



Original Contribution

PHYLOGENETIC STUDY ON THE EXPRESSION OF HUMAN HISTO-BLOOD GROUP ANTIGENS A AND B IN VERTEBRATE LIVER

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ABSTRACT

The work was aimed at performing a comparative phylogenetic study of the liver expression of the human histo-blood group antigens (HBGA) A and B in different vertebrates. Nine species of free-living adult organisms from Classes *Chondrichthyes*, *Osteichthyes*, *Amphibia*, *Reptilia*, *Aves* and *Mammalia* were studied. The biotin-streptavidin-peroxidase system with primary monoclonal antibodies to human HBGA A and B was applied on paraffin sections. Non-specific binding was excluded by inhibition tests. Hepatocytes of all vertebrates studied did not express HBGA. Epithelial cells of bile ducts stained positive. The staining was localized mainly in cell cytoplasm and rarely on cellular membranes. Red blood cells, like hepatocytes, also tested negative. The endothelial cells of blood vessels in the liver of *E. orbicularis* expressed HBGA. Our study presents for the first time evidence for a cell-specific pattern of human HBGA expression in nine different species belonging to six vertebrate classes. This expression pattern might be related to the different embryologic origin of cells and structures. Hence HBGA tissue expression might reflect the level of cell differentiation and specialization in the course of embryogenesis and phylogenesis, rather than the evolutionary rank of the vertebrate.

Key words: Histo-blood group antigens, Liver, Bile ducts, Vertebrates, Immunohistochemistry.

INTRODUCTION

Human blood group antigens A and B are genetically determined carbohydrate moieties in glycoproteins or glycolipids on cell membranes (1, 2, 3). Apart from their presence on red blood cell membranes they are also widely distributed in human tissues and organs for which they are known as histo-blood group antigens (HBGA). The primary ABO-gene products are the glycosyltransferase enzymes, which regulate the stepwise elongation of a common oligosaccharide chain by the addition of the so-called immunodominant sugar residues: L-fucose (determining H-specificity), N-acetyl-galactosamine (A-specificity) and D-galactose (B-specificity) (4, 5).

ABO HBGA are important markers in blood transfusions, organ transplantations and play vital role in forensic and anthropological investigations (6, 7, 3). Their role as receptors for pathogens, tumor-

associated antigens and adhesion molecules are still being studied (8, 9, 10).

At the moment HBGA function in animal tissues is still sketchy. For example, there have been few probing investigations on the evolutionary aspect of their expression in various animal tissues. Haemagglutination and biochemical assays have been used to examine the antigens in domestic and laboratory animals, in higher mammals and in animal cell lines (11, 12, 13). Very few information from immunocytological studies on lower vertebrates are available. At present immunohistochemistry has been used to study the HBGA expression in the liver and bile ducts from members of the *Pisces* and *Amphibia* classes. Such investigations could possibly contribute to the elucidation of the evolutionary trends in HBGA tissue distribution and cellular localization, as well as open up knowledge about their complex immunobiological functions.

The aim of the present study was to examine the expression of human HBGA A and B in liver of some free-living vertebrates from Class *Chondrichthyes* through *Mammalia*.

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MATERIALS AND METHODS.

All test materials in this investigation were free-living vertebrate animals found in South Bulgaria. They comprised nine different species groups. Each species group had at least five adult individuals. Livers from them were obtained under ether anesthesia in line with the National Ethical Board Recommendations. Paraffin sections from these livers were then made.

The biological identities of the test materials are shown as follows:

- Class *Chondrichthyes*, suborder *Selachoidea*, family *Squalidae*, genus *Squalus*, species - *Squalus acanthias*;
- Class *Osteichthyes*, order *Cypriniformes*, family *Cyprinidae*, genus *Cyprinus*, species *Cyprinus carpio*; genus *Carassius*, species - *Carassius auratus gibelio*;
- Class *Amphibia*, order *Anura*, genus *Bufo*, species - *Bufo viridis*; order *Urodela*, family *Salamander*, genus *Triturus*, species - *Triturus cristatus*;
- Class *Reptilia*, order *Squamata*, suborder *Lacertilia*, family *Lacertidae*, genus *Lacerta*, species - *Lacerta viridis*; order *Chelonia*, family *Emydidae*, genus *Emys*, species - *Emys orbicularis*;
- Class *Aves*, order *Oscines*, family *Ploceidae*, genus *Passer*, species - *Passer montanus*;
- Class *Mammalia*, order *Rodentia*, family *Muridae*, genus *Apodemus*, species *Apodemus flaviacollis*.

