To estimate health risk, the authors combine the probability maps with population data. Although the estimated population affected may be high owing to mitigation efforts or model false positives, such estimates can be valuable tools for prioritizing action. In Bangladesh, where arsenic poisoning is perhaps most acute, a random survey to establish nationwide trends in arsenic concentration, completed in 2000 (*8*), provided a foundation for impact estimates and more targeted studies. China is 67 times the size of Bangladesh, with 9 times the population, but arsenic occurrence is likely much less pervasive. Nationwide monitoring activities are under way (*2*, *6*), but risk maps could guide more targeted screening. Risk maps may also prompt private well-testing initiatives (*12*) or public pressure for mitigation in affected areas.

Historically, arsenic in groundwater has tended to be detected only after diagnosis of health effects (*1*, *5*). In areas where such effects have not yet been identified, predictive models will enable action before the appearance of symptoms, which can require decades of chronic exposure to manifest. The ability to predict contamination of water with arsenic and potentially other poisons is also important where groundwater is not currently used for drinking and agriculture but may be used in the future. In Bangladesh (*13*), widespread installation of wells began in the 1970s to mitigate the problem of microbial contamination of surface water. Arsenic was not widely recognized as a natural groundwater contaminant, and its concentrations were not measured when wells were installed. Understanding of the conditions that threaten the quality of water sources can help to better predict, identify, and prevent threats in areas where little is known about groundwater quality.

Recognition of the extent of the problem is, however, only the first step toward relief of health impacts. Arsenic concentrations can vary widely on the scale of tens of meters. These small-scale patterns cannot be predicted from surface features that vary on the scale of kilometers. This variability is the result of a complex set of hydrological, geological, and biogeochemical factors that affect release of arsenic from sediments and the movement of dissolved arsenic through aquifers. Understanding this complexity, along with social and economic factors, is critical for identifying appropriate mitigation options. In Bangladesh, despite decades of hydrogeochemical investigations and screening of about half of the existing wells (*14*), tens of millions of people continue to be exposed (*15*), illustrating the challenges in moving from identification to mitigation.

Many arsenic-affected basins, deltas, and floodplains are among the most populated in the world; humans have fundamentally changed these landscapes and altered their hydrology. Understanding the anthropogenic factors that affect natural hydrological and biogeochemical processes will be critical in predicting where arsenic and other problems exist, determining how they will evolve in the future, and implementing effective mitigation strategies to improve the health and livelihood of millions of people worldwide.

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MEDICINE

Gene Therapy That Works

Gene therapy trials show a beneficial effect in children suffering from a neurodegenerative disorder or an immunodeficiency disease.

Inder M. Verma

The concept of gene therapy is disarmingly simple: Introduce a healthy gene in a patient and its product should alleviate the defect caused by a faulty gene or slow the progression of disease (*1*). Why then, over the past three decades, have there been so few clinical successes in treating patients with this approach? A major obstacle has been the delivery of genes to the appropriate cell, tissue, and organ. How does one introduce a gene into the brain with trillions of cells, or the liver with billions of cells, or the rare hematopoietic adult stem cell that has the potential to populate all lineages of lymphoid and myeloid cells? Much effort has been devoted to finding ways to efficiently deliver a therapeutic gene to the desired cell type, resulting in sustained production of the gene product, ideally through the entire life of the recipient, without unwanted side effects like genotoxicity or unsettling the immune balance (*2*). On pages 864 and 865 in this issue, Biffi *et al*. (*3*) and Aiuti *et al*. (*4*) report encouraging results using lentivirus-mediated gene therapy to treat children with rare genetic defects.

