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PERSPECTIVES: MICROBIOLOGY

TB Vaccines: Global Solutions for Global Problems

Douglas B. Young and Brian D. Robertson

Tuberculosis remains one of the 10 most important causes of premature mortality worldwide, claiming over 2 million lives every year. In spite of dramatic successes in control of the disease in prosperous countries, geography is an unreliable correlate of protection. Recent sporadic increases in the incidence of tuberculosis (particularly drug-resistant forms of the disease)—most strikingly in New York City—attest to the ability of the causative pathogen, *Mycobacterium tuberculosis*, to exploit new opportunities for travel and immigration and to seek out the disadvantaged pockets in all societies. Control of tuberculosis must be at the global level, with the best prospects offered by improved diagnosis and treatment together with prevention by effective vaccination (*1*). The search for an effective tuberculosis vaccine has challenged and frustrated generations of scientists. In their report on page 1520 of this issue, Behr *et al.* (*2*) exploit state-of-the-art DNA microarray technology to provide new insights into this longstanding problem.

The current tuberculosis vaccine—the Bacille Calmette and Guérin, BCG—was derived from an isolate of *Mycobacterium bovis* (which causes bovine tuberculosis) that had been attenuated by laboratory passage in the early years of this century. It has had a chequered history in efficacy trials, providing more than 70% protection in trials in the United Kingdom, no significant protection in South India, and intermediate levels in a range of other studies in different countries. Behr and Small have previously suggested that differences in efficacy might have arisen as a result of changes in the vaccine strain over time (*3*). Their new study provides further support for this notion, demonstrating that the subculture of BCG in different laboratories resulted in a series of genetic deletions and the evolution of a number of BCG substrains. The authors compared the genomes of *M. bovis* and contemporary BCG strains with that of a virulent reference *M. tuberculosis* strain, using comparative hybridization to a DNA microarray. They found that Calmette and Guérin "lost" a 10-kilobase fragment from the *M. bovis* progenitor strain during the initial process of attenuation. A second fragment was deleted in the late 1920s, and three additional fragments were selectively deleted during subsequent passage in separate laboratories to generate the current diaspora of BCG substrains. Although there is as yet no direct evidence to confirm Behr and Small's hypothesis that these deletions are responsible for changes in vaccine efficacy, the results provide a rational starting point for attempts to generate—or perhaps regenerate—a better BCG vaccine.

A tuberculosis patient in a Victorian hospital.

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These observations represent an important addition to knowledge of BCG deletions originally identified in previous groundbreaking publications (*4*, *5*). A key aspect of the present study is that it provides the first such analysis at a whole-genome level. The authors have used information from the recently completed genome sequence of *M. tuberculosis* (*6*) to construct a DNA microarray in which almost every open reading frame is displayed. This has allowed a global analysis of genetic differences between *M. tuberculosis*, *M. bovis*, and the various BCG substrains. Almost 100 *M. tuberculosis* genes were "missing" from all of the *M. bovis* isolates that were examined. Earlier sequence-based analysis of a limited set of genes demonstrated a remarkably high degree of genetic conservation between *M. bovis* and *M. tuberculosis* (*7*). The new findings suggest that genetic diversity amongst members of the *M. tuberculosis* complex (comprising *M. tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti*) may in fact be much greater than previously anticipated, and that gene deletion, rather than point mutation, may be a key source of this variation. Currently, information provided by the microarray approach is "one-way," identifying *M. bovis* deletions relative to the framework provided by the *M. tuberculosis* genome. Sequence analysis of the genome of *M. bovis* is currently under way (*8*), and identification of regions present in *M. bovis* but absent from *M. tuberculosis* will also be of considerable interest in the context of understanding the evolution of the bovine and human pathogens. As in the case of the BCG vaccines, functional analysis of the "missing" genes may provide important insights into mycobacterial physiology.

In addition to the genotypic analysis described by Behr *et al.*, the microarray approach is also applicable to the study of temporal changes in global patterns of gene expression. This is accomplished with the use of RNA isolated from organisms grown under different conditions and has been successfully applied to the investigation of global changes in gene expression associated with the shift from fermentation to respiration in yeast (*9*). This approach has also yielded information about gene expression

patterns during different growth phases of *Haemophilus influenzae* and *Streptococcus pneumoniae* (*10*). This, however, is only the beginning. As microarrays and their associated technology get cheaper and easier to use, they will become a standard laboratory tool, considered in the same way as other hybridization methods, such as the blotting techniques pioneered by Ed Southern.

