mous mutations in CovS[10]. A more robust study of other rheumatogenic serotypes is required before conclusions can be drawn.

The precise reason why one strain is associated with a specific severe or debilitating complication is unknown. Do strains with the ITP profile induce ARF or toxic shock because they are better equipped to escape the throat and cause systemic infections, or do they express a unique sequela-specific gene product? Upregulation of secreted streptococcal superantigens that can induce a shower of cytokines could initiate toxic shock, whereas localized hyperproduction of HA with the potential to engage CD44 receptors on memory T cells could dysregulate immune responses to infection.

Clearly, however, host factors dramatically influence the outcome of infections by a particular virotype. Although local increases of ARF, acute streptococcal glomerulonephritis and invasive disease have been witnessed over time, they are still rare relative to pharyngitis and impetigo, even when virulent clones predominate. Are low-grade throat infections the source of ITP *Streptococcus* that cause the more than 40% of cases of necrotizing fasciitis where no external wound is observed[5]?

Future challenges include understanding why children fail to develop protective immunity and often experience recurrent pharyngitis. Such studies should help inform the development of vaccines that will reduce colonization of tonsils, and eliminate dreadful forms of streptococcal disease associated with transcriptome shifts.

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Inserting optimism into gene therapy

Luigi Naldini

A small clinical trial for an immunodeficiency disease provides a new way to think about gene therapy (pages 401–409).

Gene therapy has been successful in clinical trials in children affected by rare inherited disorders that cause severe combined immunodeficiency (SCID)[1–3]. A retroviral vector was used to replace a functional copy of the defective gene in some hematopoietic stem and progenitor cells (HSPCs), which reconstituted the lymphoid system and largely cured the immunodeficiency. Sadly, some of the children treated for the X-linked form of SCID were later diagnosed with leukemia consequent to oncogene activation at a vector insertion site[4].

Although no adverse effects have been reported in trials with another type of SCID (ADA-SCID), this setback has raised serious concerns about the safety of the technique. A study in this issue is likely to spur new debate about the use of retroviral vectors for gene therapy.

Grez, von Kalle and colleagues used HSPC gene therapy to correct another rare immunodeficiency, chronic granulomatous disease (CGD), caused by defective phagocytes in the myeloid lineages[5]. As with the X-SCID trial, insertional mutagenesis occurred at a high frequency in the two individuals tested—but in the new study, the consequence was apparently to reinforce the therapeutic benefit. Some vector insertions activated genes that prompted proliferation and differentiation of a set of cells that were able to reconstitute the immune function. The findings raise challenging ethical questions and have profound implications for the future design of HSPC gene therapy.

CGD results from mutations in the NADPH oxidase complex, which phagocytes normally harness to kill microbes—a process gone awry in this severe disease. CGD is often treated by transplant of allogeneic HSPCs; but for individuals with CGD who lack human leukocute antigen (HLA)-matched donors, gene therapy with autologous HSPCs may be the only treatment option.

In the SCID trials, the success of gene therapy was facilitated by the selective growth advantage that corrected lymphocytes gained over their defective counterparts. Because the inherited mutations block lymphocyte maturation and growth, the progeny of a few gene-corrected HSPCs could reconstitute the immune system. In contrast, no growth advantage of gene-corrected myeloid progenitors was observed in preclinical studies for CGD. This was expected because the NADPH complex does not influence the growth of myeloid progenitors. Furthermore, phagocytes are short-lived and need to be continuously replaced. Thus, HSPC gene therapy has represented a challenge[6].

The present study took advantage of a conditioning regimen introduced in the ADA-SCID trial[2] to enhance engraftment of gene-corrected HSPCs. The authors sought to correct the most common defect in CGD, mutations in the gene encoding the gp91^{phox} subunit of the NADPH oxidase complex. A functional gp91^{phox} gene was introduced into HSPCs from two individuals with CGD using a retroviral vector selected for robust activity in HSPCs[7]. The individuals were then treated with a myelotoxic drug to make space in the bone marrow before infusion of the vector-treated cells. The expectation was to reach sufficient engraftment of gene-corrected HSPCs to guarantee a steady supply of functional phagocytes to the individuals' blood and tissues.

