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TISSUE EXPRESSION OF *A* AND *B* BLOOD GROUP ANTIGENS IN SMALL INTESTINES OF EURASIAN TREE SPARROW (*PASSER MONTANUS*) AND GOLDFINCH (*CARDUELIS CARDUELIS*)

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Abstract

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Tissue localization, molecular mechanisms, functions and importance of the A and B blood group antigens (BGA) in the evolution of vertebrate animals have not been fully clarified. Their exploration can elucidate those aspects and their significance in the evolutionary process. In this work, for the first time, we studied the tissue expression of A and B BGA on the length of the intestines of the class Aves - *Passer montanus* and *Carduelis carduelis*. We applied biotin-srteptavidin-peroxidase system on paraffin cuts. As primary antibodies for immune histochemical study we used monoclonal antibodies against human A and B BGA.

Along the length of the intestine we proved a modulating expression of the antigens searched. The epithelial cells of lamina epithelialis are permanently positivized and the glands in lamina propria. Near the end of the small intestines, the intensity of immune peroxidase reaction decreases. At the end of the organ of *Carduelis carduelis* the expression of A and B blood group antigens disappears. These results can hardly be explained with species specificity and evolutionary level of the animals. Or rather, the possible leading role has the selective inclusion of genetically encoded glycosyltransferases responsible for blood group expression, with no clear limiting factors and unlocking mechanism of their biosynthesis, either.

Key words: A and B blood group antigens, tissue expression, Aves

Introduction

A and B BGA are genetically determined glycoproteins and glycosphingolipids. The glycosyltransferases responsible for their biosynthesis are the first gene product of blood group genes (Cartron, 1996). It turns out that except in human erythrocyte membranes they are expressed by cells of many cell types and secretions not only in human and also in representatives of vertebrates. Their tissue localization, molecular mechanisms, functions and importance in vertebrates' evolution have not been fully clarified yet. Exploration of ABO system can elucidate these aspects. There are many studies on humans, and except them the most numerous are these of the digestive tract in primates (Dabelsteen and Mackenzie, 1976; Nehlsen-Cannarella and Bohn, 1987; Ohshima et al., 1988; Oriol et al.,1999 etc.) and less in other mammals (Oriol,1987; Hanagata et al.,1990; King and Kelly,1991; Oriol et al.,1994;

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Bouhours et al.,1995; Karlsson et al.,1997, etc.). The studies on A and B blood group antigens in representatives of birds are extremely limited and mainly about some types of poultry. It has been proved a weak expression of ABH antigens in epithelial cells of the ostrich and emu producing exocrine glands. There are no data the tissue expression of A and B blood group antigens to have been studied in the organs of other wild birds and even less of the Eurasian tree sparrow (*Passer montanus*) and goldfinch (*Carduelis carduelis*). The purpose of this study was to examine the expression of A and B blood group antigens on the length of the intestines of the birds *Passer montanus* and *Carduelis carduelis*.

Materials and Methods

The objects of this study were free-living, sexually mature individuals of the class of birds Aves. Animals relate to the family Weavers (*Ploceidae*) Eurasian tree sparrow (*Passer montanus*), and the Passerine family Goldfinch (*Carduelis carduelis*), and they were caught in locations around the city of Plovdiv.

We studied the small intestine, as we took material from its beginning, medium and end. The pieces were taken from the animals under ether narcosis in accordance with regulations for humane treatment in experiments with laboratory and wild animals. We used 3 -5 individuals of each species. Tissue material was fixed in 10% neutral formalin for 12 hours. Inclusion in paraffin was made by the methods of Volkova and Eletski (1991). The paraffin cuts were 5-7 microns thick. On prepared paraffin cuts we applied biotin-streptavidin-peroxidase system (DAKO LSAB kit). As primary antibodies for the immune histochemical study we used monoclonal antibodies against human A and B antigens, while as substrate chromogen - 2% solution of amino-ethyl-carbazole (AEC). We did a contra-coloration with hematoxylin by Mayer. The presence of red-brown granules in the cell cytoplasm or plasma membrane of cells was assumed as a positive reaction. A semi-quantitative scale was used for recording the results: (+ +) 80-100% positive cells, (+) 40-50% positive cells; (+/-) 10-15% positive cells, (-) no positive cells. Negative controls were made for each cut as well. For exclusion of non-specific activity of monoclonal antibodies in animal tissues we did inhibition tests.

Results and Discussion

The immune histochemical study on tissue cuts from the different parts of the intestine of individuals of Eurasian tree sparrow (*Passer montanus*) and Goldfinch (*Carduelis carduelis*) showed the following results: In the intestinal epithelium of both species, there were permanently A and B BGA in the tunica mucosa.

