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Gen therapy for diabetes type 1

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What is gene therapy?

Gene therapy is a technique for correcting defective genes responsible for disease development. The most common approach for correcting faulty genes is to insert a “normal” gene into the genome to replace an “abnormal” disease-causing gene.

OBJECTIVES : The aim of this work is know the general appropriate modifications of viral vector to lead correctly the genetic material to interest tissue and describe the AAV virus to understand how it has been used in experiments to cure Diabetes mellitus type 1.

Type 1 Diabetis Mellitus

Diabetes Mellitus type 1 is a disease caused by an autoimmune destruction of the insulin-producing ß-cells of the islets of Langerhans in the pancreas resulting in absolute insulin deficiency. Disease causes a chronically raised blood glucose concentration, which can be causes subsequent complications.

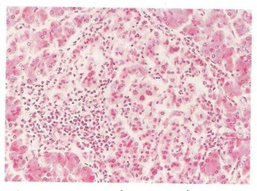


Figure 1. Insulinitis. Chronic inflammatory cell infiltrate [3]

Although the causes of *type 1* diabetes are not fully understood, several environmental factors such as viral infection may induce the β-cell–destructive immune response in genetically susceptible hosts.
Islet-resident APC (DC or macrophages) shifts its phenotype into activators of an inflammatory response, and they migrate from islet environment toward peripheral lymphoid organs.
Once there, APC eventually encounter the autoreactive T cells.

Genetic susceptibility to *type 1* diabetes is most closely associated with HLA genes. Clas II HLAs (HLA-D) play a role in presenting foreign and self-antigens to T-helpers lymphocytes and therefore in initiating the autoimmune process. [3]

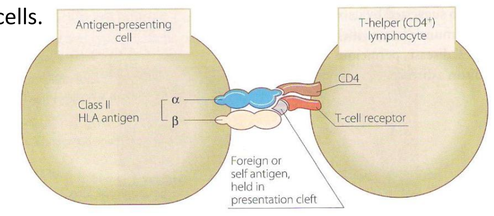


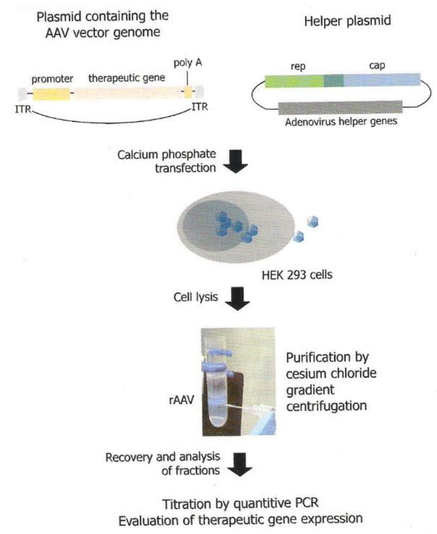
Figure 2. Antigen associated with class II HLA is presented to T cell. [3]

Adeno-associated virus

(AAVs) are small 4.7 kb near single-stranded (ss) DNA Dependoviruses that belong to the Parvoviridae family. [1]

Advantages of use AAV as a vector :

- Lack of any associated human disease.
- Broad host range.
- Ability to infect growth-arrested cells.
- Ability to carry non viral regulatory sequences without interference from the viral genome.
- No superinfection immunity associated.



The efficiency of AAV infection has been improved through the use of certain serotypes of AAV that have different tropisms in vivo.

Figure 3. Production of AAV vectors. The two ITRs are the only AAV sequences preserved in the vectors, while transcriptional cassette (promoter + gene + polyadenylation site) substitutes the rest of the genome. [1]

Gene therapy treatments for type 1 diabetes

PREVENTIVE

Preventive gene therapy is based on exploit immunoregulatory networks to promote hyporesponsiveness of autoaggressive immune cells as a viable means of improving or restoring normoglycemia.

ADJUNTIVE

Adjuntive gene therapies are based on reducing immune reactions or inducing immune tolerance in transplantation.

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CURATIVE

Patients with an advanced stage of Diabetes *type 1* cannot be restore normal insulin level production by preventive gene therapy, due to the high destruction of β-cell. Recovery from type 1 diabetes requires β -cell generation.
One approach to do so is by genetically engineering the endocrine pancreas in vivo to express factors that induce ß-cell replication and neogenesis and counteract the immune response.

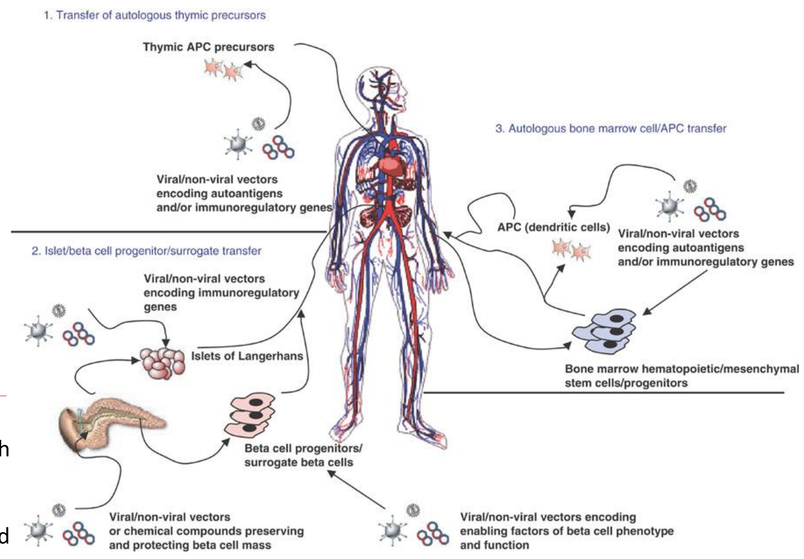

Figure 4. Gene and cell therapy strategies for type 1 diabetes. A number of strategies can be employed alone or in combination. Extracted from Gene- and cell-based therapeutics for type I diabetes mellitus. Gene therapy,2003;10.

