Evaluation of Human Blood-Type A Transgenic Mice

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Introduction

The discovery of the ABO-blood system by Karl Landsteiner in 1901 made the medical world aware of the cause of tragedies during the processes of blood transfusion and more relevantly, organ transplantation.[i] Since ABO-incompatible transplants introduce foreign antigens into the recipient, a phenomenon known as "hyperacute antibody-mediated rejection" will occur in which the recipient's antibodies will attack the new organ and result in its failure. Under these circumstances, ABO-incompatible organ transplants pose dangerous risks to the recipients' well-being; therefore, doctors all over the globe avoid this type of procedure.

Recipient ABO-blood type:	ABO-antigen present:	Antibodies produced:	Compatible organ donor blood groups:	Incompatible organ donor blood groups:
O	—	anti-A, anti-B	O	A, B, AB
A	A	anti-B	O, A	B, AB
B	B	anti-A	O, A	A, AB
AB	A, B	—	O, A, B, AB	—

[i] "**Landsteiner, Karl**." *Encyclopædia Britannica*. 2008. Encyclopædia Britannica Online. 8 Aug. 2008 <http://original.britannica.com/eb/article-9047068>.

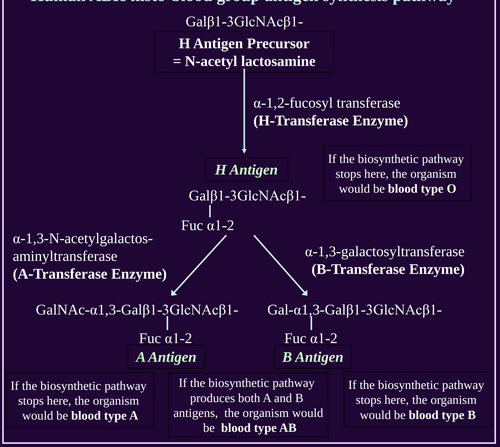
Meanwhile, many patients on lengthy waiting lists have died because of organ shortage and many ABO-incompatible organs have gone to waste. In 2001, Dr. Lori West made a significant advance in attempting to remedy this problem by successfully performing ABO-incompatible heart transplants in infants.[ii] It is known that the initial success of the transplants was due to the lack of antibody development in infants, but the maintenance of the transplants as the patients aged was surprising. The immunological mechanism by which this occurred was, and remains, a puzzle. The field of applications is vast – a similar situation induced in adult patients could save many lives and resources.

However, first, a method needs to be developed to ascertain the mechanism of ABO-incompatible transplantation tolerance. Since the risk and moral implications of human trials are extensive, an animal model is desirable. A mouse model is chosen for its versatility and accessibility. The ABO-blood system is not present in the mouse; to make the model functional, two of the human ABO-blood group genes, A- and H-genes, were transferred into the mice. The H-gene produces an enzyme that modifies carbohydrates into the precursor molecule for the blood group antigens; the A-gene produces an enzyme that modifies this carbohydrate into the A-antigen. Despite that the B-antigen's absence in the model, which renders it an incomplete representation of the ABO-blood system, the A-antigen alone is sufficient for experiments involving ABO-incompatible transplants as the two antigens operate in the same manner.

[ii] West LJ, et al. ABO-incompatible heart transplantation in infants. N Engl J Med. 2001 Mar 15;344(11):793-800.

Diagram 1

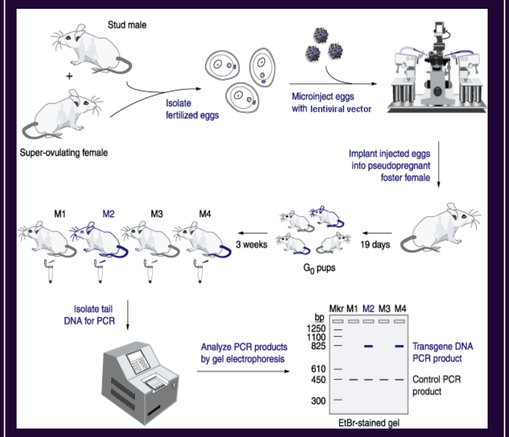
Human ABH histo-blood group antigen synthesis pathway



Introduction (continued)

The incorporation of the A- and H-genes was achieved through lentiviral transgenesis. Four transgenic mice were bred with non-transgenic mice. The resulting offspring was then screened for the presence human transgenes A and H in their genome. Successful transgenic mice were bred together for several generations to produce pups with increased purity and stability of the transgenes in their genome. After several rounds of breeding, the West lab now has four stable lines of transgenic mice. The tissues used in this project were derived from second and third filial transgenic mice.