Indeed, after recovery from the conditioning, phagocytes carrying the new gp91^{phox} gene appeared in the blood of both individuals. These cells showed correction of the biochemical defect and could kill microbes. Unexpectedly, the percentage of gene-corrected neutrophils progressively increased later after transplant and then stabilized until the latest observation (16 months after gene therapy). Total blood and bone marrow cell counts, however, remained within the normal

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range throughout follow up. What's more, both individuals appeared to benefit, clearing up deep-tissue infections, and they have since been free from severe infections.

The authors tracked, to an unprecedented scale and sophistication, the hematopoiesis of the transplant. Taking advantage of the vector insertion site, which uniquely marks each cell before the transplant, they monitored the contribution of individual progenitors to the mature cell pool.

This analysis showed that the delayed expansion of gene-corrected neutrophils resulted from the selective outgrowth of progenitors carrying a vector insertion that activated one of three genes (two related genes for zinc-finger transcription factors, *MDS1-EVI1* and *PRDM16*, and the *SETBP1* gene; **Fig. 1**). These are well-known cancer-associated genes involved in chromosomal translocations characteristic of human leukemias[8]. Yet most clonal outgrowths marked by insertion at these genes exhausted within a few months. Had it not been for the unique set of integrations uncovered in these clones, one would have considered their delayed contribution to hematopoiesis as part of a physiological succession of different progenitors.

The therapy seems to have induced proliferation and differentiation of myeloid cells, without prompting invasive or malignant features. This balance may be achieved because insertional activation of *EVI1*, *PRDM16* and *SETBP1* occurred in committed myeloid progenitors, with the result of expanding their progeny. Because these cells have lost self-renewal capacity early in the haematopoietic hierarchy, their outgrowth may be self-limited.

That notion gains strength from findings in mice and primates that parallel those in the current study. For instance, activation of the *Mds-Evi1* and *Prdm16* genes is the most common mechanism of *in vitro* immortalization of mouse myeloid progenitors by insertional mutagenesis; upon transplant, however, these cells do not engraft and are not leukemogenic[9]. Likewise, progenitors carrying retroviral insertion at the *MDS-EVIl* locus were the most frequent contributors to hematopoiesis in gene therapy–treated nonhuman primates, without evidence of continued cell expansion[10].

The high rate of vector integration at the same locus, as observed in this study, is unprecedented. The targeted genes may be hot spots of retroviral integration in HSPCs. Because retroviral integration occurs throughout the genome, however, the findings suggest instead that insertional mutagenesis saturates the human genome in the cell population contributing to the graft—and that some insertions prevail in the host by specifically promoting progenitor proliferation. This behavior sounds a note of caution, adding to the evidence that insertional gene activation seems to have a robust capacity for influencing the engraftment and output of hematopoietic progenitors[11].

