Cells of Porcine Epidermis and Corneal Epithelium Are Not Recognized by Human Natural Anti-α-galactoside IgG

E. HRDLIČKOVÁ-CELA[1, 2], K. SMETANA, Jr.[1, 3], J. PLZÁK[1, 4], Z. HOLÍKOVÁ[1, 3, 5], S. ANDRÉ[6], M. HŘEBÍČEK[7], K. HODAŇOVÁ[7], B. DVOŘÁNKOVÁ[2, 8], J. MOTLÍK[3, 9], H.-J. GABIUS[6]

[1]Charles University, 1st Faculty of Medicine, Institute of Anatomy, Prague, Czech Republic
[2]Charles University, 1st Faculty of Medicine, Department of Ophthalmology, Prague, Czech Republic
[3]Research Center for Cell Therapy and Tissue Repair, Prague, Czech Republic
[4]Charles University, 1st Faculty of Medicine, Department of Otorhinolaryngology, Head and Neck Surgery, Prague, Czech Republic
[5]Charles University, 2nd Faculty of Medicine, Department of Dermatology, Prague, Czech Republic
[6]Ludwig-Maximilians-University, Faculty of Veterinary Medicine, Institute of Physiological Chemistry, Munich, Germany
[7]Charles University, 1st Faculty of Medicine, Institute of Inherited Metabolic Disorders, Prague, Czech Republic
[8]Charles University, 3rd Faculty of Medicine, Department of Burn Surgery, Prague, Czech Republic
[9]Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Liběchov, Czech Republic

Abstract. Human natural antibodies against Galα1,3Gal-R are mainly responsible for hyperacute rejection of xenografts transplanted to the human host. In addition to the anti-α-Gal activity, human serum also contains anti-β-Gal IgG fractions. Employing biotinylated IgG subfractions with anti-α- and anti-β-Gal activity purified from human natural IgG, we have studied expression of reactive epitopes in porcine and human skin, porcine cultured keratinocytes and porcine and human cornea, porcine liver and human lacrimal gland, tear fluid and capillaries. No reactivity of porcine and human epidermis as well as anterior corneal epithelium was observed for human anti-α-Gal IgG. Serving as positive control, porcine capillaries gave the expected signal with the anti-α-Gal antibody. The anti-β-Gal subfraction recognized cell nuclei in the epidermis of both these species. The pig liver cells interacted with antibodies against α- and β-galactosides like cells of the human lacrimal gland. α-galactoside-reactive glycoproteins were also detected in the human tear fluid. The carbohydrate specificity of the reaction was ascertained by using melibiose as competitive sugar for α-galactoside-mediated binding. These results reveal the presentation of Galα1,3Gal in epithelial cells of human lacrimal gland, its biosynthetic origin being unclear. With respect to a potential clinical perspective, the given results facilitate consideration of the use of porcine epidermal cells in engineering of non-permanent wound covers to improve treatment.

Received May 23, 2001. Accepted August 30, 2001.

This study was supported by the Ministry of Education, Youth and Sport of the Czech Republic (Grants No. LN00A065 and No. MSM 111100005).

Corresponding author: Karel Smetana, Jr., Charles University, 1st Faculty of Medicine, Institute of Anatomy, U nemocnice 3, 128 00 Prague 2, Czech Republic. E-mail: ksmet@lf1.cuni.cz.

Abbreviations: Gal – galactoside; PBS – phosphate-buffered saline.

It is well known that approximately one percent of the circulating human IgG is directed against α-galactosyl epitopes of general structure Galα1,3Gal-R, the so-called Galili antigen, which occurs in mammals except Old World monkeys, apes and humans (Galili et al., 1988a). Compared to natural antibodies against carbohydrate epitopes of A or B histo-blood group antigens these antibodies are not present in neonates and can be detected after the colonization of the intestine with bacterial flora (Wiener, 1951). Interestingly, the titre of these natural antibodies significantly increases with bacterial/parasitic antigenic challenge, a proven target of the antibodies (Springer and Horton, 1969; Galili et al., 1988b; Avila et al., 1989). The abundance of the polyclonal antibody against α-Gal autoreactivity to human tissues was postulated to contribute to autoimmune diseases such as thyroiditis. In this case, α-Gal epitopes were found on normal as well as autoimmune human thyroid cells, rendering this explanation rather unlikely (Thall et al., 1991). In a different context, the presence of this carbohydrate antigen is unquestionably crucial. Its presentation on surfaces of animal endothelial cells represents the main barrier for the use of animal, mainly porcine, organs in xenotransplantation (Bach et al., 1995; Cooper and Oriol, 1997). The porcine vascular endothelium is damaged by exposure

to the antibody and the organ is eventually destroyed during hyperacute rejection (Cooper 1996; Cooper and Oriol, 1997).