For scientists in the field of gene therapy, good news, tinged with occasional setbacks, has been trickling in over the past decade, starting with the successful clinical trials of children with X-linked severe combined immunodeficiency disease (SCID) (*5*). Currently, more than 1700 clinical trials are under way worldwide, drawing on a wide array of gene therapy approaches for both acquired and inherited diseases (*6*). The approach involves genetically engineering a virus so that it infects a target cell to deliver a gene, but does not cause disease. Retroviruses (such as lentiviruses) integrate their genetic material, including the new gene, in to the host cell genome. Such transduced host cells are transplanted back into the patient and proliferate with the correct gene, producing healthy cells (see the figure). Biffi *et al*. and Aiuti *et al*. provide new hope to children with metachromatic leu-

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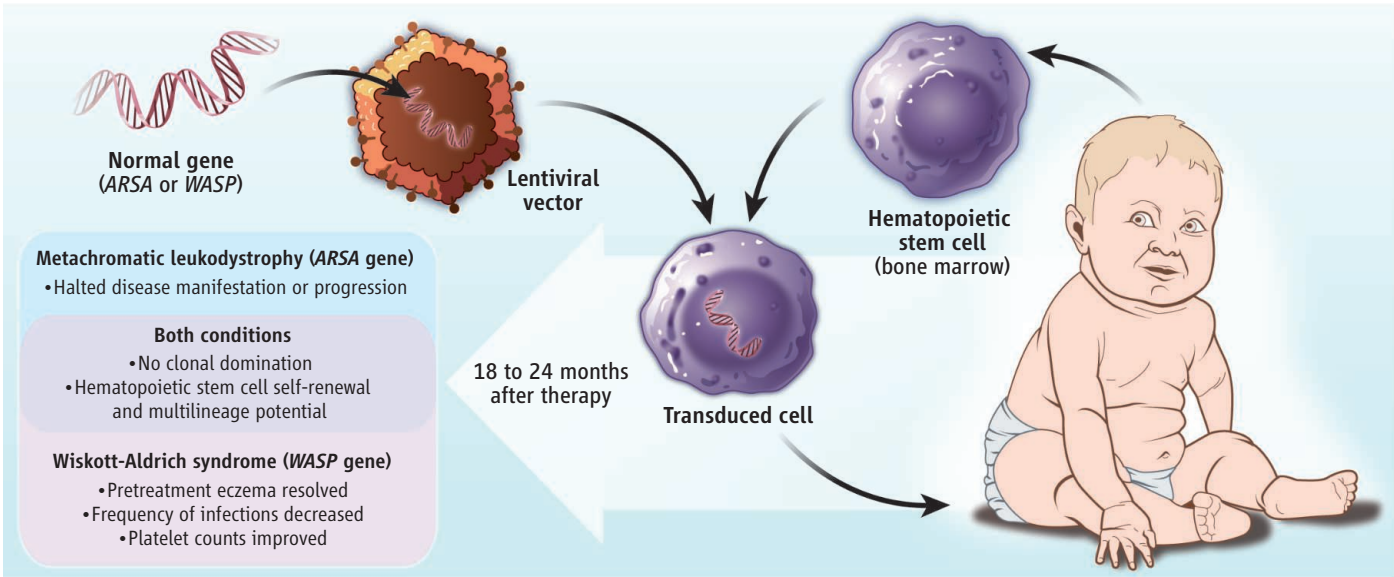

Clinical benefit. Children with the rare genetic diseases shown were treated with gene therapy. The approach delivered a normal gene, whose product halted or slowed gene progression up to 2 years after treatment.

kodystrophy (MLD) and Wiskott-Aldrich syndrome (WAS), respectively, both genetic defects that result in a deficiency of proteins essential for the early years of life and lack any effective treatments. MLD is an autosomal recessive lysosomal storage disease caused by mutations in the *ARSA* gene, leading to a deficiency of the enzyme arylsulfatase A, and hence the buildup of toxic sulfatide, causing widespread demyelination and neurodegeneration. Children with this disease appear healthy at birth, but gradually lose their cognitive and motor skills, with no possibility of arresting the neurodegenerative process. Children born with WAS, an X-linked primary immunodeficiency, lack WASP, a protein that regulates the cytoskeleton. Its loss leads to a faulty immune system that makes them vulnerable to the development of infections, autoimmune diseases, and cancer, as well as causing a defect in platelets that results in frequent bleeding. Bone marrow transplantations, when feasible, have proved to be a successful therapeutic approach for these two diseases (*7*). So, in 2010, clinical trials were initiated using lentiviral vectors to transfer functional *ARSA* and *WASP* genes in bone marrow–derived hematopoietic stem cells (expressing the marker $CD34^+$) from 16 patients, 6 of whom suffered from WAS and 10 from MLD. The studies of Biffi *et al*. and Aiuti *et al*. report results from three patients from each group, for whom sufficient time has passed since administration of the therapy to allow conclusions to be drawn regarding its safety and efficacy.

