Musings

Musings on genome medicine: gene therapy

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Abstract

Though the field has moved with glacial speed, gene therapies have been carried out successfully in patients with bone marrow disorders including immune deficiencies. The field may be poised to move forward more rapidly, but many barriers have yet to be surmounted.

Discoveries of restriction enzymes by Hamilton Smith and Daniel Nathans [1], and reverse transcriptase by Howard Temin [2] and David Baltimore [3] in the 1970s set the stage for an explosion in molecular and clinical genetics. Armed with a sufficient amount of the mRNA product of a gene such as the beta globin gene, investigators could now produce a radioactive DNA copy (cDNA) of the mRNA and use it as a valuable probe of gene expression. Moreover, the globin cDNA was functional. Cloned into an expression plasmid [4] and transduced into an appropriate cell, the cDNA would produce mature beta globin mRNA, and cDNAs could be manipulated at will in what became the era of recombinant DNA technology.

The new discoveries were greeted with enthusiasm by most biologists and clinical investigators but with mounting horror and suspicion by many members of the public and their elected officials, as well as some academics. Frankensteins were thought to be loose in biomedical laboratories; monsters would be created; plagues of vicious E. coli would be loosed on an innocent population; mad scientists would forever contaminate the food supply; the new genetics would lead to a resurgence of social Darwinism. The specter of Nazi medicine roiled some faculty meetings and communities in which the science was rapidly advancing. Rules and restrictions were demanded that would deliberately inhibit the work. To their credit, leaders of this new genetics revolution met in Asilomar California [5], where they established laboratory standards intended to reassure themselves, their colleagues, and the general population that gene manipulation could be rendered safe and useful.

While eager clinical investigators hoped to apply the new genetics in the treatment of inherited diseases, cooler heads recognized that the technology was not sufficiently powerful or predictable. The more cautious advised the National Institutes of Health to be very wary of human application because the biological 'rules of the game' had not yet been established. That did not stop an American clinical entrepreneur from injecting beta globin cDNA into the bone marrows of thalassemic patients in Italy and Israel. His reward was failure and opprobrium [6], and academic medicine encountered a Congress increasingly determined to surround clinical research with an ever-growing net of regulation.

Following the unfortunate thalassemia contretemps, scientists focused on a fundamental problem. How could a cDNA be introduced efficiently into a rare, quiescent, mammalian cell, such as a bone marrow stem cell, remain potentially active, and be sufficiently expressed when that stem cell developed into a hematopoietic precursor and subsequently fully differentiated functional blood cell? Furthermore, could a defective gene be actually replaced in human cells in a targeted fashion by a normal counterpart and still maintain high transcription efficiency [7], or would such 'plug and socket' technology be so inefficient that correction would be impossible? Instead, could cDNAs such as a beta globin cDNA be carried into the target cell chromosomes on the back of a virus, such as a retrovirus, and could the transduced sequence express its mature mRNA regardless of its genomic location?

The idea of a retrovirus as a gene-transfer agent was first seen as dangerously oncogenic, until 1983 and 1984 when Mann, Mulligan and Baltimore [8] devised cell lines that would produce replication-defective retroviruses that still exploited the capacity of the viruses to incorporate themselves efficiently in the DNA of dividing cells. For the most part, the modified retroviral vectors infected human cells at comfortingly low multiplicities of infection.

Shortly after this, Williams and Mulligan [9] and Dick and Bernstein [10], and their colleagues, showed that murine bone marrow cells could be transduced with defective retroviruses carrying cDNAs, and that mature nucleated blood cells would carry the foreign cDNA for weeks, proving that the murine hematopoietic stem cell, despite its very low rate of division, could be so transduced. But the percentage of infected cells was very low and expression of the transferred gene was vestigial. The results suggested that successful gene transfer with cDNAs borne on replication-defective retroviruses would require high recombinant viral titer, cell culture systems that would encourage stem cell division without differentiation, and a setting in which target cells would have a selective advantage following gene transfer. The latter requirement proved to be an Achilles heel.

The entire field of gene therapy was energized by the findings of Williams and Dick and their colleagues [9,10], but the barriers to translation and clinical application were soon found to be almost insurmountable. The low level of gene transfer and expression was extremely discouraging. The slow pace prompted Orkin and Motulsky [11] to lower their expectations for immediate clinical application, and focus instead on solving the basic technical and biological problems. In an attempt to gain some clinical traction, Blaese and his colleagues [12] introduced retroviral cDNA *ex vivo* into the mature T cells of patients with immunodeficiency due to mutations in adenosine deaminase. They used a modified retrovirus bearing a normal adenosine deaminase cDNA. The treatment provided little if any clinical benefit, and the risk of malignancy was obviously high because the T cells were influenced to divide in culture in order to enhance transduction. The approach was soon abandoned.

Concerns about unwieldy and potentially unsafe clinical research protocols began to mount in the United States. Four levels of review, the Recombinant DNA Advisory Committee, individual institutional review boards, individual institutional biosafety committees, and the Food and Drug Administration all established barriers that slowed the pace of gene therapy clinical research to a crawl. This necessary regulatory environment was onerous enough, but it became even more obstructive when investigators at the University of Pennsylvania performed a study of gene replacement in a rare metabolic disorder using adenovirus as a vector in order to infect non-dividing liver cells. One young adult with the disease died after a high titer of virus was administered [13]. An investigation revealed that the gene therapists had a financial stake in the company that produced the vector. That revelation initiated an even higher burden of regulation and added massively to a growing concern about conflict of interest in clinical research - a conflict that continues to roil academic waters to this day.