Diagram 2



Aims

Although both transgenes were transferred into the genome of the four transgenic mouse lines, their expression pattern in different organs had not yet been characterized. Moreover, the presence of the A antigen was not confirmed.

This project focuses on evaluating the expression pattern of A- and H- genes in four tissues of the four transgenic mouse lines.

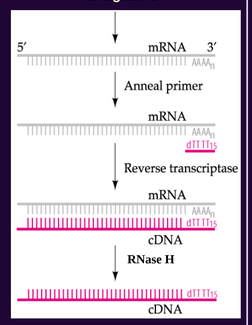
The expression of the actual human blood-type A antigen is tested through immunohistochemistry staining.

Methods

RNA Isolation – mouse tissues (liver, heart, spleen, testes) are collected and homogenized in TriZol solution. Chloroform is used to separate the phases, and RNA is precipitated from the aqueous phase using isopropanol. After centrifugation, the RNA, now visible as a pellet, is washed with ethanol and re-suspended in water.

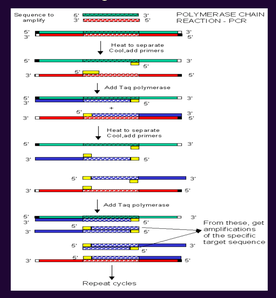
After the **RNA is treated with DNase** (an enzyme that removes DNA contamination), **complementary DNA** (cDNA) is synthesized. The transcribed RNA (mRNA) is the target; oligo-dT primers are used to bind specifically to the poly-A tail found only in mRNA. The enzyme **reverse transcriptase** uses this primer to start construction of the cDNA by incorporating deoxynucleotide triphosphates (dNTPs) complementary to the mRNA template.

Diagram 3



Methods (continued)

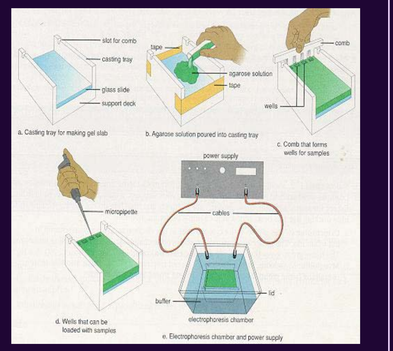
Diagram 4



The **Polymerase Chain Reaction** (PCR) amplifies the amount of DNA to increase its utility and visibility. The cDNA (water is the negative control while a plasmid containing the target genes is the positive control) is added to gene specific primers, dNTPs, a reaction buffer, and Taq DNA polymerase, and is then heated in cycles of varying temperatures. At 94°C, the cDNA is denatured; at 56°C, the primers bind to the single-stranded cDNA; at 72°C, the DNA polymerase synthesizes the complementary strand of DNA. With each cycle, the amount of DNA is doubled.

Gel electrophoresis examines the PCR products of the A- and H-genes. A gel made using 1.5% agarose and Tris-acetate-EDTA buffer separates the DNA of interest by molecular size. Added ethidium bromide binds to DNA and fluoresces under ultraviolet light so that the DNA in the gel can be visualized. The PCR products are loaded into wells in the gel and a voltage is applied in the electrophoresis chamber. After the samples travel a sufficient distance, the gel is placed under UV light and photographed. Bands appear if the transgenes are expressed.

Diagram 5

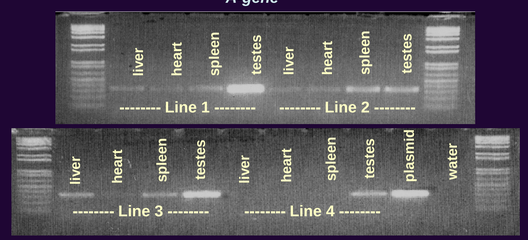


For **immunohistochemistry staining**, thin, frozen sections of transgenic mouse tissues are labelled with lectin from *Helix pomatia,* an enzyme-conjugated protein, that agglutinates with human A antigen. A light brown colour on the sections indicates the presence of the A antigen.

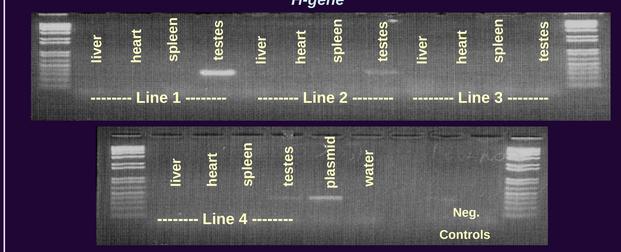
Results

Gel electrophoresis:

A-gene



H-gene



The presence of banks along each column of the gel denote that the gene is expressed in the tissue of that transgenic mouse line. The brighter the band, the stronger the gene expression.

