

# Profile of Dan Littman, winner of the 2016 Vilcek Prize in Biomedical Science

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The 2016 Vilcek Prize in Biomedical Science has been awarded to Dan Littman, Professor of Molecular Immunology at New York University's Skirball Institute. The aim of the Vilcek Prizes, awarded each year since 2006, is to recognize the significant contributions of immigrant professionals to biomedical science in the United States and to help raise public awareness of the important role played by foreign-born scientists in sustaining the nation's leading role in the international scientific enterprise.

The Vilcek Prize includes an award of \$100,000 and recognizes contributions made by immigrant scientists at the pinnacle of their careers. Since 2009, the Vilcek Foundation has also awarded Prizes for Creative Promise in Biomedical Science to a younger generation of foreign-born scientists; candidates for this prize must be 38 years of age or younger at the time of selection. Currently, the Foundation bestows three annual Prizes for Creative Promise in Biomedical Science, each accompanied by a \$50,000 award. All told, 11 scientists have been awarded Vilcek Prizes and 12 have been honored with Prizes for Creative Promise in Biomedical Science through 2015.

The Vilcek Foundation Prizes are among the few laurels earmarked to reinforce the crucial role of immigrant scientists in sustaining the scientific leadership of the United States on the world stage. Another unique aspect of the prizes are the accompanying prizes awarded to outstanding foreign-born artists working in the United States. Throughout history, transformative science and flourishing arts have borne witness to the greatness of civilizations and nations across the world. The Vilcek Foundation Prizes serve as a shining testament to the rich contribution of immigrants to science and arts in the United States.

## Pioneering Insights into Immunity: Dan Littman

In an unassertive red-brick building on a quiet, tree-lined street on Harvard University's leafy campus in Cambridge, Massachusetts, a team of researchers is trying to endow mice with a special trait: the ability to fend off HIV. The hope is that the mouse model will pave the way for the transplantation of engineered blood stem cells that can spawn virus-resistant immune

cells in people, a feat that might help counter one of humanity's most fearsome scourges. Fraught with challenges, the approach turns on a powerful tool to precisely edit the human genome. For the Harvard team, the target of the tool is a gene called *CCR5*, which encodes a protein that adorns the surface of the immune sentinels targeted by the virus. If successful, the effort might someday help engineer HIV resistance in some people.

Such technical advances to combat HIV owe a debt to a momentous scientific discovery made nearly two decades ago by Dan Littman, a professor of molecular immunology at New York University's Skirball Institute and member of the National Academy of Sciences. Littman's finding that HIV uses *CCR5* as a handhold on human immune cells transformed researchers' understanding of the pathogenesis of the virus and paved the way for experimental approaches aimed at curbing its spread. Over a career spanning nearly three decades and a wide array of themes in immunology, Littman has unraveled intricately braided molecular pathways that control the identity of immune cells, the specificity of immune responses, the ability of immune cells to turn against their hosts, and the interplay between immune cells and the human body's microbial denizens. For his sprawling work on the staggering complexity of the immune system, Littman has earned many laurels, including memberships in the United States National Academy of Sciences, the National Academy of Medicine, and the American Academy of Arts and Sciences.

Littman was born and raised in Bucharest, Romania in the 1950s, a few years before the harsh political regime of the socialist leader Nicolae Ceaușescu cast a bleak shadow over the Eastern bloc nation. Although



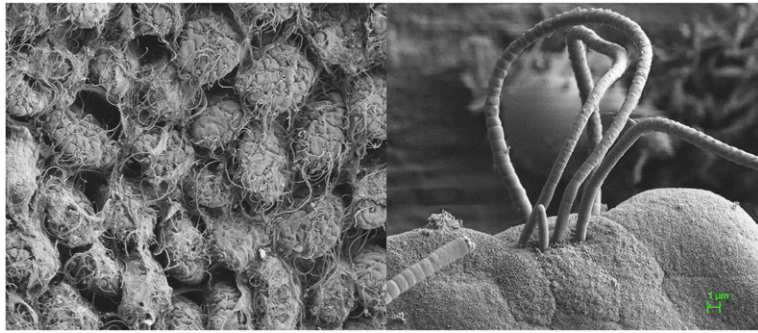
Dan Littman. Image courtesy of Peter Hurley (photographer).

