# Histochemical Analysis of the Chemical Structure of Blood-group-related Carbohydrate Chains in Human Pancreas

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## INTRODUCTION

In a series of previous studies, we have developed the lectin staining method in combination with exo- and endoglycosidase digestion procedures to localize and analyze the definite chemical structures of blood-group-related carbohydrate chains in human pancreas and submandibular glands (Ito et al. 1987, 1988a, b, 1989a, b). The results of these previous studies suggested that type 1, type 2 and O-glycosidically linked, type 3 based ABH antigens are secreted in pancreatic acinar cells. In the present study, we developed immunostaining method using several monoclonal antibodies(MoAbs) against blood-group-related antigens combined with exoglycosidase digestion procedures to provide more definite information as to tissue distribution and regulation of blood group related antigens in human pancreas.

## MATERIALS AND METHODS

Pancreatic tissues were obtained at autopsy cases. They were fixed in 10% or 10% neutralized formalin and embedded in paraffin and sectioned serially at 4 μm. The typing of donor blood group for ABO and Lewis was performed by routine hemagglutination testing of the donor's blood samples. MoAbs directed to A, B and H antigens were purchased from Dako(Santa Barbara CA, USA), MoAbs against $Le^a$ and $Le^b$ antigens were from Biotest Diagnostic(Frankfurt/Main, West Germany) and MoAbs against X, Y and precursor type 1 chain were from Cambridge Research Lab(Cambridge Mass, USA). They were used at dilution of x50-x10.

Tissue sections were de-waxed, hydrated and incubated in 0.1 M phosphate buffer pH 7.2, containing 0.2 M NaCl(PBS) three times(each for 3 min) at room temperature and then incubated in PBS containing 1mg/ml of bovine serum albumin for 20 min. After being blotted dry with filter paper, the tissue sections were incubated with MoAb at 0° C for 4 hr. Tissue sites reactive with MoAb were visualized with the streptavidinbiotin peroxidase complex method, using StreptAvidin Immunostaining Kit(BioGenex Lab., Dublin CA, USA). Finally, the sections were counter-stained with hematoxylin, dehydrated and mounted in balsam. Exoglycosidases used and digestion procedures of tissue sections were described in previous papers(Ito et al. 1987, 1988a, b, 1989a).

## RESULTS AND DISCUSSION

Immunoperoxidase staining with MoAb against blood-group-related antigens showed that A, B, H, $Le^b$ and Y antigens are secreted in acinar cells but not centroacinar cells, intercalated duct cells and islet cells of Langerhans. X antigen was often detected in acinar cells

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whereas $Le^a$ antigen was mainly localized on the surface membrane of centroacinar cells and intercalated duct cells. These results were obtained irrespective of the secretor status of the tissue donors. Expression of $Le^b$ antigen in nonsecretors has been reported in some tissues and explained by the ability of the H gene-specified α-2-L-fucosyltransferase to use both type 1 and type 2 acceptor substance (Mollicone et al. 1988). In one Le(a-b-) individual, $Le^a$ antigen was not detected in any cells while MoAb against $Le^b$ antigen weakly reacted with acinar cells. The reason for such a discrepancy between the genotype and expression of antigens is not known. Such an unexpected reaction may be due to secondary to cross-reactions of the MoAb against $Le^b$ antigen used. Since weak reactivity with the MoAb against type 1 precursor chain was occasionally detected in acinar cells from nonsecretor but not secretor individuals, fucosylation of the type 1 chain may be in part under the control of the Se gene. Previous studies suggested that fucosylation of O-glycosidically linked, type 3 precursor chain was also under the control of the Se gene(Ito et al. 1988b; 1989b). It may be presumed that both the H and Se gene-specified α-2-L-fucosyltransferases are expressed in pancreatic acinar cells and the enzyme coded by the H gene has lower affinity for the type 1 and type 3 precursor chains than that coded by the Se gene.

As reported previously(Ito et al. 1986), some clusters of acini elaborated A or B antigen, whereas others did not, and the distribution pattern of H antigen is exactly the reverse of that of A or B antigen. The results of the present study further demonstrated that the pattern of distribution of $Le^b$ and Y antigen is likewise the reverse of that of A or B antigen. Thus, cell clusters producing the $Le^b$ and Y antigen correspond well with those producing H antigen.

Although $Le^b$ and Y antigens could not be detected in cells producing A or B antigen, prior α-N-acetylgalactosaminidase or α-galactosidase digestion revealed the reactivity with MoAbs against both $Le^b$ and Y antigens as well as H antigen in cells producing A or B antigen. These results suggest that difucosyl A and B antigens($ALe^b$, AY, $BLe^b$, BY) are produced in acinar cells along with the type 1, type 2 and type 3 based A and B antigens.

Since the $Le^b$ structure, once formed, is not an acceptor for the A or B gene-specified transferase, it has been presumed that the $ALe^b$ and $BLe^b$ structure are constructed by the fucosylation of the C-4 position of GlcNAc of the type 1 based A or B antigen(Watkins, 1978). The process is catalized by α-4-L-fucosyltransferase coded by the Le gene. Similar mechanism may be operating in the synthesis of AY and BY structures(Lloyd, 1987). In this case, it is presumed that type 2 based A or B antigens are first produced and then they are fucosylated by α-3-L-fucosyltransferase coded by the X gene to give the AY and BY structures. Since the distribution of difucosyl A or B antigen is strictly confined to those producing A or B antigen and the reverse of that of $Le^b$ and Y antigen, the mechanism proposed by Watkins(1978) and Lloyd(1987) may be operating in pancreatic acinar cells. Thus, the type 1 and type 2 based A or B antigen may be served as the precursor of difucosyl A or B antigen.

Sialidase digestion imparted the reactivity with MoAb against X antigen on the membrane of centroacinar and intercalated duct cells from some but not all the individuals examined. In some individuals, staining patterns with MoAb against the X antigen are quite similar to those with MoAb against $Le^a$ antigen after the enzyme digestion. These results suggest that X antigen is criptic in its sialylated form in these tissue sites. In one Le(a-b-) individual, sialylated type 1

chain was localized on the surface membrane of centroacinar and intercalated duct cells. The type 1 chain cannot be fucosylated to the $Le^a$ antigen because of the absence of the Le gene and instead, they are sialylated to be a criptic form in the Le(a-b-) individual.

Sialidase digestion also imparted the reactivity with MoAb against the type 1 precursor chain in islet cells of Langerhans from some individuals. Since sialidase digestion elicited the reactivity of islet cells with soybean agglutinin and peanut agglutinin which recognizes the type 2 and type 3 precursor chain, respectively(Ito et al. 1987), three types of precursor chains are produced and converted in their criptic forms by sialylation in these cells.

The results of the present study as well as previous ones demonstrated that the distribution pattern of blood group antigen is very complex in human pancreas; their expression is under the control of the ABO, H, Se, Le and X genes in acinar cells while in centroacinar cells and intercalated duct cells, only the Le and X genes are operating. In islet cells of Langerhans, these genes are not functioning although precursor chains may be produced and converted to their criptic forms by sialylation. These differences in the ability to express blood-group-related antigens are presumed to be related to the differences in function of these different cell types.

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