Phylogeny and Ontogeny of Blood Group antigens. A study by Polychromatic Tissue Immunofluorescence of ABH and Related Antigens.

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CHEMISTRY AND GENETICS

ABH antigens can be built on six different precursor chains:

Type 1....βGal(1→3)βGlcNAc-R
Type 2....βGal(1→4)βGlcNAc-R
Type 3....βGal(1→3)αGalNAc-R
Type 4....βGal(1→3)βGalNAc-R
Type 5....βGal(1→3)βGal-R
Type 6....βGal(1→4)βGlc-R

Each of these chains can be transformed into the different monofucosylated and difucosylated A, B or H structures, by addition of terminal fucose, galactose or N-acetylgalactosamine residues (Oriol et al. 1986). Addition of these sugars is performed by the products of specific genes which work at three different levels (Table 1). At the first level, addition of fucose to subterminal N-acetylglucosamine, by products of the Lewis or X genes, produces the monofucosylated Le^a or X antigens on type 1 or type 2 chains respectively. At the second level, addition of another fucose, α(1→2) linked to terminal galactose of any of the six precursors, makes the monofucosylated H antigens. Addition of both fucose residues, at levels 1 and 2, makes the difucosylated Le^b or Y structures. Finally, at the 3rd level, addition of N-acetylgalactosamine or galactose in α(1→3) to any of the H structures makes the A or B antigenic determinants.

Level	Gene	Enzyme	Structure
3	ABO	A or B	**αGalNAc** or **αGal**
2	Hh;Sese	H or SE	**αFuc** → βGal
1	Xx;Lele	X or Le	**αFuc** → βGlcNAc
			R

Table 1. Genes, enzymes and chemical structures resulting from interactions of the products of ***ABO, Hh, Sese, Lele*** and ***Xx*** genetic systems at three different levels of terminal oligosaccharides.

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PHYLOGENY

ABH and related oligosaccharide antigenic determinants are widely distributed in nature.

An "A like" antigen was found in digestive secretions and on cortical granules of *Xaenopus leavis* eggs. The X or Le^x antigen (also known as SSEA1) was found in venom glands of poisonous snakes (Oriol et al. unpublished results). But most of these glycoconjugate structures in lower vertebrates or even in invertebrates have been detected only serologically, they have not been purified and their chemical structures are not well defined as yet.

Rat. Chemical analysis of glycolipids extracted from rat intestinal mucosa has enabled characterization of the terminal tetrasaccharide of the A antigen: αGalNAc(1→3)[αFuc(1→2)]βGal(1→3)βGlcNAc-R (Breimer et al. 1982). This antigen was present in the small intestine of certain rat strains, whereas other strains expressed only the H antigen αFuc(1→2)βGal(1→3/4)βGlcNAc-R, in the small intestine (Breimer et al. 1980). Unlike this, the A antigen was expressed in the colonic mucosa of all rat strains. Therefore, in the rat, A antigenic determinant expression is under polymorphic genetic control, restricted to the small intestinal mucosa alone. None of the strains of rat tested expressed A on the oral mucosa, but H and B were found in this area in all the rats examined (Reibel et al. 1984 and Reibel 1987). B and H antigens were also found in some primary sensory neurons of the rat (Mollicone et al. 1985a, Dodd et al. 1985), but no ABO related structures have been described so far in rodent erythrocytes or vascular endothelium.

Rabbit. Expression of the A antigen is polymorphic in the digestive mucosa of rabbits. There are A^+ rabbits, expressing the A antigen and A^- rabbits, which only have the precursor H antigen (Oriol et al. 1977). All rabbits express the B antigen in digestive mucosa irrespective of their A genotype, including homozygous A^+/A^+ rabbits. This suggests that A and B antigens do not behave like alleles in rabbits.

Some primary sensory cells of the rabbit also express A, B or H antigens. Erythrocytes of this species do not express the normal fucosylated ABH antigens, but they have an unfucosylated linear "B like" structure (Hanfland et al. 1981).

Pig. A and O pigs have been defined serologically using red cells, but the antigenic structures responsible for these serological reactions are probably circulating glycosphingolipids adsorbed onto red cells. Incubation of red cells from an O pig, in plasma of an A pig, transformed the O cells into A cells and viceversa, incubation of A red cells in O plasma resulted in a significant loss of A antigen (Oriol 1987). The digestive mucosa has large amounts of A antigen in A pigs and H antigen in O pigs.

