ABH-RELATED ANTIGENS PARTICIPATE IN THE SPERMATOGENESIS OF CATS AND RATS

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The ABH antigens were expressed in the secretory cells of many mammals(1), including humans. Although the allelic cDNA of ABO blood group had been cloned and sequenced(2), no clear explanation of the biological significance of the ABH and related antigens has been proposed. In this study we examined the distribution of ABH related antigens in the urogenital organs from cats and rats in order to pro mote better understanding on the biological significance of the antigens, using monoclonal antibodies (MoAbs) against the ABH and related antigens, and lectins. The results obtained from the present study suggested that the ABH and related antigens may play certain roles in the processes of development of spermatogenic system. In view of forensic practice, the importance of species identification prior to ABO blood grouping from seminal, saliva and urinal stains are stressed on the basis of the present results.

Materials and Methods

Tissue specimens of salivary glands and male reproductive organs from rats and cats were used in this study. Tissues from animals were obtained from the Institute for Experimental Animals of Shiga University of Medical Science. The specimens were fixed in 10% formalin, embedded in paraffin and sectioned serially at 5μm. The staining procedures were described previously(3), using anti A, B, H, Le^a, Le^b, Le^x, and Le^y MoAbs and Erythrina cristagalli(ECA), Helix Pomatia (HPA) and Glycine max(SBA) lectins conjugated with horseradish peroxidase. Anti A and B MoAbs were purchased from Ortho Diagnosis Systems (Raritan, N.J. USA) and anti H and Le x MoAbs were from Dako (Santa Barbara, Ca., USA). Anti Lea and Le b MoAbs were obtained from Signet Laboratories (Cambrige, MA, USA) and anti Le^y was from Nihon Koutai (Gumma, Japan). Lectins was obtained from E.Y. Laboratories (San Mateo, Ca.,USA).

Results

a) antigen expression in cats

The ABO grouping of animal individuals examined in this study could be determined by the reactivity of anti A, B and/or H MoAbs with secretory cells of salivary gland. Although all individuals of cats examined were grouped into blood group A, they were classified into two subgroups according to the difference in the distribution of A and H antigens in the submandibular gland. In one group of cats, A antigen was expressed both in the mucous and duct cells of the glands but the H antigen was found only in the duct cells. In another group, the pattern of the expression of these antigens was reverse, i.e. H antigen was expressed both in mucous and duct cells while A antigen was expressed only in the duct cells. Although similar difference in staining pattern with anti H or A MoAbs were also recognized in the serous cells of von Ebner's glands and lingual glands in the tongues, no difference was observed in the reproductive organs. The reactivity by anti A, H,Le^x and Le^y MoAbs in the collecting tubules of kidney was also recognized.

In the seminiferous tubules of cats, ECA specific for Galß(1-4)-GlcNAc (4) stained the spermatogonium, spermatocytes, spermatids and tail of elongate spermatids. The acrosome granule and nuclear cap of spermatids were clearly stained by ECA. The reactivity of SBA specific for Gal(1-3 or 1-4) GlcNAc (4) was

observed strongly in the nuclear cap and acrosome granule of the spermatids, and feebly in spermatocytes. Anti A, B, H, Le^a, Le^b, Le^x and Le^y MoAbs and HPA specific for GalNAc(4) showed no reactivity in the seminiferous tubules including the Sertoli cells. ECA showed striking staining of the epithelial cells(ECs) of the ductuli efferentes, however, the intensity is declining towards the corpus and cauda epididymis. SBA showed good reactivity with the stereocila on the free surface of ductuli efferentes and weak or feeble with the ECs of the ductus epididymidis. Anti A, H, Le^x and Le^y MoAbs stained the ECs of the ductuli efferentes and/or ampulla epididymidis, showing mosaic reactivity with the cells. Although anti A MoAb and HPA showed intense reactivity with the ECs, spermatozoa and secretory fluid in the lumen of the epididymis, anti H, Le^x and Le^y MoAbs showed no reactivity with the ECs in the corpus and cauda epidydimidis. We could not examined the reactivity by MoAbs and lectins in the prostates and seminal vesicles of cats because no tissues of the organs could be obtained.

b) antigen expression in rats

The blood groups of rats were determined to be blood group B and AB. In blood group AB rats, anti A MoAb stained secretory cells of submandibular glands and anti H MoAb stained secretory cells and duct cells. Anti B, Le^a, Le^b and Le^y MoAbs stained the duct cells of the glands. Anti Le^x MoAb showed no reactivity in the salivary glands.