Trophic wounds such as pressure and venous ulcers or diabetic foot represent a serious medical problem. Using human allogenic cells, tissue engineering led to production of bioactive matrices as protective covers, also contributing to re-epithelization of the wound bed by eliciting production of cytokines favourable for epidermal stem cell division (comercially available products such as Apligraf and Dermagraft). As a substitute for human cells in the preparation of such biocompatible non-permanent covers, porcine epidermal cells might find a place to improve the treatment of chronic wounds. Taking stock of applying pig dermoepidermal grafts in provisional therapy of burn injuries without obvious negative side effects gives reason to test porcine epidermal cells also in this context. At any rate the expression of α-galactosides in these cells is to be evaluated concerning their potential to be a target of natural antibodies limiting applicability. The first step is visualization of the reactivity of cells with the damage-conferring human antibodies obtained by affinity chromatography of the serum. Employing the human natural anti-α- or β-Gal antibody fractions of the IgG class, we studied the presence of reactive carbohydrate epitopes in porcine and human epidermis and anterior epithelium of cornea of the human and porcine nature. Moreover, the occurrence of antibody-reactive Gal determinants in a panel of human and porcine tissues and human tear fluid was studied.

Material and Methods

Tissue and tear sample processing

The porcine epidermis was harvested using the punch-biopsy procedure from highly keratinized (foot) and poorly keratinized (snout) areas of miniature pigs (crosses of the Minnesota and Gottingen strains) after local anaesthesia. The samples of porcine liver and cornea were received post mortem. The specimens of human skin were obtained from the Department of Aesthetic Surgery (Charles University, 3rd Faculty of Medicine, Prague, Czech Republic) with the informed consent of donors. The human cornea, lacrimal gland samples and *musculus levator palpebrae* samples were received post mortem. Non-stimulated tear fluid samples were collected from healthy volunteers (N = 10), from another patient with idiopathic chronic conjunctivitis and one patient with metaherpetic keratitis. The pieces of tissue were embedded with Tissue-Tek (Sakura, Zoeterwoude, The Netherlands), incubated for 1 h at 4°C and frozen in liquid nitrogen. The tear fluid samples were also deeply frozen. All samples (tissue and tear fluid samples) were stored frozen up to further processing at –20°C.

The porcine foetal keratinocytes harvested from foetuses of 90th day of pregnancy were cultured on the surface of histological coverslips using the feeder cells – murine 3T3 fibroblasts with mitosis blocked by mitomycin C pretreatment (Sigma, Prague, Czech Republic) as described (Green et al., 1979; Dvořánková et al. 1996).

Immunohistochemical analysis of tissues

The 5–10 μm thick cryostat sections (Cryocut-E. Reichert-Jung, Wien, Austria) were fixed with 2% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.2–7.4) and washed carefully with PBS. A PBS solution with 0.1% (w/v) human serum albumin (fraction V, Sigma-Aldrich, Prague, Czech Republic) was employed to block a non-specific protein binding-site solution. The α- and β-Gal-containing glycoepitopes were visualized with biotinylated human natural antibodies of the IgG class at a dilution of 10 μg/ml of PBS for 1 h at room temperature (Dong et al., 1997; Smetana et al., 1998). Isolation, subfraction and activity assays of the IgG preparations have been described in detail previously (Dong et al., 1997; Siebert et al., 2000). After careful washing with PBS, TRITC-labelled ExtrAvidin (Sigma-Aldrich, Prague, Czech Republic) was used for visualization of the immunocytochemical procedure. To assess the masking effect of N-acetylneuraminic acid at the terminal position of oligosaccharide chains on antibody binding, a part of sections were pretreated with neuraminidase applied at a dilution 1:100 recommended by the supplier for 12 h at 37°C (Institute of Epidemiology and Microbiology, Gorkij, Russia). One of the control experiments (see also section on competitive inhibition) was performed by omitting the first-step antibody or its replacement with human albumin to exclude any binding of the kit reagents to the section.