Dog. Dogs have a very weak "A-like" antigen in red cells called Tr (Bowdler et al. 1971), which is probably adsorbed at the surface of erythrocytes as the "A-like" antigen of pig erythrocytes.

Four different phenotypes have been found in dog digestive mucosa A, AY, Y and X (Oriol et al. 1975). The Y antigen of the dog corresponds to the Y antigen of man, that is the difucosylated type-2 isomer of the Le^b antigen. The serologically defined X antigen of the dog (Zweibaum et al. 1974) has the structure of the H type-2 antigen of man (Mc Kibbin et al. 1981). Therefore, from a genetic point of vue, the dog has two distinct genetic

digestive group polymorphisms, A-O and Y-O, which segregate independently (Oriol, unpublished results).

Marmoset. This small South American monkey belongs to the most primitive infraorder of higher primates, the *Platyrrhini.* All the individuals tested had A and H antigens in exocrine secretions and primary sensory cells (Mollicone et al. 1986a), but lacked these antigens on vascular endothelium and erythrocytes (Socha and Ruffie 1983).

Baboon. These animal express strong A and/or B antigens in exocrine secretions, in primary sensory cells (Mollicone et al. 1986a) and in vascular endothelium (Oriol et al. 1984). Three phenotypes A, B and AB have been described in their secretions, but the O phenotype has not been found as yet (Socha and Ruffie 1983). Expression of ABH tissue antigens in this species is intermediate between marmoset and man. They have ABH antigens on vascular endothelium as in man, but they lack ABH antigens on red cells as in lower mammals (Oriol et al. 1984).

Man. Higher antrophoid primates (Socha and Ruffie 1983) and man are the only species which express ABH antigens on red cells. Therefore the, so-called "major blood group antigens" whould better be defined as tissue antigens than as red cell antigens. In fact, they only appear on erythrocytes very late in evolution (Figure 1) (Oriol et al. 1986).

ABH	Digestive mucosae	Epidermis	Olfactory receptors	Posterior root ganglia	Vascular endothelium	Red cells
	+	+	+	−	−	−
	+	−	+	+	−	−
	+	+	+	+	−	−
	+	+	+	+	+	−
	+	+	+	+	+	+

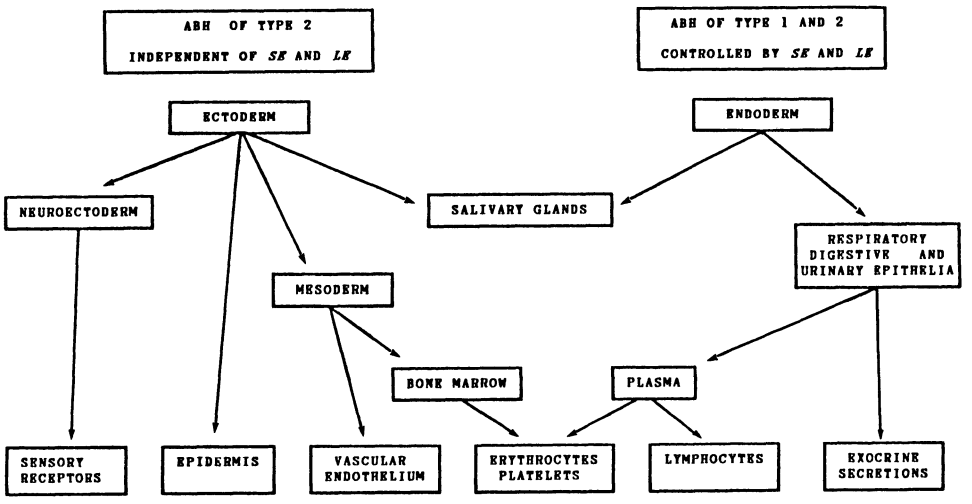
ONTOGENY

ABH antigens have been found in the earliest ascertainable stages of development of the human embryo (5th week postfertilization). At this early stage, red cells, vascular endothelium and epithelial cells of practically all organs or organ rudiments express ABH antigens in accordance with embryo ABO blood group (Szulman 1980).