ECA and SBA clearly stained the nuclear cap of spermatocytes and elongate spermatids in the seminiferous tubules, while the tail of the spermatids was not stained by these lectins. Although the intense staining by these lectins was observed in the epithelial cells of the ductuli efferentes, and in the ECs in the ampulla epididymidis, the instensity was declining towards the corpus and cauda epididymidis.

MoAbs used in this study showed no reactivity in the seminiferous tubules including the Sertoli cells. However, HPA lectin specific for GalNAc and blood group A antigen showed intensive reactivity in the testis and epidydimidis. HPA stained the spermatocytes, spermatids and tail of spermatids in the seminiferous tubules and the ECs, secretory fluid and spermatozoa in lumen from the ampulla to cauda epididymidis obtained from all the rats examined. Anti A MoAb showed weak reactivity with the ECs of epididymidis from some rats and the B and H antigens were not detected in the epididymidis of rats. Anti Le^y MoAb clearly stained the ECs of prostates and anti Le^x weakly stained the ECs of the ductus deferens. Anti H, Le^a and Le^b MoAbs showed feeble or no reactivity with the ECs of the prostates of rats. ECA and SBA stains also the ECs of the prostate. The patterns of the reactivity in the prostate varied considerably according to the MoAbs and Lectins.

Discussion

We demonstrated previously (5) that the ABH and related antigens were expressed in the salivary glands, taste bud cells and kidneys of some mammals. The antigens were also expressed in the epididymidis and prostates from rats and/or cats in this study. However, the patterns of distribution of these antigens varied considerably between animals and organs. Although Le^a, Le^b ,Le^x and/or Le^y antigens were expressed in rats as well as human tissues, Le^a and Le^b antigens were not detected in the tissues of cats. Blood group ABH antigens in human tissues are mainly carried by either type 1 or type 2 carbohydrate chains (6). The Le a and Le b antigens are type 1 and Le x and Le y antigens are type 2 based antigens, respectively. The results obtained indicate that both the type 1 and 2 carbohydrate chains of the ABH antigens were secreted in the tissues of humans and rats whereas only type 2 chain antigens were expressed in tissues of cats. The distribution patterns of ABH antigens in the submandibular gland of cats and rats were different from those of humans. In cats, the A antigen was secreted only in duct cells from one group and both in the mucous and duct cells from another group. In group AB rats, the A antigen was secreted in the mucous cells and the B antigen was secreted in duct cells. In addition, non secretor types were not observed in rats and cats examined. These results indicate that the genetic control systems of the tissue specific expression of the blood group antigens in humans might be quite

different from those of other mammalian species. On the other hand, based on the results obtained in this study, we must emphasize that the species identification prior to ABO blood grouping is indispensable because forensic materials such as the stains from saliva, urine and seminal fluid might be contaminated with the fluid from indoor pets.

Intratesticular spermatogenesis and the subsequent epididymal maturation of the sperm are hallmarked by substantial morphological and biochemical changes accompanied by important cell-surface modifications(7). Pattern of carbohydrate expression changed dramatically during the process of spermatogenesis. It is well known that SBA and ECA lectins show the specificity to the precursor structure of H antigen (4). The finding of ABH, related antigens and the precursor substance recognized by ECA and/or SBA in the reproductive organs of rats and cats may provide an important clue to the role of these antigens in the processes of spermatogenesis of these animals. It has been suggested that Le^x antigen plays a role in cell adhesion of the early mouse embryo, since the Le^x antigen appeared in the developing embryo at the compaction stage (8). Nomura et al.(9) detected blood group B active glycosphingolipids in Xenopus laevis eggs and suggested that the B antigen plays a role in the cell-adhesion process of Xenopus embryonic cells.

Summary

The distribution of ABH and related antigens in reproductive organs of male rats and cats was examined using histochemical methods. Although the reactivity of MoAbs against ABH related antigens with the tissues were different and varied among the animal species, the antigens were expressed in the epithelial cells of reproductive organs from animals inhabiting around humans. The results obtained in this study and previous ones indicate that in view of forensic practice, the species identification prior to ABO blood grouping in the stain analysis are indispensable to avoid mistyping due to contaminated materials from indoor pets.

Pattern of the reactivity with MoAbs against ABH and related antigens and lectins changed dramatically during the process of spermatogenesis. In the seminiferous tubules, the reactivity of ECA and SBA with the precursor substance was intensively observed and the reactivity was declining towards the epididymidis, while the reactivity of anti A and/or HPA in the epidydimidis was increasing. The reactivity of anti H, Le^x and Le^y MoAbs was observed in the ductuli efferentes or ampulla epididymidis of cats. The ABH and related-antigens secreted in the reproductive organs might be involved in the processes of spermatogenesis and sperm maturation of rats and cats.

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