Because the Galili antigen shares the core structure with the histo-blood B group epitope (the branching with α1,2-linked L-fucose is not present) which is known to be a glycoligand for Gal-3, we tested the possibility that Gal-3 is reactive with the core structure. Sections of the human lacrimal gland containing this glycoepitope were preincubated with label-free Gal-3 prepared and tested for activity as described previously (André et al., 1999; Plzák et al., 2000), and after extensive washing the specimens were incubated with labelled anti-α-Gal antibody as described.

The sections were mounted using Vectashield (Vector Laboratories, Burlingame, CA). An Optiphot-2 Nikon fluorescence microscope equipped with a CCD integrating camera (Cohu) and computer-assisted image analysis system LUCIA (Laboratory Imaging, Prague, Czech Republic) was used for photodocumentation.

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).

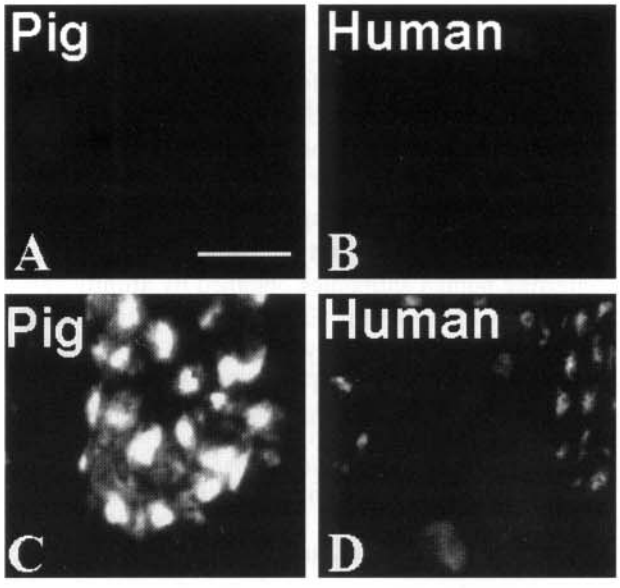

Fig. 1. Detection of glycoepitopes containing probe-reactive α-Gal (A, B) and β-Gal (C, D) moieties in pig (A, C) and human (B, D) epidermis. Scale is 20 μm.

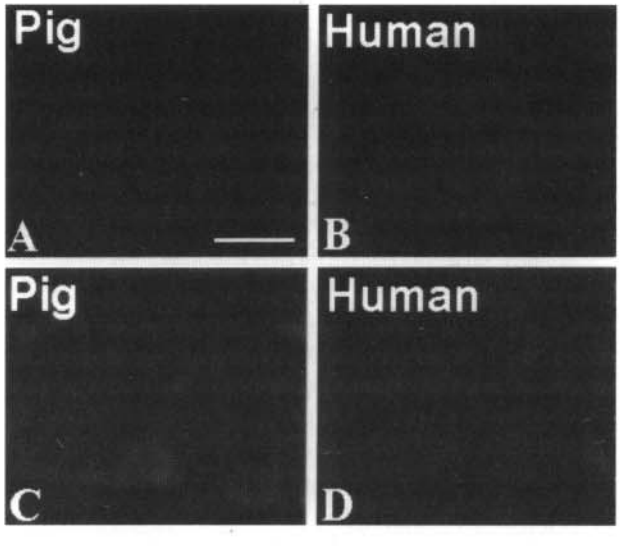

Fig. 2. Detection of glycoepitopes containing probe-reactive α-Gal (A, B) and β-Gal (C, D) moieties in pig (A, C) and human (B, D) anterior corneal epithelium. Scale is 20 μm.

