Species identification from tissue particles using lectin- and immuno-histochemical methods

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INTRODUCTION

Many kinds of forensic biological materials found at scenes of murder and traffic accidents are brought to forensic scientists for identification. Although various immunological methods and DNA techniques for the species identification from blood and bloodstains are now available, there are few investigations for blood grouping and species identification from tissue particles or tissue debris(Fechner et al 1989). In this study we report a method for species identification from tissue particles using lectin- and immuno-histochemical techniques.

MATERIALS AND METHODS

Tissue specimens from vertebrate species including fishes, frogs, saurians, chickens , mammals and primates were used in this study. The tissue specimens from primate species including 2 chimpanzees and 3 gibbons and non-primate mammals were obtained from the Primate Institute of Kyoto University and Institute for Experimental Animals of Shiga University of Medical Science. The human tissue specimens were obtained from routine autopsies. The specimens were fixed in 10% formalin, embedded in paraffin and sectioned at a thickness of 4 μm. After deparaffinizing, the sections were incubated for lectin staining and immunostaining with monoclonal antibodies, as described previously (Ito et al 1986, Nishi et al 1989). Hoseradish-peroxidase labelled Ulex europaeus agglutinin I (UEA), blood goup H specific lectin, and Griffonia simplicifolia aggulutinin I-B4(GSAI-B4), blood group B specific lectin, were purchased from E. Y. Laboratories (San Mateo CA, USA). Anti A and B antibodies were purchased from Biotest Diagnostic (Frankfurt, Germany). Anti-type II chain H antibody were obtained from Dako (Santa Barbara, USA). Sections stained with lectins or monoclonal antibodies were counterstained with hematoxylin, dehydrated and mounted in balsam .

RESULTS AND DISCUSSION

GSAIB4 and UEA used in the present study bind specifically to non-reducing terminal αGAL and αFUC residue showing blood group B and O specificity respectively (Wood et al 1979, Pereira et al 1978). Alroy et al(1987)reported that vascular endothelia from various mammalian species are selectively stained with GSAIB4 which does not usually react with human endothelia and the vascular endothelial cells of humans are consistently stained with UEA which does not bind to other mammalian endothelial cells. In the previous report(Ito et al 1990) we showed that ABO blood group antigens and UEA reactivity appeared and GSAIB4 reactivity disappeared in endothelial cells and red blood cells directly corresponding to the stage in primate evolution.

The results obtained in this study, as shown in Tab. 1, suggest that the expression of ABH antigens and carbohydrate epitopes on erythrocytes membrane and vascular endothelial cells is closely related to the evolutionary stage of vertebrate species, although epithelial cells of gastrointestinal tract and secretory cells of tissues from various vertebrate species tested in this study expressed ABO antigens.

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Red blood cells and endothelia of non-mammalian vertebrates such as saurel, yellow-tail, saurians and chickens, except those cells of frogs, showed no reactivity with GSAIB4, UEA and ABH antibodies. Epithelial cells from the gastrointestinal tract from edible frogs and bullfrogs showed good reactivity with monoclonal anti B antibody. Vascular endothelial cells also reacted with monoclonal anti A antibody and red blood cells from these frogs showed weak reactivity with anti B antibody. The erythrocytes of frogs are morphologically different from those of mammals, since the erythrocytes of frogs are nucleated cells.