Preliminary inhibition tests were done on each section to shut off nonspecific staining. Similarly, sections were initially treated, before incubation with primary antibodies, with 5M solutions of the specific immunodominant blood group sugars, N-acetylgalactosamine and D-galactose (Sigma), to inhibit nonspecific binding of monoclonal antibodies, anti-A and anti-B, respectively. The monoclonal antibodies to human HBGA A and B (National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria) were used as primary antibodies. They were culture supernatants secreted by hybridomas obtained as fusion products of mouse myeloma cell lines x.63Ag 8.653 and Sp2(O.Ag-14) with splenocytes from BALB/c mice immunized against human erythrocytes from groups A₁ and B. The antibodies were class M immunoglobulins. Negative Control (without primary antibody) was prepared for each section. Another

inhibition assay using an irrelevant primary antibody from the same Ig class was also performed to exclude “sticking” of IgM antibodies to tissue sections and to ensure the specificity of the subsequent immune reaction.

The immunohistochemical study was done using the Biotin-Streptavidin-Peroxidase labeling system (DAKO). A 2% solution of aminoethylcarbazole was used as the reaction substrate. Granular reddish-brown stain reaction was registered as positive. The nuclei were counterstained with Mayer hematoxylin.

The livers of the vertebrates studied were examined for A and B HBGA expression paying special attention to their localization in the tissues and cells of the organ. The distribution of A and B antigens in liver and bile ducts, monitored by the pattern of immunostaining (cell surface or cytoplasmic), was examined.

RESULTS

Hepatocytes of all vertebrate species and classes did not express HBGA. The same negative result was observed in red blood cells and endothelia of blood vessels within the livers. In most species epithelial cells of bile ducts stained positive for the antigens studied (**Table 1**).

Table 1. Expression of HBGA A and B in vertebrate livers

Species	Hepa toct es	Bile ducts	Interl ob. zone	Endo theli um	Lym phoc ytes
<i>S.acanthias</i>	-	-	-	-	-
<i>C.carpio</i>	-	+	-	-	-
<i>C.auratus</i>	-	+	-	-	-
<i>B. viridis</i>	-	+	+	-	-
<i>T.cristatus</i>	-	+	+	-	-
<i>L. viridis</i>	-	+	-	-	+
<i>E.orbicular is</i>	-	+	+	+	+
<i>P.motanus</i>	-	+	-	-	-
<i>A.flaviacoll is</i>	-	+	-	-	-

In the **Chondrichthyes** the livers and all cell types within the liver stained negative for the antigens. Hepatocytes in the hepatopancreas of *C. carpio* – a representative of the **Osteichthyes**– were immunonegative, but the A and B antigens were found in epithelial cells of the bile ducts (**Figure 1**). *C. auratus* showed the same pattern of reactivity. In the tailed **Amphibia**, species *T. cristatus*, the interlobular zone was positive for HBGA.

In the liver of the tailless *B. viridis* A and B antigens were seen localized in epithelial cells of bile ducts, as well as in the interstitial space among liver lobules (**Figure 2**).

Hepatocytes from the tailed **Reptilia**, *L. viridis*, stained negative for HBGA but lymphocytes within the interlobular zones were positive (**Figure 3**). In the tailless reptile, *E. orbicularis*, hepatocytes were negative too, but the interlobular area showed weak granular stains for A and B antigens (**Figure 4**). Parts of the cell membrane and cytoplasm of epithelial cells of bile ducts

stained positive. HBGA were also detected in single lymphocytes and, interestingly, in the endothelium of blood vessels (**Figure 5**).

The representatives of **Aves** - *P. montanus* - and of **Mammalia** - *A. flaviacollis* - showed similar pattern of reactivity – HBGA in epithelial cells of bile ducts and none in hepatocytes (**Figure 6**). Endothelium and red blood cells retained immunonegative reactions.

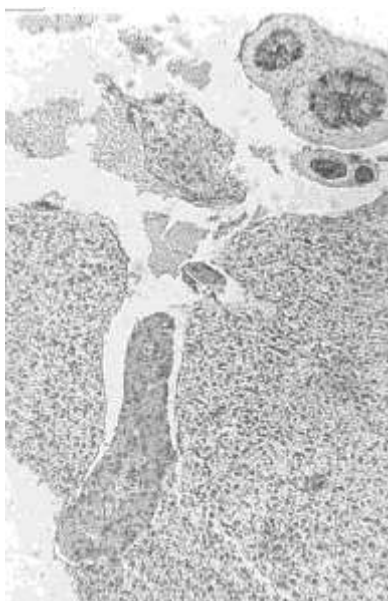


Figure 1. Expression of HBGA A in bile ducts of *C. carpio*. Biotin-Streptavidin-Peroxidase Reaction, x 200.