Biffi *et al*. found that in three presymptomatic children with late infantile MLD, treatment halted disease manifestation or progression for follow-up times ranging from 18 to 24 months, as compared to predicted disease onset in 7 to 21 months. Similarly, in three children with symptoms of WAS, Aiuti *et al*. showed that pretreatment eczema (chronic inflammation of the skin) resolved between 6 and 12 months; decreased, progressively, the frequency of infections; and improved platelet counts after gene therapy. In both clinical trials, no clonal domination was observed. Analysis of the vector insertion site in hematopoietic cells showed no preferential integrations in a particular gene locus, thus decreasing the likelihood of generating an abundance of abnormally proliferating cells. Increasing presence over time of $CD34^+$ progenitors and mature cells of myeloid and lymphoid lineages marked by identical integration sites of the delivered gene is strong evidence of self-renewal and multilineage potential of vector-transduced hematopoietic stem cells after engraftment. In this regard, the studies of Biffi *et al*. and Aiuti *et al*. are similar to those reported for two boys with X-linked adrenoleukodystrophy, a severe demyelinating disease caused by deficiency of an adenosine 5′-triphosphate transporter, where no clonal dominance was observed in lentiviral vector–transduced hematopoietic stem cells (*8*). In all three trials, partial bone marrow ablation of the patients was required to achieve maximum transplantation efficiency.

In the last 12 to 13 years, more than 50 patients affected by primary immunodeficiencies have been treated with genetically transduced autologous hematopoietic stem cells, mostly with gamma retroviral vectors (*9*). Most patients received clinical benefits, but occurrence of leukemia and myelodysplasia in some patients with SCID-X1, chronic granulomatous disease, and WAS have raised questions about their long-term safety. These adverse events have generally been ascribed to vector integration in the vicinity of specific proto-oncogenes, leading to their aberrant expression and resulting in neoplasias (*10*–*12*). The integration sites can be identified by deep sequencing of genomes, but because it is not possible to efficiently propagate clonal populations of hematopoietic stem cells, analysis can be performed only after transplantation. Although data from lentiviral-transduced hematopoietic stem cells in patients is limited, it appears not to favor any specific integration sites, though the appearance of a self-limiting dominant clone in the myeloid compartment of an individual with β(E)/β(0)-thalassemia whose hematopoietic stem cells were transduced with lentiviral vectors caused concern. In this clone, activation of the gene *HMGA2* (which encodes a transcriptional regulator) was caused by vector-mediated generation of a truncated transcript whose overexpression in mice is associated with the development of proliferative hematopoiesis and clonal expansion (*13*). Nevertheless, there was no clinical evidence supporting the existence of a preleukemic state or a substantial hematopoietic imbalance.

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Why are gamma retroviral vectors prone to increased genotoxicity? Perhaps the simplest explanation is that their genome contains long-terminal repeats, harboring intact promoter and enhancer sequences which, upon integration in the vicinity of a growth-promoting gene of the host cell, can enhance its transcription, leading to abnormal cell growth. Self-inactivating (SIN) gamma retroviral vectors are being tested in clinical trials for comparison with SIN-lentivectors in terms of their genotoxicity, efficiency of transduction, and sustained expression of the transgene. Another unknown is the conditioning regime and the precise state of the hematopoietic stem cells being transduced, which may differ from patient to patient.

With continued progress in gene therapy, scientists will likely take somatic cells from a patient, convert them into induced pluripotent stem cells, replace the defective gene "surgically" with the normal gene by homologous recombination, and differentiate them into hematopoietic stem cells, followed by conventional transplantation back into the patient. Alternatively, ways will be found to grow and proliferate hematopoietic stem cells, and bypass the need for generation of induced pluripotent stem cells. This is all good news for patients suffering from incurable genetic diseases.