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**Electron micrograph of terminal ileum of 8-wk-old Taconic C57BL/6 mice showing long filamentous bacteria with segmented filamentous bacteria morphology. Reproduced from ref. 7, with permission from Elsevier.**

his father was a physician and his mother an editor in a publishing house, it was his uncle, an engineer, who largely inspired Littman. From a young age, Littman grew increasingly fascinated by the mechanical marvels of industry and the inner workings of its machines. "My mother used to take me to the train station in Bucharest, and I would spend hours looking at steam locomotives," Littman recalls. With the dawn of the space age and the launch of Sputnik, Littman's interest in engineering deepened as he immersed himself in science fiction novels and resolved to become an aerospace engineer.

Fleeing the harsh political environment in Romania, Littman's family emigrated to the United States by way of France and Italy when he was 11 years old. After a brief sojourn in Rhode Island, the family moved to a quiet suburb of Philadelphia, where Littman completed high school. A standout in mathematics and physics, he promptly enrolled at Princeton University for a bachelor's program in aerospace engineering. "But at Princeton I realized there was a whole universe of knowledge I had not been exposed to," he says. In the summer of 1971, a year into his undergraduate studies, Littman chanced upon *The Molecular Biology of the Gene*, a gripping account of a growing field by James Watson and others that remains an unimpeachably authoritative tome decades after its first appearance. Smitten with the history of the field and the revolution it portended, Littman switched majors in his sophomore year, abandoning engineering in favor of biology.

Pursuing his newfound interest, Littman joined the laboratory of cell biologist Marc Kirschner, then newly appointed to Princeton. With Kirschner, he carried out his undergraduate thesis, unraveling the mechanics of a molecule called tubulin, found in the scaffold that composes the backbone of all living cells. Energized by the experience in Kirschner's laboratory, Littman considered a scientific career but faced a moment of doubt. "My father wanted me to follow in his footsteps, and I wanted to choose a safe professional path," he says. So he took what he perceived to be the middle road: an MD/PhD program at Washington University in St. Louis, Missouri.

The immediacy of medicine, coupled with the excitement of research, vindicated his choice. "It was tremendous to put together what I was learning from basic science in the context of disease," Littman says.

In a move that marks the beginning of his decades-long foray into immunology, in 1976 Littman joined the laboratory of a young immunologist couple, Benjamin Schwartz and Susan Cullen, who had recently arrived from the National Institutes of Health in Bethesda, Maryland, to launch a research group focused on the phenomenon of antigen presentation, which enables the immune system to recognize foreign antigens.

Around this time, immunologists Rolf Zinkernagel and Peter Doherty had shown in Nobel-winning work that T cells of the immune system identify virus-infected human cells by recognizing both viral antigens and human proteins called major histocompatibility complex (MHC) molecules. But the precise mechanism used by T cells to sample the MHC-antigen complex remained a mystery. Schwartz and Cullen set Littman on an ambitious project to unravel the interaction, and he tore into it with the enthusiasm of a fresh-faced apprentice. "I had the naive idea that by putting the viral antigen and MHC protein in lipid vesicles and monitoring whether the vesicles trigger an immune response in T cells in lab dishes, I could unlock the mystery of the interaction," recalls Littman. But the experiment was scuttled by a flaw he could not have foreseen. Because the immune system's antigen-presenting cells display only portions of viral antigens—albeit embedded in MHC molecules—to elicit an immune response from T cells, Littman's assay, devised using entire viral antigens, was doomed to fail; it would be a few years before the precise mechanism of antigen presentation came to light.

Littman's doctoral efforts may not have led to lapel-grabbing insights on immunity, but they sharpened his instincts, honed his technical skills, and prepared him for an obsessive quest to uncover the seemingly occult workings of the immune system. Decades later, the quest culminated in discoveries that transformed researchers' understanding of immune responses and set the stage for experimental treatments for HIV.

Toward the end of his doctoral studies in the late 1970s, a revolution in molecular biology was rumbling along, and Littman learned of a technical advance that enabled researchers to transfer genes into mammalian cells for fine-grained analysis of their functions. Sensing the terrain-shifting power of the technique, Littman began a postdoctoral fellowship in 1981 in the laboratory of Columbia University molecular biologist Richard Axel. In Axel's laboratory, he set out to isolate the genes for MHC proteins, thanks to the advent of monoclonal antibodies, which can serve as baits to hook individual molecules on immune cells. But when a team at Harvard beat him to the finish line in the race to clone the MHC genes, Littman shifted his focus toward molecules on the surface of T cells that are crucial to immune recognition and response. Before long, his efforts led to the identification and cloning of two major proteins that confer immunological identity to the immune system's foot soldiers: the protein CD4, which adorns the surface of helper T cells, and the protein CD8, found on killer T cells. The findings, published in a pair of reports in *Cell* in 1985, propelled Littman to scientific prominence (1, 2).