The end of the first trimester of pregnancy is marked by orderly recession of the epithelial cell wall ABH antigens of many primitive organs. Their disappearance or decrease often coincides with recognizable steps of tissue differentiation. For exemple, secretion of mucus in the gastrointestinal mucosae, transition from a solid epithelial crescent to an organ with acini in the thyroid, production of growth hormone in the anterior pituitary gland are all developmental steps related to a strong decrease in expression of ABH antigens in each of the above mentioned organs (Szulman 1964). However, this recession is not uniform in all organs. The adult human rectum has completely lost the capacity to secrete ABH antigens at both cell membrane and mucous secretions, whereas upper portions of the digestive mucosa lose part of the cell wall ABH antigens but keep the mucous ABH antigens throughout life. Red cells and vascular endothelium, by contrast, do not modify the expression of ABH throughout life.

Based on the chemical structures of the ABH antigens isolated from different tissues and their expression on individuals with different genetic backgrounds, two main kinds of genetic control of ABH and related antigens have been found in man: I. ABH antigens of type-1 and type-2 under the control of the *Se* and *Le* genes. II. Type-2 ABH antigens independent of *Se* and *Le* gene control. These tissue antigens are expected to be under *H* and *X* gene control (Figure 2).

EMBRYOLOGICAL ORIGIN OF TISSUES EXPRESSING ABH ANTIGENS



Tissue distribution for these two kinds of genetic control of ABH and related antigens is not random. Tissues of ***ectoblast*** and ***mesoblast*** origin express mainly ABH antigens independent of control by *Se* and *Le* genes, whilst tissues of ***endoblast*** origin express mainly ABH antigens under the control of *Se* and *Le* genes. However, this distribution is not an all or none phenomenon and some exceptions to this general rule have already been found (Oriol et al. 1986) .

TISSUE ABH ANTIGENS IN ADULTS

In man, the most widely distributed ABH antigens, are mesodermal type-2 antigens of erythrocyte and vascular endothelium independent of ***Se*** and ***Le*** genes. In fact, these structures are present all over the human body.

Epidermis. The human body is covered by a continuous two to four cell layer sheet of ABH positive cells which are independent of the *Se* and *Le* genes. This layer forms the most external area of the stratum granulosum, next to the stratum corneum. The only skin sample negative with anti-A, anti-B and anti-H, belonged to an H deficient individual ***(h/h)*** from Reunion Island (Le Pendu et al. 1986), suggesting that epidermal ABH antigens are under *H* locus conrol.

Primary sensory neurons. The pseudo-unipolar neurons of the posterior root ganglia have ABH antigens in the Golgi cysternae, membrane and cytoplasm. This positive reaction extends centrally to the first synapsis in the substantia gelatinosa of the posterior horn of the spinal cord (laminae II) and peripherally to the sensory receptors. Neurons of sympathic and parasympathic ganglia (Oriol et al. 1984) and the neurons of the mesencephalic nucleus of the trigeminal nerve (Mollicone et al. 1986a) are also positive. Some of the primary sensory neurons of the cranial nerves also synthesize ABH antigens i.e. olfactory, auditory (Mollicone et al. 1985a) and taste receptors. All these sensory cells expressing ABH antigens are derived either from the neural crest or ectodermal placodes. By contrast, sensory cells derived from the central nervous systems, for example the optical receptors of the retina, have no ABH antigens at all. Epithelial and endothelial cells in the cornea, which have no sensory function but are derived from the ectoderm directly overlying the optic vesicle, express ABH antigens (Salisbury and Gebhardt 1981). All ABH antigens expressed by primary sensory neurons are independent of *Se* and *Le* genes, as expected for ectodermal structures.

Digestive mucosae. This is a typical tissue of endodermal origin. All digestive epithelial cells from oral to anal mucosa express some sort of blood group related oligosaccharide antigen. Most of them express ABH and Lewis antigens under the control of *Se* and *Le* genes. However, some exceptions to this general rule have been observed in acinar cells of gastric and Brünner's glands. The surface epithelium of the duodenum and the pylorous express type 1 and type 2 ABH antigens under the control of *Le* and *Se* genes (Mollicone et al. 1985b and 1986b). By contrast, in deep gastric glands and Brünner's glands, only type-2 difucosylated antigens independent of *Se* and *Le* genes are found. These deep glands, therefore, represent an exception to the endodermal pattern of ABH antigen secretion. The remainder of the small intestine expresses ABH and Lewis antigens under the control of *Se* and *Le* genes, as does the surface of the pyloric and duodenal mucosae. The caecum and the ascending and transverse portions of the colon express these antigens under similar genetic controls. In the mucosa of the descending and sigmoideum colon, there is a progressive decrease in ABH and Le^b antigen expression. These antigens are practically absent from

normal rectal mucosa (Wiley et al. 1981). However, the Le^a antigen persists thoughout the colonic mucosa and other Lewis related antigens (of as yet unidentified structure) appear in this area (Macartney et al. 1986).