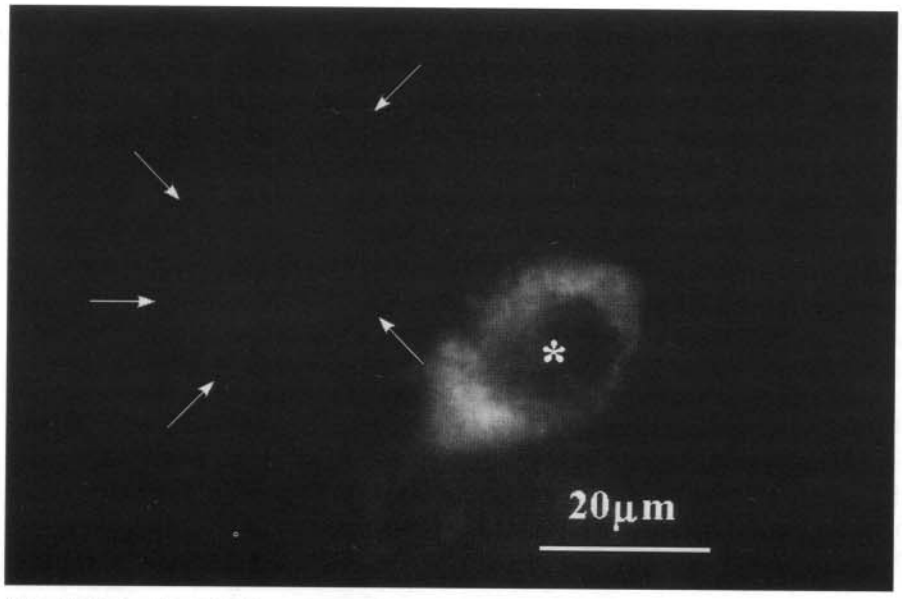

Fig. 3. Detection of glycoepitopes containing probe-reactive α-Gal in cultured pig foetal epidermal cells (position of one cell is indicated by arrows). A murine 3T3 fibroblast is marked with an asterisk. Scale is 20 μm.

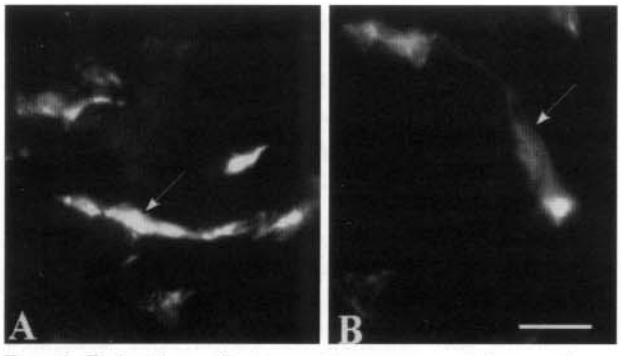

Fig. 4. Detection of glycoepitopes containing probe-reactive α-Gal (A) and β-Gal (B) moieties in pig (A) and human (B) capillaries (arrows). Bar is 20 μm.

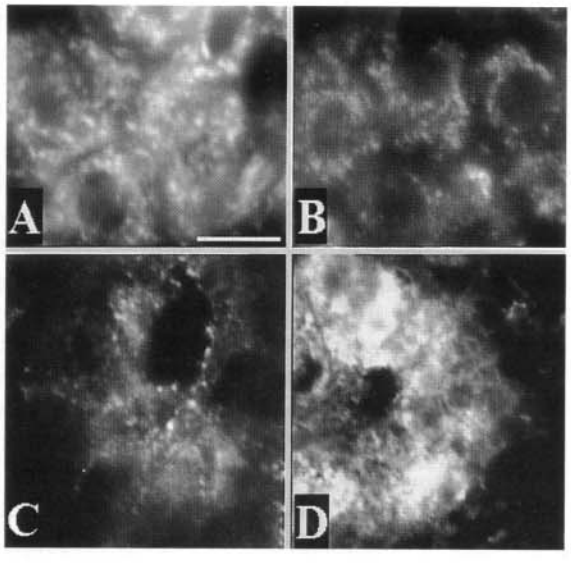

Fig. 5. Detection of glycoepitopes containing probe-reactive α-Gal (A, C) and β-Gal (B, D) moieties in pig liver (A, B) and human lacrimal gland (C, D). Bar is 20 μm.

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

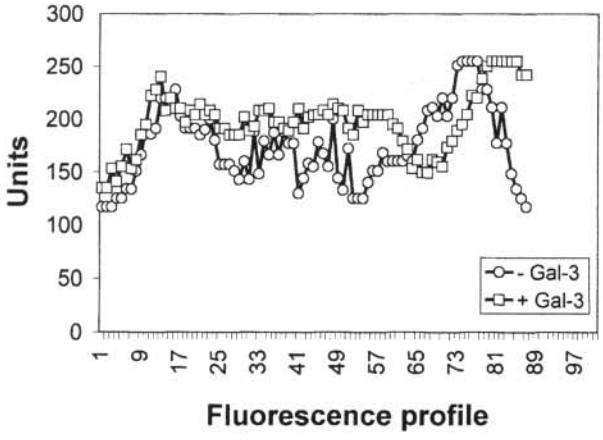

Fig. 6. Fluorescence profile for the presence of α-Gal-containing epitopes measured in cells of the lacrimal gland without and with preincubation using label-free Gal-3.

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).

