of poorly mixed sediments (*13*, *17*), or sulfide formation in sulfate-poor oceans (*6*, *18*). In all these scenarios, SRB were able to reduce available sulfate to sulfur efficiently, resulting in limited isotopic fractionation between sulfide and sulfate. But the environmental implications of these scenarios differ widely. A small fractionation caused by low oceanic sulfate concentrations would indicate low partial pressures of atmospheric oxygen, whereas a reduced fractionation effect due to rapid reduction rates at high temperatures would imply moderate to high sulfate and oxygen concentrations. Reduced fractionation in closed system sedimentary environments only indicates that the sulfate reduction rates in the sediment were higher than the diffusion rate of sulfate into the sediments. Such conditions can occur over a large range of seawater sulfate concentrations and are not diagnostic of Archean oxygen concentrations.

Comparison of sulfur isotope fractionation by SRB and the isotopic record of sulfide and sulfate minerals (*6*, *13*, *18*, *19*) could help assess the validity of the arguments made in support of the different interpretations. The kinetic isotope fractionation associated with bacterial sulfate reduction depends on parameters such as reduction pathways and rates, type and concentration of electron donors, and temperature. If we know how the isotopic fractionation is influenced by these and other parameters, this would help us understand the environmental conditions in the Archean. Canfield *et al.* (*11*) study natural bacteria populations and observe relatively large S isotope fractionation (and presumably high reduction rates) during sulfate reduction at elevated temperatures. Other systems, with different organisms or settings, indicate reduced fractionation under conditions of high reduction rates (*20*). If in the Archean, SRB were similar to the present-day natural assemblages studied by Canfield *et al.* (*11*), then arguments attributing low fractionation to high reduction rates at high temperatures would have to be discounted. Unfortunately, extensive data on organism-specific fractionation effects and their dependence on environmental parameters are still lacking. As more data such as that presented by Canfield *et al.* (*11*) become available, the formulation of a theoretical framework for the interpretation of environmental conditions at this important time of Earth's history should become possible.

Such new and innovative studies impose constraints on interpretations of the Archean S isotope record, but firm conclusions concerning the environmental evolution of the Archean Earth cannot yet be reached. To do so, we need a more reliable record of the chemistry of the atmosphere and/or oceans. As yet, data are limited, and the biological origin of many S-bearing minerals of Archean age remains questionable. Independent indicators—such as trace element concentrations and oxygen, strontium, and iron isotopes—may enable us to distinguish between sulfate and sulfide deposits of biogenic and magmatic origins and possibly between minerals that precipitated from sulfate-poor versus sulfate-rich solutions. Likewise, independent proxies for atmospheric oxygen or seawater sulfate concentrations in the Archean are needed. Sulfate concentrations in other marine minerals (such as carbonates) or distribution of redox-sensitive elements may also prove useful.