Beginning: In the paraffin cuts from *Passer montanus* in tunica mucosa, epithelial cells of lamina epithelialis, as well as intestinal glands in lamina propria, are positivized. The localization of immune peroxidase reaction is mainly cytoplasmic (Figures 1 and 2). The reaction to the B antigen is stronger. For *Carduelis carduelis*, at the beginning of the organ A and B antigens were positivized in lamina epithelialis, as well as in intestinal glands (Figure 3). The intensity in comparison with that of *Passer montanus* was weaker, and it occurred in fewer cells.

Medium: The immune peroxidase reaction in the middle of the small intestines in *Passer montanus* had a similar localization as compared with the beginning, but decreased in intensity (Figure 4). In *Carduelis carduelis* the positive reaction disappeared.

End: In the cuts from *Passer montanus* we observed a weak immune peroxidase reaction in single cells or diffusive coloration. In *Carduelis carduelis* the reaction for both studied antigens was always negative.

Tunica muscularis, tunica serosa and the displayed endotel in all layers and along the entire length of the small intestinal wall showed negative reaction to both antigens.

For the first time we found A and B blood group antigens along the length of the small intestine of *Passer montanus* and *Carduelis carduelis*. It was proved a modulating expression in endodermic epithelial cells of the lamina epithelialis and in glands of the lamina propria. The immune peroxidase reaction showed

antigenic heterogeneity and this heterogeneity was well-expressed in the initial parts of the small intestine. In the two antigens that we studied, B antigen has a higher intensity. It was found as in the number of positivized epithelial cells as in the intensity of reaction for both antigens.

Our results are similar to those of Oriol et al. (1999) where it was proved that epithelial cells, which produce exocrine secretions, express carbohydrate epitopes but in other animal species. A and B blood group antigens found in the digestive tract of vertebrates, from our previous studies, showed a high conservatism and stability in their immune peroxidase expression (Sarafian et al., 2004; Tomova et al., 2005a; 2005б). The epithelial tissue is obviously the most frequently expressing A and B blood group antigens. A similar conclusion was also reached by other scientists (Oriol et al., 1986; Clausen and Hakomori, 1989, etc.). For this reason, it is most likely that the border location of epithelial cells and their relationship to the intestinal lumen have such significance. There is no data for the exact mechanism of genetic control in the

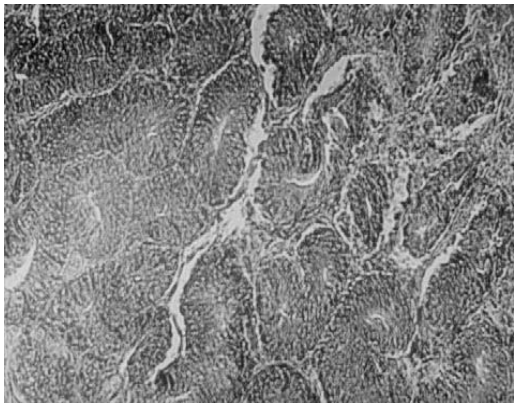

Fig. 1. Blood-group antigen A in the beginning of the intestine of *Passer montanus,* Biotin-srteptavidin-peroxidase reaction in epithelial cells, magnification x 400

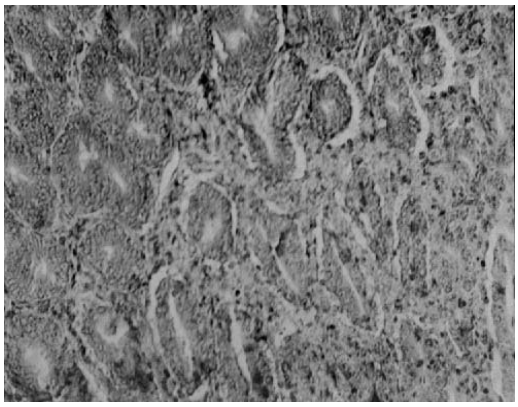

Fig. 2. Blood-group antigen B in the intestine of *Passer montanus,* Biotin-srteptavidin-peroxidase reaction in the intestine glands, magnification x 400

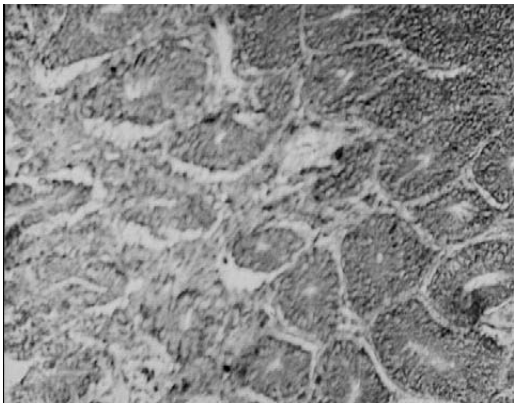

Fig. 3. Blood-group antigen A from the beginning in the intestine of *Carduelis carduelis,* Biotin-srteptavidin-peroxidase reaction in glands epithelial cells, magnification x 400

