Determining Significant Physiologic Incompatibilities

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Physiological incompatibilities could be major obstacles for clinical xenotransplantation. These metabolic variations can be regarded as adaptations of a species to its environment in the course of evolution. These developments are totally irregular and, therefore, differ from species to species, and thus from protein to protein, receptor to receptor, or enzyme to enzyme.

Molecular Incompatibilities in Discordant Models

Cells, tissues and organs function through highly specific and, in most cases, species-specific, regulatory processes. In discordant xenogeneic transplants, the function of each grafted cell depends on the extracellular signals or stimulatory commands of hormones, mediators or enzymes, which have to be cleaved from prohormones or precursors by the specific secondary mediator. These molecules need their species-specific carriers, such as albumin. Albumin varies from species to species.[1] Porcine albumin, for example, differs by a factor of 35 from the human molecule, and might therefore not be able to act as a carrier for human substrates (Table 1). Intracellular mechanisms interact as long as they are undisturbed by extracellular incompatibilities. Only few extracellular molecules respond perfectly with the foreign receptor. In most cases, they are completely inactive or induce pathological function. Where there might be full divergency of several reactants, the question arises whether the remaining products are redundant or are sufficiently overlapping to bridge the gaps provided by the absent mediators. The products of cells must be compatible with their receptors or transmitters. Interaction between the two species may even be unable to stop the process. Substrate has to be compatible with enzyme, hormone with its carrier and transmitter. The simpler the functions, the fewer regulator proteins needed, and the fewer foreign products synthesized or released, then the more likely is the outcome to be satisfactory. Some examples of potential problems will be briefly discussed.

Organ-Specific Differences

Differences between species may suggest adequate or inadequate function after xenotransplantation (Table 2). An optimal clinical situation exists, for example, for insulin and the glucose plasma level. Both are almost identical between pig and human. Therefore, cross-species regulation is likely to result in neither hyper- or hypoglycemia, nor in hyper- or hypoinsulinemia. All four major regulators of the glucose level—insulin, glucagon, adrenaline (epinephrine), and growth hormone—interact with their individual foreign receptors expressed on the islets, and guarantee normal function.

The pig heart, if size adapted, would be ideal for humans in regard to its cardiac output (Table 3) and general anatomy, despite some anatomical discrepancies concerning the valves. However, after porcine-to-human organ transplantation, human growth hormone will continue to fulfill its physiologic task, but it will not be able to downregulate in a physiologic fashion.[2] The inability of the porcine inhibitor to stop the function of human growth hormone will lead to porcine-specific proliferation of the transplanted pig organ. The resulting giant organ might not be compatible with a normal life.

Kidney regulation of fluid, electrolyte and acid-base levels, including erythropoiesis, shows

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Table 1 | **Rates of Albumin Evolution (Index of Dissimilarity, I:D:). Albumin Shows a Constant Change During Evolution**

Primate Species	ID	Canine Species	ID
Man	1.00	Dog	1.00
Gorilla	1.09	Coyote	1.06
Chimpanzee	1.14	Wolf	1.16
Oranguatan	1.22	Jackal	1.18
Baboon	1.22	Fox	1.20
Capuchin monkey	5.00		
Tupaia	11.00		
Cattle	32.00		
Pig	>35.00		

Table 2 | **Compatibility of Physiology of Organs Between Human and Pig**

	The Good	The Bad	The Ugly
Organ	Islets Heart	Kidney	Liver
Molecules	Compatible	Neutral	Incompatible
Function	Specific	No	Non-specific
Example	Isulin	Steroids Erythropoietin	Growth-Hormone Complement
Substrate	Sugar	Albumin	Cholesterol

Table 3 | **Comparison of Blood Flow (as % of Cardiac Output) Between Pig and Human**

Organ	Pig	Human
Heart	4.5	4.3
Brain	5.1	12.9
Gastrointestinal tract	18.4	20.0
Liver	26.3	21.3
Kidney	17.0	18.9
Skin	5.0	8.6

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some incompatibility in pig-to-primate models. While most electrolytes are kept within physiologic boundaries, phosphate is excreted by transgenic pig kidneys to almost undetectable levels in cynomolgus monkeys.[3] Erythropoietin has been found to be incompatible between pigs and primates. Replacement therapy in primates has had to be achieved with recombinant erythropoietin.

Each mammalian liver is able to produce more than 2500 enzymes and other proteins, including 90% of the albumin produced in the body and 95% of the complement factors. Most of these enzymes and proteins are species- and substrate-specific. Some molecules act completely differently during embryonic or fetal life than in the adult state. Due to the lack of species-specific regulator proteins, porcine complement would first lead to hemolysis of the recipient red blood cells and later to the lysis of other cells.

Some hormones, like growth hormone and gastrin,[4] are cleaved by the native liver but not by the xenografted organ. They will, therefore, circulate for a prolonged period and will maintain their function. Other molecules, and possibly some drugs, are treated differently. For example, the porcine liver metabolizes ethanol faster than its human counterpart.[5]

Vascular and Circulatory Discrepancies

Hyperacute rejection has an immunological basis, but is strongly influenced by mediators, such as prostaglandins, interleukins and adhesion molecules.[6] These vasoactive substances probably account for vasoconstriction and the cessation of blood flow. Interleukins differ greatly even between species of the same zoological family. While their detection is possible in nonspecific bioassays, the same molecule cannot be recognized by ELISAs using a highly species-specific monoclonal antibody. Beyond the taxonomic level of the zoological order, both the function and the antigenicity of interleukins are in most cases incompatible. Proteins which differ in their amino acid sequences more than 20% are usually highly antigenic, and induce secondary

antibodies that eliminate the circulating antigen before it has had the possibility of providing its action.

The way in which signals in the form of hormones, transmitters, enzymes, and growth factors are translated in a xenogeneic system has not received adequate attention. Central questions remain to be answered. How many xenogeneic hormonal and enzymatic interactions are possible between pig and human? Are they able to maintain metabolism in the foreign organ or body, and for how long? In addition, transport molecules, the most important of which is albumin, must be considered when trying to achieve long-term survival of organs from widely-divergent species.

Large quantities of foreign proteins, mediators and enzymes are released into the circulation when big organs like the liver deteriorate. The function of these liberated foreign mediators is far from being understood or even investigated. New data indicate that adhesion molecules, together with interleukins and ecosanoids, play a major role in disturbance of the microcirculation. If falsely activated, these adhesion molecules may be unable to protect endothelial cells from cellular attack and activation of blood clotting mechanisms.

Transgenic porcine organs allow prolonged function, but their products, their regulation, and their function rely on perfect interaction in this multifactorial "orchestra". About 180 million years of evolution have to be outwitted in order to create a situation in which a divergent porcine xenograft functions optimally in man.

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