Microarrays present a wonderful opportunity for exploring the regulation of gene expression at the level of the whole cell. Operons are a hallmark of prokaryotes and are easy to spot in many bacteria simply by looking at the DNA sequence to identify multiple genes with a common promoter and terminator. However, in some organisms, including *M. tuberculosis*, such signatures are difficult to find; microarrays probed with RNA or complementary DNA from *M. tuberculosis* should highlight where a linear sequence of genes is cotranscribed. Survival of successful bacterial pathogens depends on their ability to alter global patterns of gene expression, using "regulons" and "modulons" to coordinate an overall response to the changing environments encountered during infection. Interestingly, Behr *et al.* (*2*) report that although none of the open reading frames deleted during attenuation of the BCG vaccine look like classical virulence determinants, there is an overrepresentation of genes classified as transcriptional regulators—both activators and repressors—emphasizing that virulence depends not only on the presence or absence of particular gene products, but also on the way that they are controlled.

Genome-based microarray technology offers an exciting new era in molecular microbiology. As with virulence, however, future success will be determined not just by what we've got, but also by the way that we use it.

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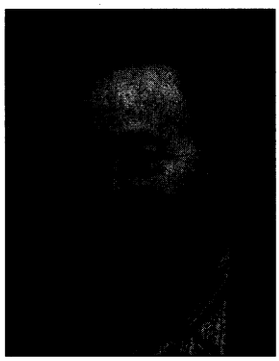
RETROSPECTIVE

Gertrude Belle Elion (1918–1999)

Michael Colvin

Gertrude Elion, a pioneer in drug development and one of only 11 women scientists to win the Nobel Prize, earned her master's degree in chemistry from New York University in 1941. She found work teaching nurses and testing food products—finding employment as a research scientist was difficult for women in those days. Her opportunity came with the arrival of the Second World War and the diminished supply of male scientists. She was hired by the Burroughs Wellcome Company to work as a research assistant in the laboratory of George Hitchings. And so began a collaboration that would last for Elion's entire career.



Elion and Hitchings started to investigate the mechanisms of purine and pyrimidine metabolism and the incorporation of these building blocks into nucleic acid. By exploring the effects of incorporating purine and pyrimidine structural analogs into DNA—a revolutionary approach—they discovered a long list of extremely important drugs: thioguanine and 6-mercaptopurine for treating acute leukemia, pyrimethamine for malaria and toxoplasmosis, and the antibacterial trimethoprim. Next came azathioprine, an immunosuppressive drug that improved the success of renal transplantation and the treatment of autoimmune disease. They developed the first truly effective antiviral compound: acyclovir for the treatment of herpesvirus infection. Further studies of the antiviral effects of pyrimidine and purine analogs led to the discovery of AZT, the first drug for HIV. These discoveries were triumphs of rational drug design based on the exploration of metabolic pathways. In recognition of their work, Elion and Hitchings (together with James Black) were awarded the 1988 Nobel Prize for Physiology or Medicine.

Worldwide recognition of Trudy Elion's role as a pioneer in rational drug design and as an articulate spokeswoman for science continued to grow even after her formal retirement from Burroughs Wellcome in 1983. To the end of her life she maintained a demanding travel schedule, which she pursued not for personal reward but for the opportunities her travels provided to promote the cause of science and to encourage those just beginning a career in research.

It was at the end of her magnificent career with Burroughs Wellcome that Trudy's association with Duke University in Durham, North Carolina, began. Trudy enthusiastically accepted an offer to join the faculty at Duke in a part-time appointment to work with and mentor medical and graduate students.

From 1983 until the end of her life, Trudy served as a mentor for many medical and graduate students in the neuro-oncology and pediatric bone marrow transplant programs. Despite her hectic travel schedule, Trudy spent a great deal of time with her students and was an inspiration to them all. During her years at Duke, she published more than 25 papers with her students and taught them invaluable lessons about how to do science and, just as important, why to do science. Trudy regularly attended the American Association for Cancer Research annual meetings where the students presented their work, and she made a point of being at their presentations whenever possible.

Most of her students are now pursuing successful careers in academic medicine. All of them remember the experience of working with a Nobel laureate who was brilliant and insightful, as expected, but who was also stimulating, supportive, and inspirational. Trudy's material rewards for this effort were meager—a small stipend and a modest office—but she never asked for more. Ironically, she was looking forward to moving into a much larger and nicer office—with two windows—but died the evening before the move.

We know that Trudy enjoyed her career with our students, and we know that her mentorship was a career highlight for them. But perhaps the greatest beneficiaries of her time at Duke were the faculty and staff, especially those of us who worked most closely with her, who were able to know and learn from a most gracious person and a brilliant and determined scientist.

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