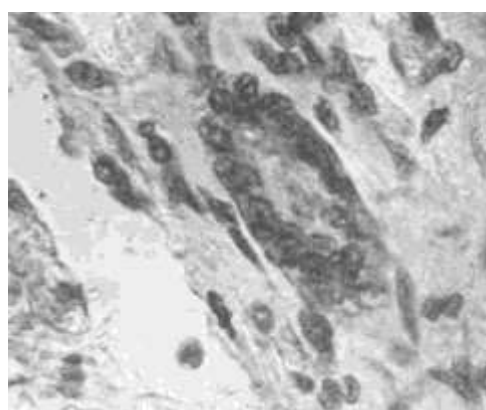


Figure 2. Expression of HBGA A in epithelial cells of a bile duct in *B. viridis*. Biotin-Streptavidin-Peroxidase Reaction, x 400.

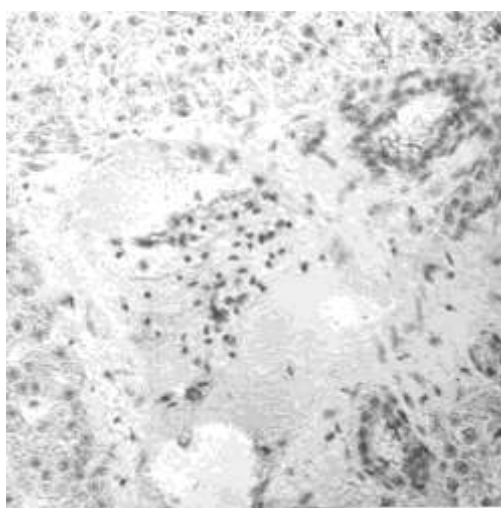


Figure 3. Expression of HBGA B in single lymphocytes in interlobular zones of *L. viridis*. Biotin-Streptavidin-Peroxidase Reaction, x 400.

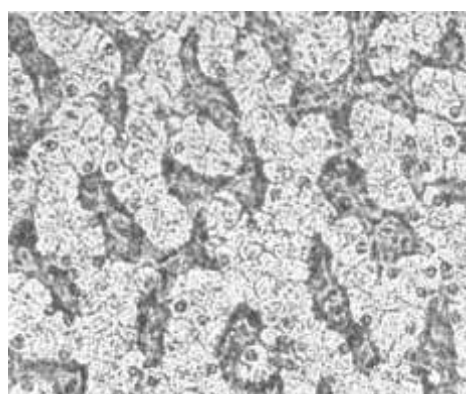


Figure 4. Expression of HBGA A in the interlobular space in the liver of *E. orbicularis*. Biotin-Streptavidin-Peroxidase Reaction, x 400.

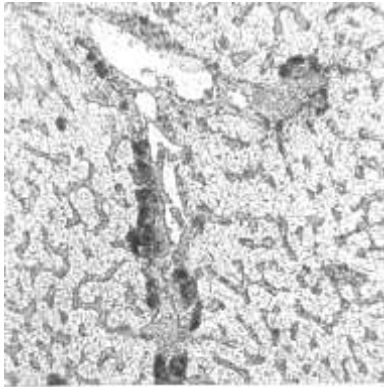


Figure 5. Expression of HBGA A in the interlobular zone and endothelium of blood vessels of *E. orbicularis*. Biotin-Streptavidin-Peroxidase Reaction, x 400.

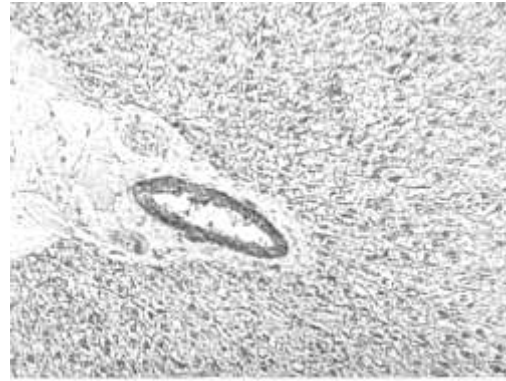


Figure 6. Expression of HBGA B in epithelial cells of a large bile duct in the liver of *A. flaviacollis*. Biotin-Streptavidin-Peroxidase Reaction, x 200.

DISCUSSION.

Knowledge about the HBGA A and B dates back to the beginning of the 20th Century. Though their structure, biochemistry and genetics have been profoundly studied, information about their immunobiological functions have been relatively few.

The carbohydrate moiety on the red cell surface could protect the cell from mechanical damage and microbial attack (14). It has been shown that blood group related glycotopes expressed by the gastrointestinal tract are potential receptors for microorganisms and parasites; they could resemble tumor antigens or lipopolysaccharides of some pathogens (15, 8).