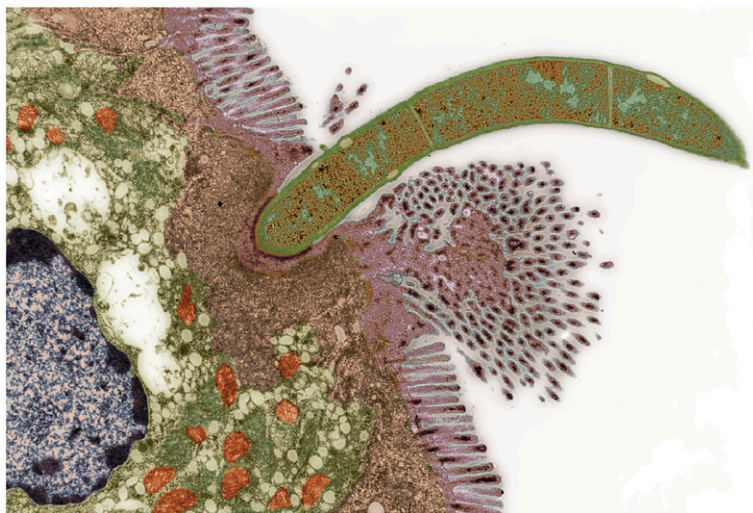
More importantly, those early years in Axel's laboratory prepared Littman for the singular findings on

HIV pathogenesis for which he is well known. By the early 1980s, researchers had found that the prime targets of HIV in the human immune system are CD4-containing helper T cells, and that the virus could glom onto CD4 molecules on T cells. But the precise handholds used by the virus to enter helper T cells were shrouded in mystery. Armed with gene-transfer tools perfected in Axel's laboratory, Littman engineered mouse cells to express human CD4 molecules on their surface and exposed the cells to HIV. The cells shrugged off the virus, suggesting that HIV needed more than just CD4 for successful entry into cells. "That set us on the course to look for the additional components for HIV entry," recalls Littman.

But Littman was not the only immunologist on the hunt for the so-called "co-receptors" of HIV. At the National Institutes of Health in Bethesda, immunologist Edward Berger and his team had discovered that a protein on the surface of T cells called CXCR4, which serves as a receptor for immune molecules called chemokines, was required for HIV to fuse with mammalian cells. In a close scientific race with at least three other research teams, including New York University microbiologist Nathaniel Landau, which ended in an ostensible tie, Littman found that a related but different chemokine receptor on T cells called CCR5, was crucial for HIV infection. "We now know that HIV enters human cells through a combination of CD4 and CCR5," says Littman.

The finding, published in *Nature* in 1996, marked a shift in the scientific understanding of HIV pathogenesis and led to the development of antiretroviral drugs against HIV (3). The CCR5-blocking drug maraviroc, manufactured by Pfizer, gained Food and Drug Administration approval in 2007 for HIV treatment in some patients; although the drug has since been supplanted by others that block protease enzymes in HIV, it remains an ingredient in some antiretroviral cocktails. More importantly, researchers soon found that natural genetic variations in CCR5 render some Caucasians largely resistant to HIV. Famous among such people, known as elite controllers, is the Berlin patient, an American who contracted HIV but is considered functionally cured thanks to a bone marrow transplant he received for acute myeloid leukemia from a donor with a genetic mutation in CCR5 that disables HIV infection. "That finding was proof that altering CCR5 might be a therapeutic strategy for HIV," says Littman. This proof set the stage for efforts aimed at engineering HIV resistance in people; several such efforts are ongoing, of which the CCR5 gene editing undertaken by the Harvard laboratory is but one example.

Goaded by these game-changing findings, Littman next focused on a feature of the human immune system that abets HIV infection. Dubbed dendritic cells, these immune sentinels normally aid T cells in fighting infections by presenting portions of antigens from pathogens to trigger an immune response. In the early 1990s, the late Rockefeller University immunologist Ralph Steinman stumbled on a puzzling observation: in laboratory dishes, dendritic cells greatly enhanced HIV infection of T cells while remaining uninfected themselves. Exploring the basis of this curious phenomenon, called transenhancement of



**Electron micrograph of long filamentous bacteria. Image courtesy of Dan Littman (New York University, New York, NY).**

HIV infection, which remains to be demonstrated in people, Littman found that the virus avoids replication in dendritic cells and circumvents the attendant immune response. "So HIV uses dendritic cells like a Trojan horse to infect T cells more effectively," explains Littman. Overriding the dendritic cells' resistance to viral replication results in the production of antiviral type 1 interferons and T-cell-mediated immune response against HIV. The findings, published in *Nature*, bear implications for HIV vaccine development (4).