Meanwhile, after four decades of development, the clinical application of allogeneic hematopoietic stem cells in transplantation for the treatment of congenital bone marrow diseases was moving ahead reasonably briskly (sans gigantic regulatory hurdles). This form of cell-based therapy, though initially applicable in only the 25-30% of patients with histocompatible donors, was associated with success in several patients with severe immunodeficiencies, congenital bone marrow failures of several types and even the inherited hemoglobin disorders [14]. Increasing success was also being reported with matched unrelated stem cell donors [15]. Clearly, gene therapy application lagged well behind hematopoietic stem cell treatment.

After the unfortunate event in Philadelphia, the field moldered along. Adenovirus vectors were thought to be too immunogenic to be useful but they and their cousin, the tiny adeno-associated virus, were considered to be worthy of evaluation in the transduction of non-dividing cells such as liver cells or endothelial cells. Indeed, High and her co-workers [16] have made quiet progress in the correction of canine hemophilia with adeno-associated virus. But immune responses to adeno-associated virus remain a problem. Most groups interested in hematopoietic targets or cancer vaccine protocols continue to focus on defective murine leukemia viruses or lentiviruses. The latter are thought to have a higher capacity than murine leukemia viruses to integrate within the DNA of non-dividing cells. The differences may be less striking than originally believed.

From 1985 to 2002 there was little or no progress in the United States and elsewhere. Bright young clinical investigators were gently urged to direct their energies elsewhere. Both the science and the regulatory apparatus seemed to be daunting, and the funding was fragmented and difficult to obtain. But in 2002 Cavazzana-Calvo and his colleagues [17] blew new life into the field when they made the startling announcement that they had successfully treated nine of ten patients with X-linked severe combined immunodeficiency (SCID), utilizing a fairly standard murine leukemia viral vector that carried the common gamma chain of the interleukin-2 receptor into autologous bone marrow cells. Thrasher and his colleagues [18] subsequently confirmed these results in another study. Shortly thereafter, however, cynicism returned when the authors reported that several of the patients had developed T cell leukemia. Careful work by the investigators revealed that the long

terminal repeat (LTR) of the vector may have a predilection for the LMO2 proto-oncogene on chromosome 11 [19,20]. But even random integration of the LTR at the LMO2 site would favor selection of such cells. The LMO2 proto-oncogene is often activated by translocation (11:14) in T cell leukemia. Clearly, the gene-corrected immunocytes had a survival advantage, but the malignant T cells had an even greater survival advantage as well as a growth advantage. What was once a promising new start for gene therapy became an enormous set-back. Vector safety had always been a pressing issue - now it had become a yawning chasm: of 20 patients with X-linked SCID treated by gene transfer in these two trials, 18 are currently alive and with good immune reconstitution, but five have experienced a serious side-effect. Of these five children, one died of therapy-related leukemia, and one died of complications of a subsequent stem cell transplant that was performed as a result of the failure of gene therapy. The others are in remission.

Of further concern have been the results of retroviral correction of oxidase deficiency in chronic granulomatous disease. In two well-described cases, correction of granulocyte oxidase deficiency has been achieved but at the cost of clonal proliferation of cells activated at the sites of the MDS-1-EVI1, PRDM16 or SETBP1 proto-oncogenes [21]. This result represents a clear leukemogenic hazard. Furthermore, other *in vitro* studies have demonstrated similar insertions by lentiviruses bearing beta globin genes. Finally, Williams and his co-workers [22] have temporarily discontinued their pioneering work on the correction of the deficiency of DNA repair pathways in hematopoietic stem cells of patients with Fanconi anemia, because they are concerned that current vectors that permit rescue of those cells may induce insertional mutagenesis and leukemia in the treated patients, and that focus should be on developing methods of expanding deficient hematopoietic stem cells in this disease.

Despite these serious setbacks there have been some recent bright lights. In 2008, Maguire [23] and Bainbridge [24] and their colleagues reported progress on the treatment of Leber's optic atrophy with an adeno-associated viral vector applied to the retina. And retrovirally transfected epidermal stem cells have been grown into keratinocytes to correct the lesions of epidermolysis bullosum [25]. More follow-up is needed but the initial results hold promise for local applications of gene transfer. An encouraging report emerged at a recent annual meeting of the European Society of Gene and Cell Therapy. Lentivirus vectors have been used to transduce hematopoietic stem cells in X-linked adrenoleukodystrophy, a progressive demyelinating disease that causes severe debilitation by early teenage years. Long-term and stable gene modification has been observed in 20% of myeloid cells, well within the range to reverse phenotypes in some red cell and myeloid disorders. Finally, Auiti and his colleagues have recently reported highly encouraging results in the treatment of adenosine deaminase deficiency [26,27].

Thus, gene therapy of hematopoietic diseases with retroviruses lumbers in choppy straits twixt the Scylla of insufficient gene transfer and the Charybdis of leukemia, while the therapy of metabolic and coagulation disorders with adeno- and adeno-associated viruses is blunted by immune reponses to the vectors. To committed gene therapists, these are simply the challenges that they have always faced, while those who are engaged in stem cell transplantation, or in finding better halfway measures that support afflicted patients, work as best they can, all the while hoping that the holy grail of gene replacement will one day become a safe reality. The future could lie in the promising field of site-specific gene correction using modified nucleases [28], and the conversion of corrected somatic cells such as fibroblasts to functioning hematopoietic stem cells [29]. Time will tell.

Abbreviations

cDNA, copy DNA; LTR, long terminal repeat; SCID, severe combined immunodeficiency.

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