at the α-Gal-reactive glycoantigen (Fig. 8).

Discussion

Fixed cells of porcine epidermis, including cultured epidermal cells, and of anterior corneal epithelium were not reactive for anti-α-Gal using labelled natural human IgG without and after neuraminidase pretreatment. The possibility for false negativity of this observation e.g. due to a lack of probe activity could be excluded with a positive signal of the marker binding to porcine endothelium and liver cells, which are known as carriers of the Galili antigen, the docking epitope for anti-α-Gal antibodies (Vaughan et al., 1994). Moreover, the reactivity of the anti-β-Gal antibody fraction in human and pig epidermal cells underscores the absence of anti-α-Gal reactivity in these cells. The accessibility of sugar epitopes for anti-β-Gal antibodies in the epithelium of porcine vessels was greatly improved by neuraminidase pretreatment, corroborating recent data published by Lucq et al. (2000).

The human lacrimal gland expressed both studied glycoepitopes, i.e. α- and β-Gal reactive with human natural antibodies. α-Gal-containing glycoproteins can evidently be secreted into tear fluid. Since the antibody reactivity was significantly inhibited with the competitive sugar inhibitor melibiose, the carbohydrate specificity of the reaction within the immune recognition of α-Gal was clearly ascertained. This observation is supported by previous work noting α-Gal-containing deposits on contact lens surfaces by lectin histochemistry (Klotz et al., 1987). The molecular weight of band(s) positive for α-Gal presentation corresponded to that of tear lactoferrin or products of its enzymatic digestion (Kuizenga et al., 1991; Vorland, 1999). The