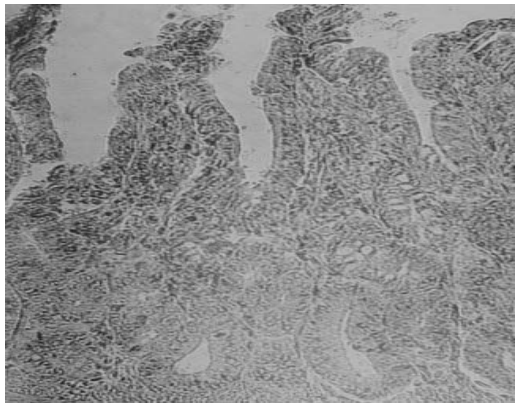

Fig. 4. Blood-group antigen A from the end intestine of *Passer montanus,* Biotin-srteptavidin-peroxidase reaction in epithelial cells, magnification x 400

studied antigens in the animals subjected to this study to have been studied. We assume that the laid genetic program can be performed fully or be interrupted in some stages of their biosynthetic route as a result of the effect of complex reasons. They can be internal, which relate to changes in the course of determination and specialization in different cells, and external, which are related to intercellular communication, intracellular matrix or other molecules that are carriers of its own or foreign genetic program. This probably is the reason to monitor the antigenic modulation of blood-group epitopes along the length of the intestines of high intensity to their full extinction or preservation, and this reflects the high dynamic of blood-group expression and cellular localization.

The intensity of immune peroxidase reaction decreases near the end of the intestine. In *Carduelis carduelis* we found its total lack at the end of the organ. Unlikely, in earlier studies we found that in representatives of tailless amphibians - *Bufo viridis* and *Rana ridibunda*, the antigen expression of the searched epitopes is the same to the end of the intestine (Tomova and Sarafian, 2005[b]). Other results also show different localization and intensity of carbohydrate epitopes along the length of the intestinal mucosa (Mollicone et al., 1985; Oriol, 1987; Ohshima et al., 1988; Mak and Lieber, 2000). The presence of modulating expression of A and B blood group antigens along the length of the intestine in various representatives of vertebrates shows the lack of relation between the antigen expression and species specificity. Finding the searched antigens in the intestinal mucosa of representatives from different classes vertebrate animals also shows a lack of dependency with the evolutionary level of the animals. We suppose that the presence or the disappearance of immune peroxidase reaction in the intestinal mucosa is related to the mechanisms of their genetic expression. The most probably, what is important is the selective inclusion of the genetically-encoded glycosyltransferases responsible for blood group expression. In some species, gene activity can be blocked in whole sections of the digestive tract, and it explains the negative reaction at the end of the intestine in the two bird species that we studied. Limiting factors, as well as unlocking mechanisms of their biochemical biosynthesis, are not clear.

Concerning the presence of heterogeneity in the expression of the two antigens, other authors also proved its presence but in other species of vertebrates. Turcot et al. (2003) investigated gastro-intestinal wall of BDIX rats and the breeds DA and WKY, as they proved A antigen throughout the mucosa, and B antigen - only in surface cells. Heterogeneity of both antigens expression, regarding the intensity, cannot be explained at this stage. In the expression in small intestines of rats BDIX, despite the presence of mRNA for ABO gene, B antigen is not found and it suggests that expression of B antigen is regulated at post-transcriptional level.

The heterogeneity of expression regarding the intensity for both antigens in the intestinal wall cannot be explained at this stage. There is no data about the biochemical mechanism and genetic control to have been studied. More strongly-expressed B antigenic activity could be a result of the presence of more than one B gene and only one A gene. We are going to prove the accuracy of this hypothesis in our future studies.

Conclusion

In the present study for the first time it was proved expression of A and B blood group antigens on the length of the intestinal mucosa with modulating character in representatives of *Passer montanus* and *Carduelis carduelis.* We discovered heterogeneity of the antigenic expression in regard to the intensity of both antigens in epithelial cells of the intestinal mucosa and also in the number of the positivized cells. The data obtained can be the basis for further careful studies on the genetic control and biochemical mechanism of expression of ABH blood group antigens.

This will also facilitate and clarify the significance of these antigens as the organism adaptability to external environment and to certain diseases, and the role of these glycoprotein molecules in the evolution of vertebrates.

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