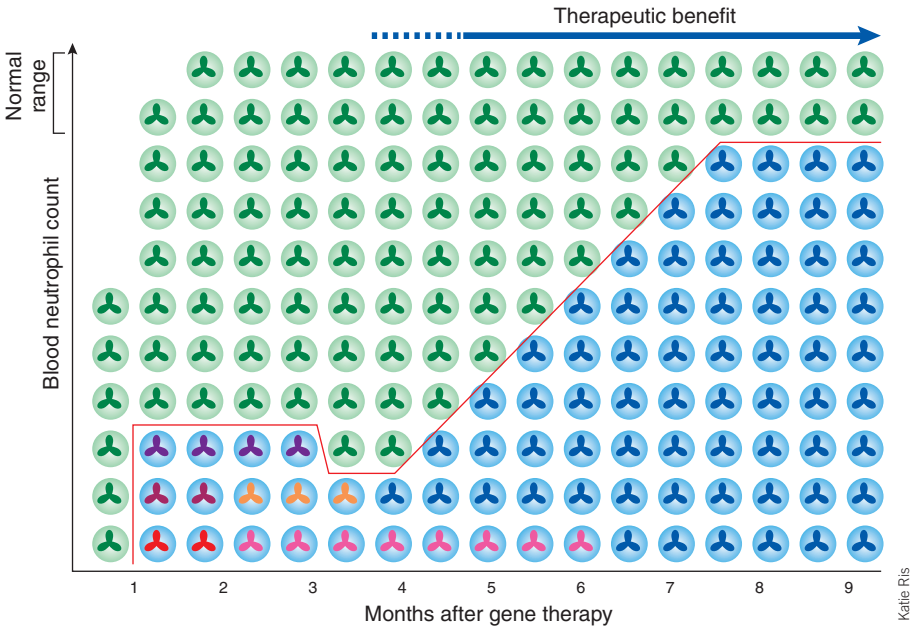

Figure 1 Gene-corrected neutrophils in the blood of an individual with CGD after HSPC gene therapy. Whereas CGD neutrophils lack NADPH activity (green cytosol), the progeny of HSPCs engrafted after gene therapy have reconstituted enzyme activity (blue cytosol) resulting from the presence of a functional gene replaced by a retroviral vector. Gene-corrected cells differ for the site of vector insertion (nucleus in different color), reflecting the origin from distinct progenitors. Early after gene therapy, many progenitors contribute to the neutrophil pool but become exhausted with time. At later times, gene-corrected cells from a unique progenitor progressively expand until accounting for the majority of circulating cells. In these cells, the vector integrated near a growth-promoting gene which is activated by insertional mutagenesis and confers a growth advantage. Total neutrophil number does not increase with time, suggesting that the expanding cells are subjected to normal control. Because the clinical benefit is dependent on the number of circulating gene-corrected cells (gray line), the expansion enables efficacious and sustained correction of the disease.

The most troubling finding of the study is probably that a single clone (carrying an *EVI1* insertion) came to dominate the myeloid compartment in one individual. Expansion of this clone did not trespass the normal boundaries of the myeloid pool, and its progeny remained dependent on growth factors and unable to self-renew *in vitro*—suggesting a benign nature.

The authors provided some evidence that the dominant clone may have originated from a primitive progenitor, or stem cell, with the capacity to give rise to more cell types than the other transduced cells. The optimist would point out that such a cell may provide gene-corrected cells in the long term and potentially guarantee continued therapeutic benefit. Possibly, *EVI1* activation would selectively expand its myeloid output or skew differentiation of the cell progeny toward it. A more realistic view, however, would caution that such a clone may have expanded at the expense of all the others, because it still had or regained self-renewing capacity, and may represent a premalignant state with the potential to evolve into full-blown leukemia. Only long-term follow up of the patients will determine the true safety of this protocol.

It is possible that, in the absence of such clonal expansions, there would be insufficient numbers of gene-corrected cells to reach a sustained therapeutic effect. Thus, we find for the first time a silver lining on the dark cloud of insertional mutagenesis. We now face the dilemma of whether we should continue exploiting this effect as a therapeutic option for CGD before we find safer ways to treat the disease.

We should also ask why similar expansions of myeloid progenitors were not observed in other gene therapy trials. Although the gene transfer procedures are similar in all these trials, we can point to subtle differences that may have been crucial for the different behavior of the HSPCs. One difference may be the

vector. The vector used in the CGD study had much higher transcriptional activity in HSPCs than the vector used in most other trials[7]. Although this strong activity probably allowed better reconstitution of the NADPH enzyme activity, it also may have provided a greater capacity to transactivate cellular genes at the integration site, and thus a higher chance of oncogene activation. The vector could also possibly confer some specificity to the target gene and host cell undergoing activation.

The source of transduced cells also may make a difference. The CGD study used growth factor–mobilized peripheral blood HSPCs, which are more enriched in activated myeloid progenitors than the bone marrow cells used in other trials. Thus, a different array of progenitor subsets, and possibly a specific activation state, may have influenced the gene transfer frequency and the integration site selection to favor cells more susceptible to *EVI1*-induced immortalization.