References and Notes

1. J. W. Schopf, *Earth's Earliest Biosphere: Its Origin and Evolution* (Princeton Univ. Press, Princeton, NJ, 1983), and references therein.
2. S. L. Miller and L. E. Orgel, *The Origins of Life on Earth* (Prentice-Hall, Englewood Cliffs, NJ, 1974).
3. H. D. Holland, *The Chemical Evolution of the Atmosphere and Ocean* (Princeton Univ. Press, Princeton, NJ, 1984), and references therein.
4. D. J. DeMaris *et al.*, *Nature* **359**, 605 (1992).
5. H. Ohmoto, in *Early Organic Evolution: Implications for Mineral and Energy Resources*, M. Schidlowski *et al.*, Eds. (Springer-Verlag, Berlin, 1992), pp. 378–397.
6. E. M. Cameron, *Nature* **296**, 145 (1982).
7. G. W. Skyring and T. H. Donnelly, *Precambrian Res.* **17**, 41 (1982); D. E. Canfield, *Nature* **396**, 450 (1998).
8. R. A. Berner, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **75**, 97 (1989); A. D. Lasaga, *Am. J. Sci.* **289**, 411 (1989); S. J. Carpenter and K. C. Lohmann, *Geochim. Cosmochim. Acta* **61**, 4831 (1997); P. Brimblecomb and A. Y. Lein, in *Evolution of the Global Biogeochemical Sulphur Cycle, Scope 39* (Wiley, New York, 1989).
9. H. Strauss, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **132**, 97 (1997).
10. $\delta^{34}S$ data for the Archean are summarized by H. Ohmoto and P. F. Robert [*Nature* **328**, 244 (1987)], I. B. Lambert and T. H. Donnelly [in *Stable Isotopes and Fluid Processes in Mineralisation*, H. K. Herbert and S. E. Ho, Eds. (Spec. Publ. 23, University of Western Australia, Nedlands, Australia, 1990), pp. 260–268], and J. M. Hayes, I. B. Lambert, and H. Strauss [in *Proterozoic Biosphere: A Multidisciplinary Study*, J. W. Schoff and C. Klein, Eds. (Cambridge Univ. Press, Cambridge, 1992), pp. 129–134].
11. D. E. Canfield, K. S. Habicht, B. Thamdrup, *Science* **288**, 658 (2000).
12. K. Hattori, F. A. Campbell, H. R. Krouse, *Nature* **302**, 323 (1983); J. C. G. Walker and P. Brimblecombe, *Precambr. Res.* **28**, 205 (1985).
13. H. Ohmoto, T. Kakegawa, D. R. Lowe, *Science* **262**, 555 (1993).
14. J. Monster *et al.*, *Geochim. Cosmochim. Acta* **43**, 405 (1979); I. B. Lambert *et al.*, *Nature* **276**, 808 (1978).
15. P. A. Trudinger, in *Early Organic Evolution: Implications for Mineral and Energy Resources*, M. Schidlowski *et al.*, Eds. (Springer-Verlag, Berlin, 1992), pp. 367–377.
16. T. Kakegawa, H. Kawai, H. Ohmoto, *Geochim. Cosmochim. Acta* **62**, 3205 (1999).
17. H. Ohmoto and R. P. Felder, *Nature* **328**, 244 (1987).
18. M. Schidlowski, J. M. Hays, I. R. Kaplan, in (*1*), pp. 149–186; A. M. Goodwin, J. Monster, H. G. Thode, *Econ. Geol.* **71**, 870 (1976).
19. K. Hattori, H. R. Krouse, F. A. Campbell, *Science* **221**, 549 (1983); K. S. Habicht and D. E. Canfield, *Nature* **382**, 342 (1996).
20. I. R. Kaplan and S. C. Rittenberg, *J. Gen. Microbiol.* **34**, 195 (1964); A. W. L. Kemp and H. G. Thode, *Geochim. Cosmochim. Acta* **32**, 71 (1968); L. A. Chambers, P. A. Trudinger, J. W. Smith, M. S. Burns, *Can. J. Microbiol.* **21**, 1602 (1975); P. Aharon and F. Baoshun, *Geochim. Cosmochim. Acta* **64**, 233 (2000).

PERSPECTIVES: GENE THERAPY

The Best of Times, the Worst of Times

W. French Anderson

According to recent press coverage, gene therapy has fallen on hard times. If one were to believe the news media, gene therapy is both a scientific failure and unsafe. Is this gloomy picture true? Fortunately, no. The paper by Cavazzana-Calvo *et al.* (*1*) on page 669 of this issue provides an example of the exciting results that are starting to be obtained in human gene therapy clinical trials. The authors have successfully treated with gene therapy (for at least up to 10 months) two infants suffering from inherited severe combined immunodeficiency (SCID).

Mutations in several different genes of immune cells can result in SCID. The first gene therapy trial almost 10 years ago treated two girls suffering from a type of SCID caused by a deficiency in the enzyme adenosine deaminase (ADA). In the new study, Cavazzana-Calvo *et al.* treat patients with an X-linked form of SCID (SCID-X1) caused by a mutation in the gene encoding the γc subunit, a component of certain cytokine receptors. After several years of preclinical studies, these investigators have carried out a clinical trial with two SCID-X1 patients, ages 11 and 8 months. They took hematopoietic stem cells (which expressed the surface marker CD34 and were capable of differentiating into all types of blood cells) from the infants' bone marrow and incubated the cells ex vivo with a retroviral vector carrying the γc cDNA. The transduced stem cells were then transfused back into the SCID-X1 patients. The authors present data from 10 months of follow-up and the results are very encouraging. Ten months after receiving transduced stem