The evolutionary aspect of human HBGA has not been thoroughly studied. For example, no systematic immunohistochemical studies of HBGA expression in liver and bile ducts of lower vertebrates are currently available. Our present investigation has studied the liver expression of A and B HBGA in nine species of free-living vertebrates from six different classes. We were able to map the localization of these antigens. They were found all the time in epithelial cells of bile ducts, but absent in hepatocytes. Our results so far coincide with the data published on humans and some other mammals (2, 16, 17, 18). HBGA were not found in the livers of **Chondrichthyes**. It is quite possible that this class of **Pisces**, being evolutionarily the most ancient, could lack ABH antigens expression. The evolutionary higher class, the **Osteichthyes**, has already acquired the

possibility to produce HBGA in epithelial cells.

A and B antigens were detected using the same technique and primary antibodies as were used in human liver and this, interestingly, showed a similar pattern of reactivity. Both fetal and healthy adult humans do not express HBGA in hepatocytes. This reaction could be due to the early morphological and functional maturation of hepatocytes occurring during 5th to 8th week of gestation (17, 19). The lack of HBGA in hepatocytes could be due to repression of the relevant genes in both prenatal and adult stages of the organism. The appearance of the A and B antigens in human hepatocytes obtained from hepatocellular carcinoma cell lines suggests HBGA as tumor-associated antigens. It could also reveal the possibility of gene de-repression, cell de-differentiation and glycosyltransferase activation (20, 16). On the other hand, the permanent lack of HBGA in hepatocytes of all examined species is a unifying fact suggesting consistent specialization of that particular cell type in the whole vertebrate world. That finding of ours supports the idea that cellular HBGA expression reflects a specific and unique model of maturation in each organ (1).

The presence of A and B antigens in bile ducts suggests that HBGA could be produced by epithelial cells and are possibly secreted in ductal lumina. There had not been any data on HBGA expression in these studied animal species but similar results regarding the presence of the immunodominant residue Gal β 1-4GlcNAc in other mammals have been reported (18). In humans the same

pattern of reactivity in bile ducts has been observed (17). Endodermal ependymocytes express A and B antigens not only along the gastrointestinal tract, but also in the bile epithelium. It is known that endodermal derivatives (like bile duct epithelium) express more frequently HBGA than ectodermal and mesodermal derivatives (2, 17). Our previous parallel studies on the tissue localization of A and B antigens in animals and humans suggested a tendency for stable expression in endodermal derivatives (21, 22). The present investigation confirms that observation as HBGA are found in bile duct epithelium only, and not in hepatocytes and blood vessels. Authors examining the ontogenetic expression of ABH antigens in human tissues, suppose that they could serve as early markers of endothelial differentiation of mesenchymal cells (23). Phylogenetically the first animal class in which we discovered HBGA in endothelial cells is the **Reptilia** (*E. orbicularis*). A possible explanation of our finding could be the well-known fact that order **Chelonina**, and family **Emydidae** in particular, are believed to be a blind branch in vertebrate evolution (24).

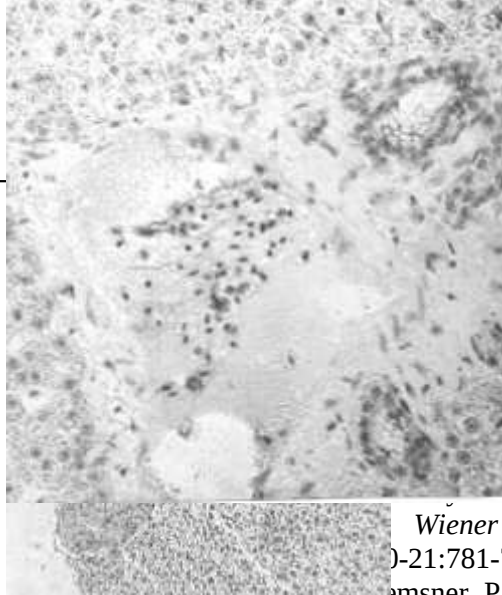
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To conclude, our data present the first comparative evidence for the expression of HBGA in the liver of nine free-living vertebrate species belonging to six classes. It could be suggested that the immunobiological role of blood group glycotopes in animal liver is possibly related to cell differentiation as negative hepatocytes are a highly differentiated cell type. The different expression pattern in hepatocytes and epithelial cells of bile ducts might be related to the different embryologic origin of cells and structures. HBGA tissue expression could reflect the level of cell differentiation and specialization in the course of embryogenesis and phylogenesis, rather than the evolutionary rank of the vertebrate.

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