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CHEMISTRY

Nucleation from Solution

Computer simulations of crystal nucleation provide evidence for two-step nucleation.

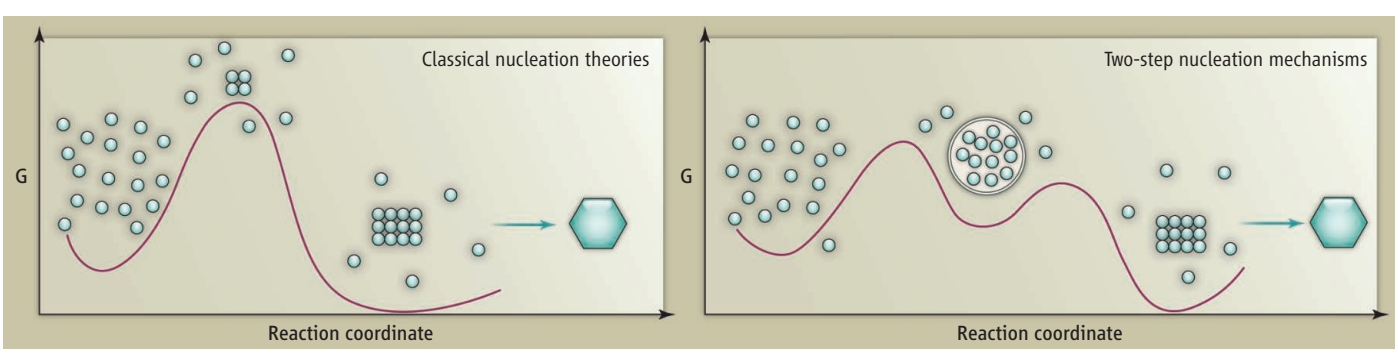
Allan S. Myerson and Bernhardt L. Trout

The formation of crystalline solids from solution is fundamental to many natural and industrial processes. Crystallization may even be the key to the formation of life itself (*1*). The crystallization process begins with nucleation, which plays a central role in determining the structure and size distribution of the crystals. In the past decade, experimental and molecular modeling studies of ionic materials such as calcium carbonate, proteins, and organic molecular crystals has suggested that nucleation of solids from solution does not proceed via the classical pathway but follows much more complex routes. These routes are generally referred to as two-step nucleation, but actually encompass a number of potential mechanisms (*2*–*4*). On page 885 of this issue, Wallace *et al*. (*5*) use molecular simulations to probe $CaCO_3$ nucleation. The results provide evidence for a dense liquid-liquid phase in which solvated $CaCO_3$ clusters come together during nucleation.

Calcium carbonate nucleation and crystallization have been studied intensively because of their importance in many biomineralization processes. Recent experimental studies (*6*) have provided evidence for stable $CaCO_3$ clusters with diameters as large as 2 nm. The authors suggest that these large clusters coalesce and then rearrange to form nuclei. Other experimental studies with cryotransmission electron microscopy and scanning electron microscopy have reported both stable amorphous $CaCO_3$ clusters and $CaCO_3$ as a dense liquid phase. In all cases, some type of amorphous or liquid like precursor is observed. It is difficult, however, to separate nucleation from the growth process in these experimental studies.

This difficulty is avoided in computer simulations, which allow molecular events to be analyzed in detail in a way that is currently unfeasible experimentally. In their molecular simulations of $CaCO_3$ nucleation, Wallace *et al*. provide evidence for a dense liquid-liquid phase in which solvated $CaCO_3$ clusters come together during the nucleation process (see the figure). There are two important points to note about this result. First, solvent effects may govern nucleation, yet are often ignored (*7*). Second, this and other simulation studies over the past decade indicate that liquid-like or amorphous precursors and even crystal-like configurations that differ from the final crystal are essential steps in the nucleation process (*8*–*10*).

How crystals nucleate. According to classical nucleation theory, nucleation proceeds as a one–step process (**left**). Recent experimental and computational studies have pointed to more complex mechanisms, referred to as two-step nucleation (**right**). Using computer simulations, Wallace *et al*. provide support for two-step nucleation and show that solvation plays a key role in this process.



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Gene Therapy That Works

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