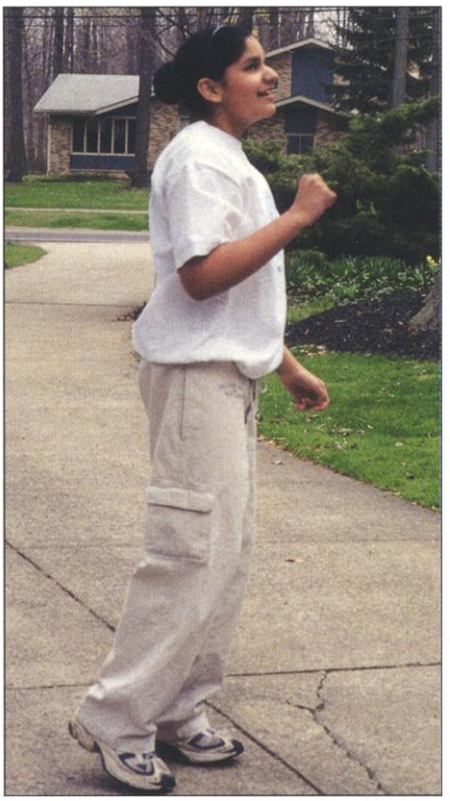
The author is at the Norris Cancer Center, Room 6316, University of Southern California, Keck School of Medicine, 1441 Eastlake Avenue, Los Angeles, CA 90033, USA. E-mail: sdiaz@genome2.hsc.usc.edu

cells, the numbers of T, B, and natural killer (NK) cells of the immune system were normal, as were a number of measures of immune function (such as specific responses to antigen). Clinically, the two patients improved considerably and were able to leave protective isolation in the hospital after 3 months and have been at home ever since. Clearly, longer follow-up is necessary and more patients need to be treated, but the initial data strongly suggest that SCID-X1 can be successfully treated by retroviral-mediated gene therapy.

Why are the results of Cavazzana-Calvo *et al.* more encouraging than those from the earlier gene therapy experiments that treated ADA-deficient SCID patients (*2*–*6*)? In the first clinical protocol, the investigators inserted a normal copy of the gene encoding ADA (carried in a retroviral vector) into mature T lymphocytes (*2*). Later protocols attempted to transfer the same gene into bone marrow stem cells (*3*–*5*), which would differentiate into T lymphocytes capable of responding to new antigens. Cavazzana-Calvo and coworkers used a Moloney-derived retroviral vector (MFG) to deliver the therapeutic gene to the SCID-X1 infants. MFG is an improvement over the earliest retroviral vectors and may be more effective for expressing genes in T cells, but the vector itself could not be the major reason for success; MFG has been used in a number of trials without significant efficacy. Certainly, the transduction conditions of the new study are far superior to those of the early 1990s. Of most significance, perhaps, is the inclusion of Flt3 (a factor that greatly enhances stem cell growth in culture) along with other growth factors in the medium for culturing bone marrow stem cells, and the use of fibronectin-coated culture vessels. The levels of gene transduction obtained by Cavazzana-Calvo *et al.* are, consequently, much higher than those obtained in earlier studies.

Unlike the earlier gene therapy trials that treated ADA-deficient SCID patients, Cavazzana-Calvo and colleagues did not have to administer PEG-ADA—a polyethylene glycol–conjugated ADA enzyme preparation that reduces the levels of the toxic molecule deoxyadenosine in ADA-deficient patients—to the SCID-X1 infants. The concomitant administration of PEG-ADA is believed to lessen the potential growth advantage of ADA gene–corrected cells (*7*). Finally, SCID-X1 can result in a more profound deficiency in T cells than ADA-deficient SCID; therefore, the positive selection for gene-corrected T cells may have been more vigorous in the SCID-X1 patients.

