

AMGP "M.U.G."

"Monthly Update in Genetics"

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Edited by Matt Taylor, MD, Director Adult Clinical Genetics

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Introduction to Gene Therapy:

One of the promises of the genetic/genomics revolution has been that ultimately **gene therapy** will provide the solution to many (some say all) our medical woes. Running on essentially enthusiasm alone this belief has held true for decades, in spite of the relatively disappointing results to date in this field. To be sure there are a host of practical problems associated with directly correcting mutations at the level of the genome, including:

- **Selection of a suitable vector for delivery**
- **Targeting the gene therapy to the appropriate cells**
- **Avoiding insertional mutagenesis**
- **Regulation of the inserted vector (avoid over/under expression)**
- **Sustained expression of the desired gene product or effective suppression of the host's mutated gene(s)**
- **Avoidance of germline integration**

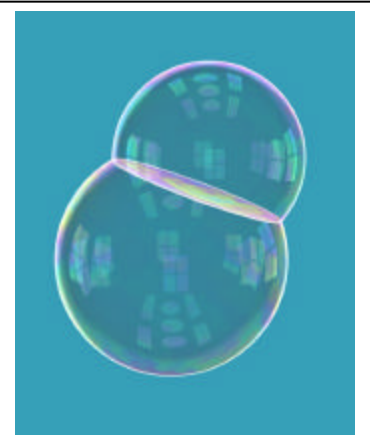
This month I focus briefly a report highlighting a recent success in an animal model of a human disease. Tempering this encouraging data are concerns raised by the finding of leukemic disease in one of 11 boys treated with gene therapy in France.

CONTENTS

FOCUS GENE Rx

Gene Rx for Mucopolysaccharidosis Type VII

Complication of Gene Rx for SCI D..."Time for the boy to get back in the bubble?"



Insertional Mutagenesis:

The creation of new potentially deleterious mutations due to the insertion of a gene-therapy vector INTO an existing gene in the host genome.

PRACTICE SAFE GENETICS: The intent of this newsletter is to increase your familiarity with clinical genetics. Genetic evaluations and testing raise many issues and are often most appropriately addressed in a genetics clinic setting. Please feel free to contact me with any clinical questions that arise as you care for your patients.

matthew.taylor@uchsc.edu

MUCOPOLYSACCHARIDOSES (MPS) AND GENE THERAPY:

The **MPS** are a collection of lysosomal storage diseases caused reduced activity of a collection of enzymes integral to the degradation of mucopolysaccharides. Mucopolysaccharides (glycosaminoglycans) are extracellular proteins secreted by cells that contribute to the makeup of connective tissue (including cartilage and vessel walls). They are complicated compounds comprised of long sugar chains bound to specific core proteins within complex macromolecules called proteoglycans. The degradation of these protein complexes involves a number of sequential breakdown reactions, and occurs largely in the lysosomes.

The **MPS** diseases are a group of recessive (mostly autosomal) diseases that present with physical features (coarse facies, short stature), skeletal anomalies, mental retardation, and often a reduced life span. The overall frequency is estimated to be 1:27,000 live births and **Hurler (MPS I)** and **Hunter (MPS II, X-linked)** are the two most striking in phenotype; and were likely last presented to you in medical school. A tragedy of these diseases is that the diagnosis may not be made until an age of 2-5 years (even in some of the more severe cases) allowing sufficient time for the birth of another affected sibling.

MPS VII (Sly Syndrome) is due to deficient **B-glucuronidase** activity and presents with retarded growth, bony disease, facial dysmorphisms, hepatosplenomegaly, cardiac valvular disease, and mental retardation. Ponder's group [Ponder et al. PNAS 2002;99:13102-107] studied **MPS VII** dogs (pictured on previous page) and developed a gene transfer technique to correct the **B-glu** deficiency.

As an aside, you may wonder why **Sickle Cell Anemia** has not yet been "cured" by gene therapy, as the underlying genetic defect has been known for years. Diseases where a mutation causes a **novel function** of a protein (e.g. polymerization under low oxygen tension) are inherently much harder to target by gene therapy, as you must **suppress/eliminate the expression/function of the mutated protein**. Autosomal recessive, enzyme deficiencies (like the **MPSs**) **only** require that you **replace** the deficient enzyme, and replacement of missing function is much simpler than removal of aberrant function. The **overly simple** analogy: it is much easier to replace a **missing egg**, than it is to clean up the mess made by a **broken egg**.

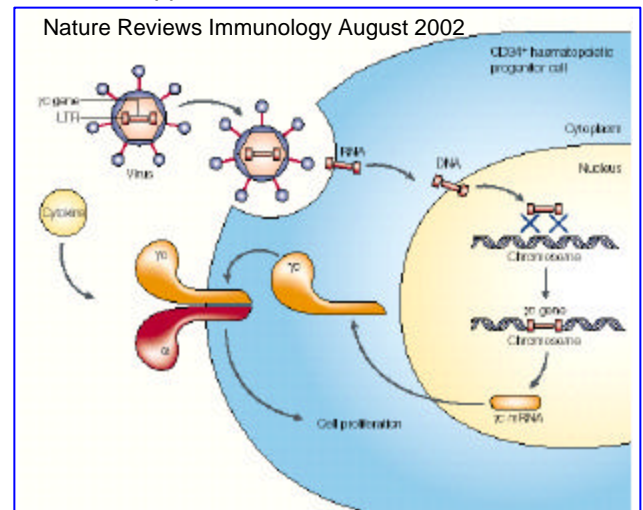
In short (e.g. to avoid the molecular biology details) this group used a retroviral vector containing a replacement **B-glu** gene. At age 2-3 days of life seven dogs were given the gene-therapy drug; comparisons with control dogs were done. The figure on the previous page shows two dogs; the one standing is 17-months old and received gene-therapy at birth. This dog has nearly fully mobility (less bony disease) and has very few signs of the **MPS VII** phenotype. Contrast that with the 5-month old untreated dog that can no longer walk. There is evidence of continued **B-**

glu expression after 14 months and the sperm analysis (in only two animals) has not shown evidence of germline integration.

SEVERE COMBINED IMMUNODEFICIENCY (SCID) AND GENE THERAPY:

As with **MPS**, **SCID** is not a single disorder, but rather a group of diseases characterized by absent (or blocked) T-Cell development. Devoid of a cellular immune response, patients are vulnerable to recurrent and life-threatening infections. In 1990 French Anderson and others replace the **adenosine deaminase** gene in a group of SCID patients. Definite, but transitory expression of the recombinant gene failed to provide long-term immune system reconstitution.

Ten years later Cavazzana-Calvo et al [Science 2000;288:669-72] reported a more sustained success in two infants with **X-linked SCID**. As the gene therapy expression was long-term and improved the clinical status this achievement has been hailed as the first unequivocal proof-of-principal in humans that gene-therapy has a "curative" role in human disease. The figure below (from an excellent review article) outlines the approach to treatment of **X-linked SCID**.



The celebration of this milestone was dampened this summer with a report that one of eleven patients in a **SCID** protocol had developed a leukemia-like process resulting from an expanded T-cell clone. The T-cell clone has been proven to contain the gene-therapy transgene raising the question of whether the leukemic process was caused by **insertional mutagenesis**. Whether **insertional mutagenesis** was responsible for this adverse event remains to be proven, but the implications to future studies are considerable.

At the **American Society of Human Genetics Meeting** (Oct 2002) data was presented on a human Phase I trial to correct hemophilia with Gene Therapy. While not yet commonplace in clinical care we can expect to learn much from the incremental successes and setbacks from these ongoing endeavors which are crossing the scientific lines from fantasy to reality.