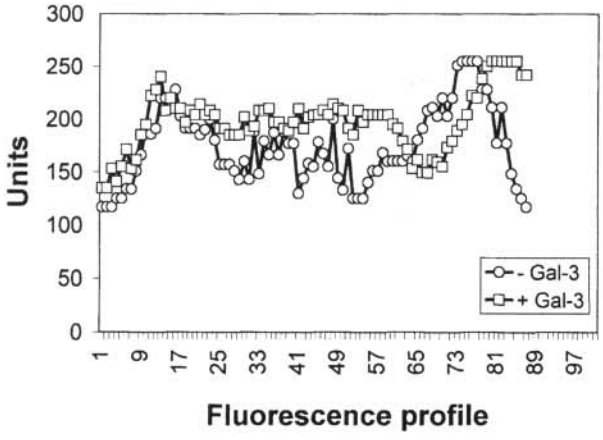

Fig. 6. Fluorescence profile for the presence of α-Gal-containing epitopes measured in cells of the lacrimal gland without and with preincubation using label-free Gal-3.

Fig. 7. Presence of α-Gal-containing glycoepitopes in tear fluid collected from donors with idiopathic chronic conjunctivitis (1), postherpetic lesion (2) and from two healthy persons (3, 4) without or with competitive inhibition by melibiose (Galα1,6Glc).

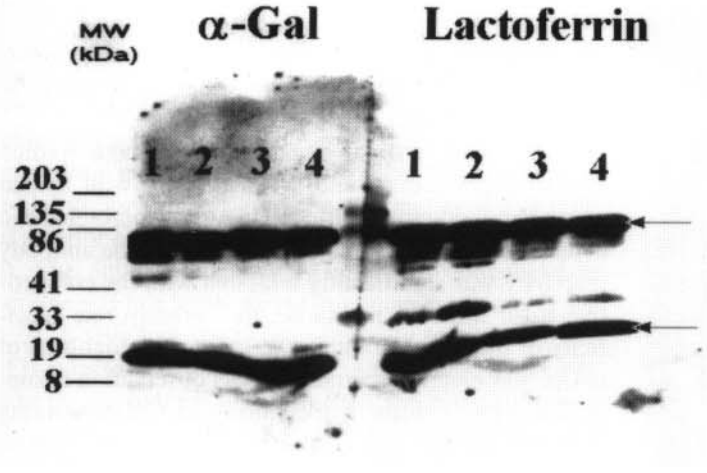

Fig. 8. Presence of α-Gal-containing glycoproteins and lactoferrin in tear fluid collected from four healthy persons (1–4). The arrows indicate position of bands positive for both antibodies, i.e. anti-α-Gal and anti-lactoferrin.

presence of IgG-reactive α-Gal in tear fluid of healthy volunteers indicates the physiological occurrence of this glycoepitope in healthy people with no signs of an autoimmune disorder. The functional consequences of the α-Gal presence in tear fluid are not yet clear but a protective role blocking bacterial adhesion to the eye surface could be of considerable significance. In line with this assumption, the complex mixture of milk oligosaccharides has been inferred to inhibit the docking of pathogenic bacteria to the susceptible cells (Kunz and Rudolff, 1993; Nascimento de Araujo and Giogliano, 2000). Lactoferrin as well as lactalbumin also exert direct bactericidal activity (Ellison et al., 1988; Hakansson et al., 2000). Moreover, material from a patient with a chronically inflamed eye surface contained no band recognized by human natural anti-α-Gal antibody in the same position as that from healthy donors. Our preliminary studies revealed the absence of Gal-3 in the tear fluid from people without eye problems and a high content of this lipopolysaccharide-binding lectin in tear fluid from inflamed eyes. An α-Gal-containing glycoprotein(s) was detected in tear fluid from normal eyes and not in the tear samples from inflamed eyes. This result points to the possibility of an interaction of α-Gal with Gal-3. However, the preincubation of lacrimal gland sections with label-free Gal-3 for epitope masking had no inhibitory effect on anti-α-Gal binding to lacrimal gland cells. Further explanation could be the absence of α-Gal in these individual donors or a breakdown of an anti-α-Gal-reactive epitope by glycosidases produced by pathogens.

Concerning cellular reactivity, the porcine corneal epithelium was negative for Galα1,3Gal structures, which are known to be abundantly expressed on cells of non-primate grafts, consequently causing an immunological barrier between humans or other Old World primates and non-primate mammals and preventing xenografting. These findings raise the question whether it might be possible to use pig cornea and the epithelial cell layer in clinical medicine, as viewed from the perspective of α-Gal.

In conclusion, this study demonstrated the absence of α-Gal epitopes (so-called Galili antigen) in porcine epidermal cells and corneal epithelium *in situ* or cultured *in vitro*. This result is an essential step to testing porcine epidermal cells in the development of non-permanent tissue-engineered devices improving the healing process of skin defects. The presence of α-Gal in human tear fluid adds the evidence that α-Gal could be present in human glycoproteins, as seen in human tumour samples or inflammation (Bjerrun and Schafer-Nielsen, 1986; Tremont-Lukats et al., 1996; Kayser et al., 1998; Kayser et al., 2000), probably as a product of aberrant galactosylation or glycolytic degradation.

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