The findings of Grez, von Kalle and colleagues strengthen the emerging concept that the type and frequency of side effects of retroviral insertional mutagenesis depend highly on context. The study also highlights the need to pay more attention to all the variables in gene transfer protocols besides the disease type and transgene function. Rather than dismissing gene therapy for falling short of its early enthusiastic promises, we should start to recognize the medical advances and the scientific insights born out of its painful coming of age.

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Fighting infections with vitamin D

Michael Zasloff

Sunlight can treat tuberculosis, a phenomenon observed more than a century ago. The mechanism now becomes more clear, and it involves induction of a microbe-fighting peptide by vitamin D.

In 1895, Niels Finsen of Denmark found an effective way to treat tuberculosis. He exposed individuals with tuberculosis of the skin—lupus vulgaris—to high-intensity light produced from an electric arc lamp. Exposing a small area of affected skin to intense light produced moderate sunburn. The superficial skin layers subsequently peeled away—leaving normal, healthy skin underneath. Phototherapy cured or substantially improved the disease in about 95% of affected people, and by the 1920s sun exposure for the treatment of pulmonary tuberculosis had become routine.

Finsen was awarded a Nobel Prize in 1903 for his treatment, preceding Robert Koch who claimed the prize in 1905 for identifying the causative agent of tuberculosis, and Selman Waksman, who won in 1952 for his discovery of streptomycin, the first antibiotic to cure the disease.

More than a century after Finsen's discovery, we are beginning to understand how sunlight helps us battle tuberculosis and other microbes. In a recent issue of *Science*, Liu *et al.*[1] propose that sunlight, by stimulating the synthesis of vitamin D, upregulates the expression of a microbe-fighting peptide.

Most multicellular organisms produce antimicrobial peptides (AMPs) and proteins, which can kill viruses, fungi, protozoa, bacteria and other microbes. In people, AMPs are produced on epithelial surfaces and within circulating white cells[2,3]. Some AMPs are expressed constitutively, and others are expressed in response to stimuli such as tissue injury (through interleukin-1 and other cytokines) or microbial components (such as lipopolysaccharide). Amongst the better studied of the inducible AMPs are human β-defensins 2 and 3 and LL-37 (also known as cathelicidin)[4].

In addition to their anti-infective activities, AMPs such as LL-37 help orchestrate the ensuing wound-repair process. LL-37 stimulates local angiogenesis and synergizes with the epidermal growth factor receptor to promote epithelial growth. LL-37 can also attract monocytes and neutrophils through FMLP receptors on these cells[2,3].

Recent studies of the gene encoding LL-37 have revealed that it contains sites for the vitamin D receptor (VDR)[5–7]. The active form of vitamin D—1,25-D3—boosts levels of LL-37 in human neutrophils. 1,25-D3 also induces expression of LL-37 in keratinocytes in tissue culture and after topical administration onto the skin of human subjects[8].

Why should an antimicrobial peptide be induced by vitamin D, a product of sunlight?

Sunlight, especially within the UVB spectrum, 'burns' skin, by damaging the lipids and DNA of epidermal cells[9]. At the same time, sunlight also induces the synthesis of two powerful immunosuppressants within the skin, vitamin D and α-MSH. α-MSH is believed to exert its anti-inflammatory effects by inhibiting the activation of NF-κB in a wide spectrum of tissues. Vitamin D depresses the activity of Langerhans cells, and inhibits the induction of T helper type 1 cells and the expression of major histocompatibility complex (MHC) class II proteins on antigen-presenting cells.

We assume that UVB-induced immunosuppression evolved to control the intensity of inflammation (for example, pain, redness, epidermal damage) caused by UVB-provoked injury. But suppressing inflammation also makes us more vulnerable to infection, especially in a setting where the skin has been damaged. To balance this compromise in host defense, vitamin D stimulates the synthesis of the potent antimicrobial peptide LL-37 in skin and circulating phagocytic cells.

Liu *et al.* did not set out to explain the antimicrobial properties of sunlight. The authors were exploring the response of human immune cells to activation by the Toll-like receptor (TLR) 2/1, which senses molecules derived from pathogens. They observed that activated monocytes or macrophages could

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