Epithelial cells from the gastrointestinal tract from mice, rats and rabbits showed good reactivity with ABH antibodies. Secretory cells of the salivary glands , acinar cells of the pancreas , epithelial cells of gastrointestinal tract and biliary duct epithelium from cats, dogs, prosimians (common tupai, grand galagoand, ring-tailed lemur) and new world monkeys (common marmoset, cotton-top tamarin, common squirred monkey and central american spider monkey) showed good reactivity with monoclonal A, B or H antibody. The blood group of individuals of these animals could be determined , acording to the reactivity of these cells with the antibodies. UEA and GSAIB4 showed various reactivity with secretory cells and characteristic of blood group specific lectins. Vascular endothelial cells and erythrocytes from non-primate mammals, such as cow, deer, pig, wild boar and other mammals described above showed no reactivity with monoclonal ABH antibodies. In the evolutionary stage of non-primate mammals and lower primate species, such as prosimians and new world monkeys, GSAIB4 binding to erythrocytes and endothelial cells appears irrespective of animal species. These results suggest that α-galactosyl epitopes consititute a substantial proportion of carbohydrate residues on those cells of non-primate mammals and lower primates. The reactivity of GSAIB4 to these cells is not due to terminal αGal residues of the B antigen, since blood group ABH antigens are not expressed in these cells of non-primate mammals and lower primate species.

In old world monkeys (japanese monkey, crab-eating monkey, rhesus monkey, savanna monkey and hamadryas baboon) ABH antigens are expressed in secretory cells, as in non-primate mammals and lower primate species, in endothelial cells but not yet in erythrocytes. Usually, UEA and GSAI-B4 did not react with endothelial cells from old world monkeys except those individuals whose endothelial cells reacted with anti H or B antibody.

In anthropoid apes(chimpanzee and gibbon), both red blood cells and endothelial cells expressed ABH antigen as in humans. The secretory cells also showed good reactivity with ABH antibodies. Although UEA reacted with endothelial cells from two chimpanzees, which were both typed as blood group A, as found in of humans, UEA did not show reactivity with the cells of 3 gibbons which were typed as blood group AB. This results indicate that the ability of endothelial cells to show reactivity with UEA seems to be possessed only at the stage of the highest primates such as chimpanzee and humans.

In humans and chimpanzees UEA reactivity is recognized on erythrocytes and endothelial cells irrespective of the ABO group of the individuals. Although Petryniak and Goldstein(1986) demonstrated that UEA showed good affinity for the type II chain H antigen, the UEA reactivity with erythrocytes and endothelial cells is not due to αFuc of type II chain H antigen, because monoclonal anti type II chain H antibody used in this study hardly reacted with erythrocytes and endothelial cells from blood group A, B and AB individuals except blood group A_2 individuals(Nishi et al 1989).

It seems that both the changing of carbohydrate epitopes and the development of ABH antigen expression on red blood cells and endothelial cells are regarded as an important evolutionary event in vertebrate species.

We reported(Fechner et al 1989) that ABH antigens and human hemoglobin antigen can be reliably detected in the mummified tissues particles for a long period of time by means of immunohistochemical methods using ABH antibodies and anti human hemoglobin. Since blood vessels are present in all tissues and are expected to be found in putrified and/or mummified tissues, the histochemical method described in this study seems to be useful for the forensic practice.

Table 1.
The expression of ABH antigens and lectin binding in red blood ells, endothelial cells and body secretions of various animal species

	Red Blood Cells			Endothelial Cells			Secretions
	A, B, H	GSAIB4	UEA	A, B, H	GSAIB4	UEA	A, B, H
Humans	+	-	+	+	-	+	+
Anthropoid apes							
Chimpanzee	+	-	+	+	-	+	+
Gibbon	+	-	-	+	-	-	+
Old world monkeys	-	-	-	+	-/+*	-/+*	+
New world monkeys	-	+	-	-	+	-	+
Prosimians	-	+	-	-	+	-	+
Nonprimate mammals	-	+	-	-	+	-	+
Nonmammalian Vertebrates							
chicken	-	-	-	-	-	-	n.d
saurian	-	-	-	-	-	-	n.d
frog	+	+	-	+	-	-	+
fish	-	-	-	-	-	-	+

*: Cells from B and O typed individuals reacted with the blood group B and H specific lectins respectively. n.d: not done.

CONCLUSION

The results obtained in this study show that the species identification from tissue particles can be reliably performed by lectin- and immuno-histochemial methods using GSAIB4, UEA and blood group ABH antibodies.

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