Insulin and Glucokinase gene transfer to skeletal muscle corrects diabetes in dogs

This approach, demonstrates that it is possible to generate a “glucose sensor” in skeletal muscle through coexpression of **glucokinase** and **insulin**. The genes were one-time intramuscular administration in diabetic dogs through adeno-associated viral vectors of serotype 1.

The liver enzyme **glucokinase**, compared to muscle glucokinase II in the skeletal muscle is not inhibited by glucose-6-phosphate, and shows kinetic cooperation with glucose. When expressed in skeletal muscle of transgenic mice, glucokinase remains active in the cytosol and facilitates glucose uptake only when blood glucose is high. However, during diabetes, constant basal levels of **insulin** are required to ensure the presence of GLUT4 on the cell membrane.

Therefore, the regulation of glycemia was achieved by coexpression in skeletal muscle of glucokinase and low levels of insulin. [2]

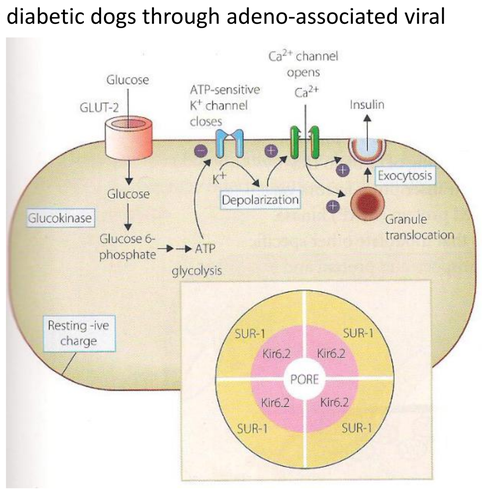

Figure 5. The mechanism of glucose-stimulated insulin secretion from the β-cell. [3]

Five diabetic dogs were treated with one of the following:

A. Insulin, administered twice daily. (Fig .6)
B. Intramuscular injection of the glucokinase gene alone (AAV1-Gck). (Fig.7)
C. Intramuscular injection of the insulin gene alone (AAV1-Ins) (Fig.8)
D. Intramuscular injection of both the insulin and glucokinase genes(AAV1-Ins/GcK).(Fig 9)

RESULTS

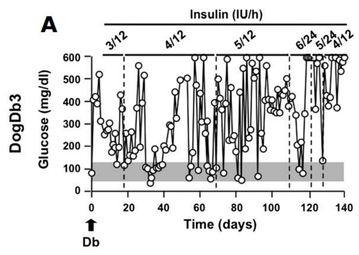

Figure 6. The dogs on **twice daily insulin** were better controlled but remained hyperglycemic. [2]

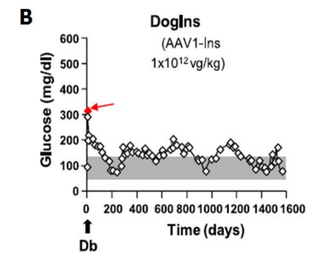

Figure 7. Expression of insulin alone. The ability of dispose glucose was only moderately improved. [2]

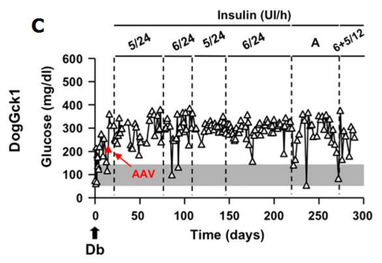

Figure 8. **Expression of glucokinase alone** caused hyperglycemia in overload glucose conditions. [2]

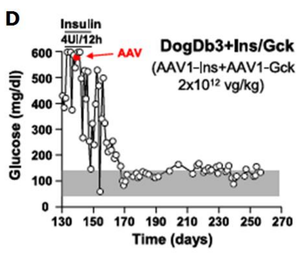

Figure 9. **Expression of glucokinase and insulin.** dogs quickly regained normoglicemia. [2]

CONCLUSIONS : In reference to the cure of type 1 diabetes, the authors demonstrate for the first time in a large animal model that this gene therapy approach has a beneficial therapeutic effect for up to 4 years. This is a major advance in the field of gene therapy for DM. rAAV-based gene transfer vectors represent one of the most promising gene therapy systems and gain increasing popularity.

BIBLIOGRAPHY : [1] Giacca M. “Methods for Gene Delibery”. In: Giacca M.*Gene therapy*. 1st ed.Springer;2010, pg. 47-137.
[2] Bosch F, Callejas D, Mann J, et al. Treatment of diabetes and long-term survival after insulin and glucokinase gene therapy.Diabetes,2003;62.
[3] Williams G, Pickup J. *Handbook of Diabetes*. 3rd ed. Blackwell Publishing; 2004.