The majority of ADA-deficient SCID patients treated with gene-corrected stem cells have not been significantly helped. But the very first ADA-deficient SCID patient, a 4-year-old girl who received only gene-corrected mature T cells, and not stem cells, has thrived (*2*, *8*, *9*) (see the figure). She received 11 infusions from September 1990 to August 1992 and has maintained a circulating level of 20 to 25% gene-corrected T cells and a normal life-style with amelioration of her disease symptoms. Her partial response to PEG-ADA treatment before gene therapy had not provided her with an adequate immune system; nonetheless, we have felt it wise to continue treating her with PEG-ADA as a safety-net.



CREDIT: VAN DE SILVA

Ashanti de Silva. Now 13, Ashanti was the first patient to be treated with gene therapy. She received infusions of T cells that had been transduced with a gene for ADA (an enzyme that she lacks), resulting in an amelioration of the symptoms of her severe combined immunodeficiency.

Careful monitoring indicates that the vigor of her immune response has gradually diminished over the past several years, but it still remains in the normal range, albeit at the low end. But, as she only received mature gene-corrected T cells that, unlike stem cells, cannot be educated to respond to new antigens, how has she been able to generate an immune response to the new antigens that she encounters constantly? One explanation is provided by the data obtained in the first 6 months of treatment [see figure 1A of (*2*)]. When only a small number of gene-corrected T cells were infused (less than 1% of the total T cell population), the total number of T cells tripled from 500 to 1500 per microliter of blood. When several infusions were missed because of technical problems, the T cell number plummeted back to 500. When infusions of gene-corrected T cells were resumed, the total T cell number again rose rapidly, this time to over 2000. One could speculate that it requires only a threshold level of "normal" (that is, gene-corrected) T cells in the lymphoid tissues to provide a microenvironment that allows the noncorrected (both immature and mature) T cells to function normally. Thus, her immature T cells may be able to differentiate in lymphoid tissue, and thereby provide her with immune protection against new antigens, as long as the "normal" mature T cells are maintained above a critical level.

The successful treatment of the first gene therapy patient suggests that the positive results of Cavazzana-Calvo *et al.* may continue over the long term. The gene-corrected stem cells of the two SCID-X1 infants should continue to experience a positive selection in the patients so that even if some cells have their γc gene silenced over time, others will expand to maintain the immune status of the patients.

In addition to the success achieved with gene therapy for the treatment of SCID, recent publications suggest progress in the treatment of hemophilia (*10*) and in the growth of new blood vessels to treat cardiovascular disease (*11*). Furthermore, early data demonstrate headway in the development of gene-based vaccines for treating several chronic infectious diseases and some types of cancer.

The field of gene therapy has been criticized for promising too much and providing too little during its first 10 years of existence. But gene therapy, like every other major new technology, takes time to develop. Antibiotics, monoclonal antibodies, organ transplants, to name just a few areas of medicine, have taken many years to mature. Major new technologies in every field, such as the manned rocket to the moon, had failures and disappointments. Early hopes are always frustrated by the many incremental steps necessary to produce "success." Gene therapy will succeed with time. And it is important that it does succeed, because no other area of medicine holds as much promise for providing cures for the many devastating diseases that now ravage humankind.

References

1. M. Cavazzana-Calvo *et al.*, *Science* **288**, 669 (2000).
2. R. M. Blaese *et al.*, *Science* **270**, 475 (1995).
3. C. Bordignon *et al.*, *Science* **270**, 470 (1995).
4. D. B. Kohn *et al.*, *Nature Med.* **1**, 1017 (1995).
5. P. M. Hoogerbrugge *et al.*, *Gene Ther.* **3**, 179 (1996).
6. M. Onodera *et al.*, *Blood* **91**, 30 (1998).
7. D. B. Kohn *et al.*, *Nature Med.* **4**, 775 (1998).
8. W. F. Anderson, *Science* **256**, 808 (1992).
9. C. A. Mullen *et al.*, *Hum. Gene Ther.* **7**, 1123 (1996).
10. M. A. Kay *et al.*, *Nature Genet.* **24**, 257 (2000).
11. M. Isner and T. Asahara, *J. Clin. Invest.* **103**, 1231 (1999).