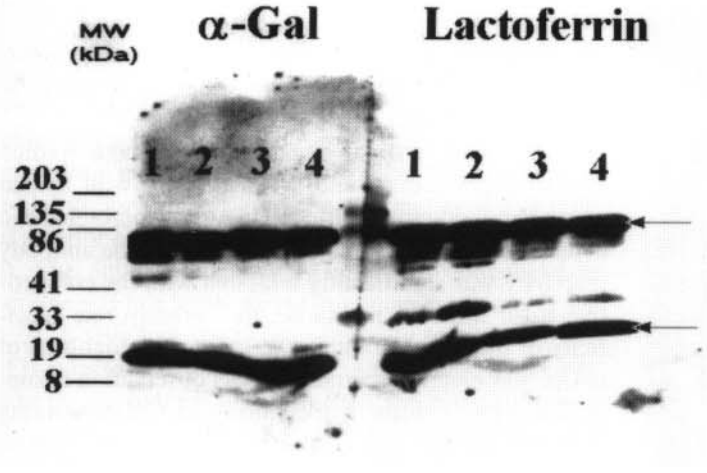

Fig. 8. Presence of α-Gal-containing glycoproteins and lactoferrin in tear fluid collected from four healthy persons (1–4). The arrows indicate position of bands positive for both antibodies, i.e. anti-α-Gal and anti-lactoferrin.

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.

Acknowledgements

The authors are grateful to Mrs. Eva Vancová for excellent technical assistance.

References

André, S., Kojima, S., Yanazaki, N., Fink, C., Kaltner, H., Kayser, K., Gabius, H.-J. (1999) Galectins-1 and -3 and their ligands in tumor biology. *J. Cancer Res. Clin. Oncol.* **125,** 461-474.

Avila, J. L., Rojas, M., Galili, U. (1989) Immunogenic Galα1-3Gal carbohydrate epitopes are present on pathogenic American Trypanosoma and Leishmania. *J. Immunol.* **142,** 2828-2834.

Bach, F. H., Auchincloss, H. J., Robson, S. C. (1995) Xenotransplantation. In: *Transplantation Immunology,* eds. Bach, F. H., Auchincloss, H. J., p. 305, Wiley-Liss, New York.

Bjerrum, O. J., Schafer-Nielsen, C. (1986) Buffer systems and transfer parameters of semidry electroblotting with a horizontal apparatus. In: *Electrophoresis '86,* ed. Dunn, M. J., p. 315, VCH Publishers, Deerfield Beach, Fl.

Cooper, D. K. C. (1996) Xenotransplantation - state of art. *Front. Biosci.* **1,** 248-265.

Cooper, D. K. C., Oriol, R. (1997) Glycobiology in xenotransplantation research. In: *Glycosciences. Status and Perspectives,* p. 531, eds. Gabius, H.-J., Gabius, S., Chapman & Hall, London.

Dong, X., André, S., Hofer, B., Kayser, K., Gabius, H.-J. (1997) Disease type associated increases of the plasma levels and ligand expression for natural α- or β-galactoside-binding immunoglobulin G subfractions in patients with lung cancer. *Int. J. Oncol.* **10,** 709-719.

Dvořánková, B., Smetana, K. Jr., Vacík, J., Jelínková, M. (1996) Cultivation of keratinocytes on polyHEMA and their migration after inversion. *Folia Biol. (Praha)* **42,** 83-86.

Ellison, R. T. III, Giehl, T. J., LaForce, F. M. (1988) Damage of the outer membrane of enteric Gram-negative bacteria by lactoferrin and transferrin. *Infect. Immun.* **56,** 2774-2781.

Galili, U., Shohet, S. B., Kobrin, E., Stults, C. L., Macher, B. A. (1988a) Man, apes, and Old World monkeys differ from other mammals in the expression of α-galactosyl epitopes on nucleated cells. *J. Biol. Chem.* **263,** 17755-17762.

Galili, U., Mandrell, R. E., Hamadeh, R. M., Shohet, S. B., Griffiss, J. M. (1988b) Interaction between human natural anti-α-galactosyl immunoglobulin G and bacteria of the human flora. *Infect. Immun.* **56,** 1730-1737.

Green, H., Kehinde, O., Thomas, J. (1979) Growth of cultured human epidermal cells into multiple epithelia suitable for grafting. *Proc. Natl. Acad. Sci. USA* **76,** 5665-5668.

Hakansson, A., Svensson, M., Mossberg, A. K., Sabharwal, H., Linse, S., Lazou, I., Lonnerdal, B., Svanborg, C. (2000) A folding variant of α-lactalbumin with bactericidal activity against *Streptococcus pneumoniae. Mol. Microbiol.* **35,** 589-600.