Kidney. This organ contains structures from two embryonic origins. Glomeruli and proximal and distal convoluted tubules derive from the local mesenchima and express ABH and related antigens independent of *Se* and *Le* genes, whereas the urinary epithelium (collecting ducts, calyces and ureter) derive from the ureteral bud which has its origin close to the cloaca of the primitive digestive tube. These cells express ABH antigens under the control of *Se* and *Le* genes. Nevertheless, although all kidney cells follow these general rules, each portion of the nephron has its own particular expression of ABH and related structures. The endothelial cells of glomeruli and vessels and the epithelial cells of some distal tubules express ABH antigens independent of *Se* and *Le* genes (Hinglais et al. 1981). The epithelial cells of proximal convoluted tubules and the descending limb of the Henle loop are devoid of ABH antigens, but they contain large amounts of the X and Le^x antigen. This X antigen is probably under the control of the same ***X*** gene controlling the synthesis of the α-3-fucosyltransferase in serum since the proximal convoluted tubules of a young female, whose serum was devoid of α-3-fucosyltransferase, did not contain any detectable X antigen. The ascending portion of the Henle loop and the distal convoluted tubule secrete the Tamm-Horsfall glycoprotein. Finally, the Lewis antigens, under the control of the product of the Lewis gene, are only found in some distal convoluted tubules and the urinary epithelium (Oriol et al. 1980).

Type 3 antigens. Type-3 antigens contain an internal αGalNAc, which is the terminal structure of A antigens. Therefore, they have been described in A individuals as repetitive A structures and they contribute to the A_1-A_2 difference on red cells (Clausen et al. 1985, 1986). They have been found in all cells expressing A antigens, although in nucleated cells they are restricted to the area of the Golgi appararus (Le Pendu et al. 1986). The fact that the anti-A type-3 antibodies only stain the Golgi apparatus suggests that the terminal portion of type-3 structures might either be cut off, by specific glycosidases, or masked by the elongation of the oligosaccharide chains during their transit through the Golgi cysternae.

Type 4 antigens. An A type 4 heptaglycosylceramide has been isolated from human kidneys and represents the major blood group related glycolipid structure of this organ (Breimer and Samuelsson 1986).

Type 5 and 6 antigens. They have been found in exocrine secretions like milk or urine. Type 6 chains are probably the core of the ABH antigens found in the gastric surface epithelium of nonsecretors.

POLYCHROMATIC IMMUNOFLUORESCENCE

ABH and related antigens were located on tissues with specific antibodies or lectins labelled with one of the following three fluorochromes: a. fluorescein isothiocyanate (FITC); b. tetramethyl rhodamine isothioryanate (TRITC); c. amino methil coumarin acetic acid (AMCA). This last fluorochrome has been recently commercialized by Bio-Carb (Khalfan et al. 1986). All three fluorochromes can be used with the same light source, but each has different excitation and emission optima. Therefore, specific filtersets must be used to visualize each fluorochrome, but reactions with the three

different reagents, one labelled with each fluorochrome, can be performed simultaneously on the same tissue section.

Fluorochrome	Excitation	Emission
AMCA	U.V.	blue
FITC	blue	green
TRITC	green	red

Since, with each fluorochrome, only positively labelled structures fluoresce against a black background, two or three exposures of each field, each with a different filterset, can be superimposed on the same picture. These double or triple exposures give polychromatic pictures showing the location of 2 or 3 different antigens on the same slide (Oriol et al. 1985, Mollicone et al. 1986, Le Pendu et al. 1986, Oriol 1987).

The final result can be further improved by specific yellow fluorescent staining of the DNA of nuclei. This is obtained by mounting the stained histological preparations with one drop of p-phenylenediame (1mg/ml in glycerol 90% pH 8) (Oriol and Mancilla 1983). This mounting medium enables the nuclei of all cells to be visualized in yellow with the same filterset used for FITC. In addition, it decreases the photobleaching effect undergone by fluorescein under light exposure and allows shorter exposure times.

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