# 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.**

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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.