Kayser, K., Ziehms, S., Kayser, G., André, S., Bovin, N. V., Dong, X., Kaltner, H., Gabius, H.-J. (1998) Glycohistochemical properties of malignancies of lung and pleura. *Int. J. Oncol.* **12,** 1189-1994.

Kayser, K., Seemann, C., André, S., Kugler, C., Becker, C., Dong, X., Kaltner, H., Gabius, H.-J. (2000) Association of concentration of asbestos and asbestos-like fibers with patients' survival and the binding capacity of lung parenchyma to galectin-1 and natural α-galactoside- and α-mannoside-binding immunoglobulin G subfractions from human serum. *Path. Res. Pract.* **196,** 81-87.

Klotz, S. A., Misra, R. P., Butrus, S. I. (1987) Carbohydrate deposits on the surface of worn extended-wear contact lenses. *Arch. Ophthalmol.* **105,** 947-947.

Kuizenga, A., van Haeringen, N. J., Kijlstra, A. (1991) Identification of lectin binding proteins in human tears. *Invest. Ophthalmol. Visual. Sci.* **32,** 3277-3284.

Kunz, C., Rudolff, S. (1993) Biological functions of oligosaccharides in human milk. *Acta Paediatr.* **82,** 903-912.

Laemmli, U. K. (1971) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227,** 680-685.

Lucq, J., Tixier, D., Guinalt, A. M., Greffard, A., Loisance, D., Pilatte, Y. (2000) The target antigens of naturally occurring human anti-β-galactose IgG are cryptic on porcine aortic endothelial cells. *Xenotransplantation* **7,** 3-13.

Nascimento de Araujo, A., Giogliano, L. G. (2000) Human milk fractions inhibit the adherence of diffusively adherent *Escherichia coli* (DAEC) and enteroaggregative *E. coli* (EAEC) to HeLa cells. *FEMS Microbiol. Lett.* **184,** 91-94.

Plzák, J., Smetana, K. Jr., Betka, J., Kodet, R., Kaltner, H., Gabius, H.-J. (2000) Endogenous lectins (galectins-1 and -3) as probes to detect differentiation-dependent alterations in human squamous cell carcinomas of oropharynx and larynx. *Int. J. Mol. Med.* **5,** 369-372.

Siebert, H. C., André, S., Asensio, J. L., Canada, F. J., Dong, X., Espinosa, J. F., Frank, M., Gilleron, M., Kaltner, H., Kozár, T., Bovin, N. V., von der Lieth, C.-W., Vliegenthart, J. F. G., Jiménez-Barbero, J., Gabius, H.-J. (2000) A new combined computational and NMR-spectroscopical strategy for the identification of additional conformational constraints of the bound ligand in an aprotic solvent. *CHEMBIOCHEM.* **1,** 181-185.

Smetana, K. Jr., Slavík, J., Vancová, E., Fischer, J., Liu, F.-T., Burchert, M., Dong, X., Gabius, H.-J. (1998) Fusion of macrophages on an implant surface is associated with down-regulated expression of ligands for galectin-1 and -3 in the rat. *Biomaterials* **19,** 1799-1805.

Springer, G. F., Horton, R. F. (1969) Blood group isoantibody stimulation in man by feeding blood group active bacteria. *J. Clin. Invest.* **48,** 1280-1291.

Thall, A., Etienne-Decerf, J., Winand, R. J. Galili, U. (1991) The α-galactosyl epitope on human normal and autoimmune thyroid cells. *Autoimmunity* **10,** 81-87.

Tremont-Lukats, I. W., Avila, J. L., Tapia, F., Hernandéz, D., Cáceres-Dittmar, G., Rojas, M. (1996) Abnormal expression of galactosyl (α1→3) galactose epitopes in squamous cells of the uterine cervix infected by human papilloma virus. *Pathobiology* **64,** 239-246.

Vaughan, H. A., Loveland, B. E., Sandrin, M. S. (1994) Galα1-3Gal is a major xenoepitope expressed on pig endothelial cells recognized by naturally occurring cytotoxic human antibodies. *Transplantation* **58,** 879-882.

Vorland, L. H. (1999) Lactoferrin: a multifunctional glycoprotein. *APMIS* **107,** 971-981.

Wiener, A. S. (1951) Origin of naturally occurring hemagglutinins and hemolysins: a review. *J. Immunol.* **66,** 287-295.