Results (continued)

Expression Pattern in Gel Electrophoresis of A and H-genes in 4 Tissues of 4 Transgenic Mouse Lines

Transgenic Mouse Line	DNA Type											
	Liver		Heart		Spleen		Testes		Plasmid (positive) Control		Water (negative) Control	
	A	*H*	*A*	*H*	*A*	*H*	*A*	*H*	*A*	*H*	*A*	*H*
1	+	-	+	-	+	-	+++	+++	+++	++	-	-
2	+	-	+	-	++	-	++	+				
3	++	-	+	-	++	-	+++	-				
4	-*	-	-*	-	++	-	+++	+				

Legend: - denotes no expression; + denotes weak expression; ++ denotes average expression; +++ denotes strong expression

From the gel results, it is clear that the A-gene is expressed in all the tissues of all four lines (*the lack of expression for line 4 liver and heart was likely due to human error; it has been shown in repeated tests that the A-gene is indeed expressed in all four tissues of line 4). However, the H-gene seems to be expressed only in the testes of three of the transgenic mouse lines.

Immunohistochemistry staining:

Several different types of mouse tissues were obtained for immunochemistry staining; a negative non-transgenic mouse control was tested in addition to a line of the transgenic mouse. Yellow-brown agglutination from the lectin binding to the A antigen signifies that the A antigen is present.

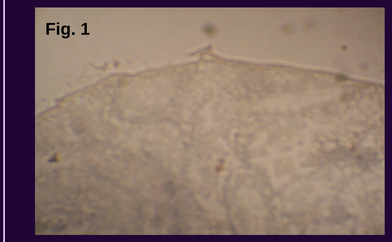


Fig. 1, taken from the testes of non-transgenic mouse control, shows no agglutination – the A antigen is absent in the tissue.

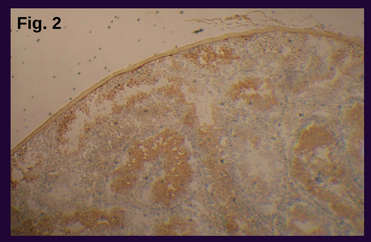


Fig. 2, taken from the testes of a line of transgenic mouse, shows agglutination – the A antigen is present in the tissue.

However, all the other tissues of the transgenic mouse tested negative (no agglutination) for the A antigen. This is consistent with the gel electrophoresis results, in which only the testes expressed both A and H-genes. Theoretically, both genes are necessary for A antigen synthesis.

Summary and Future Directions

The outcomes of these experiments, ranging from molecular biology to immunology, gives an evaluation of the double transgenic mice. The A-gene is expressed in all four tissues of the four transgenic mouse lines. However, the lack of expression of the H transgene in all but one tissue type of three lines signifies that it was not successfully transcribed. The detection of the A antigen only in the testes of a line of the transgenic mice using immunohistochemistry staining indicates the need for the presence of both transgenes for A antigen production, as the testes is the only tissue in which A and H-genes are expressed. This evidence abides by the theoretical model of biosynthesis of A antigen in mice. However, it is not sufficient for the A antigen to be present only in one organ of the mice for the purposes of establishing a mouse model for ABO-incompatibility organ transplantation.

Based on these findings, it is therefore necessary to utilize an alternative strategy to obtain H-gene expression in order to perfect an animal model for ABO-incompatible organ transplants. Currently, the most plausible idea is to import previously characterized H-gene transgenic mice and breed them with the transgenic mice we developed. The offspring should express both genes strongly, in all organs. Thus the mouse model would be complete.

The next step in this project would be to determine the mechanism by which ABO-incompatible organ transplant tolerance could be achieved. The implications of this accomplishment in the future will be momentous, as many more lives will be saved.

Acknowledgements

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Graphic References

Diagram 1 is courtesy of Xiaohu Fan; Diagram 2 is courtesy of http://www.biochem.arizona.edu/classes/bioc471/pages/Lecture19/Lecture19.html; Diagram 3 is courtesy of http://8e.devbio.com/images/ch04/0405fig2.jpg; Diagram 4 is courtesy of http://employees.csbsju.edu/hjakubowski/classes/ch331/dna/oldnalanguage.html; Diagram 5 is courtesy of http://a32.lehman.cuny.edu/molbio_course/agarose1.htm