In 1985, Littman accepted an assistant professorship in immunology at the University of California, San Francisco, eager to join his mentor Kirschner and cell biologist Bruce Alberts, who held positions there. Over the next decade, a succession of discoveries on T cells from Littman's laboratory solidified his position as a standard-bearer in the field. During this time, his team laid the groundwork for the subsequent discovery of gene switches called Runx proteins, which play a transient but crucial role in T-cell development and identity. Killer and helper T cells stem from precursors in the thymus gland that harbor both CD4 and CD8 molecules on their surface; through a process of selective elimination of the surface molecules, these precursors mature into one of the two types of T cells, namely helper and killer T cells. "The question of how this 'double-positive' cell decides to go in one direction or another remains a central one in developmental immunology," says Littman. Through brute-force genetics that took nearly a decade to bear fruit, Littman found that different Runx proteins latch onto a stretch of regulatory DNA called a silencer to suppress the expression of CD4 molecules in cells destined to become killer T cells (5). "This work provided a framework for understanding the signals important for making helper versus killer T cells," says Littman.

Years later, Littman and his postdoctoral fellows would unearth yet more fundamental insights on T cells, including a genetic switch for the development of a type of helper T cell called Th17 cells, which are



most abundant in the intestine. These cells secrete the eponymous molecule IL-17, implicated in inflammation and autoimmune disorders, such as arthritis, ankylosing spondylitis, and colitis. Antibodies against an array of proteins that influence IL-17 function have gained FDA approval for autoimmune diseases such as psoriasis. "These antibodies are tremendously effective and have led to high remission rates, validating the importance of this pathway," says Littman.

Against this backdrop, Littman's team explored the genetic control of IL-17 production by Th17 cells. Extending work begun by postdoctoral fellow Gerard Eberl, Littman and his collaborator Daniel Cua, then at the California-based DNAX Research Institute, together with postdoctoral fellows Ivaylo Ivanov, Liang Zhou, and Brent McKenzie, found that a gene switch called RORgammat controls the production of IL17 by Th17 cells, which through the small intestine. Mice in which the gene switch had been genetically ablated from intestinal T cells failed to produce IL-17 and proved more resistant to autoimmune disease than mice with the intact gene switch. The findings, published in a 2006 *Cell* article, suggested that the switch might be a drug target for such diseases (6). Preclinical trials promptly supported the notion. "Small molecule inhibitors of RORgammat have been effective in animal models of psoriasis," says Littman. And several drug companies are now pursuing ways to block the gene switch in an array of diseases; Vitae Pharmaceuticals, a Pennsylvania-based biotech firm, is conducting a phase 1 clinical trial of its RORgammat blocker for treating psoriasis.

In 1995, Littman returned to New York to accept a professorship in immunology at New York University's Skirball Institute, then a fledgling institute launched as a competitive hub for cutting-edge biomedical research in the city. Recruited by Lennart Philipson, the now-deceased founding director, Littman helped nurture immunology research at the institute, attracting several talented researchers to the university and fostering its program on molecular pathogenesis. While at Skirball, Littman probed the interaction of Th17 cells with gut microbes, which shape an ever-growing array of physiological functions. Working on a hunch that the abundance of these immune cells might be tied to the presence of certain gut microbes, postdoctoral fellow Ivanov set out to look for a link. "Ivo treated the mice with antibiotics to test whether the Th17 cells were somehow responding to the local environment in the gut, which is chock full of microbes. Sure enough, he found a link," says Littman. Before long, Littman's team also found that the place where the mice were purchased for these studies shaped the abundance of Th17 cells in the gut. Mice obtained from Taconic Farms, a purveyor of laboratory mice in upstate New York, had a wealth of Th17 cells in the small intestine, but those bought from Maine-based Jackson Laboratory, where the mice are raised

under ostensibly different conditions, had few of these cells. However, when the Jackson mice were housed in the same cage as the Taconic mice, the number of Th17 cells in their small intestine rose within weeks. Experiments in which the intestinal contents of the two groups of mice were separately transferred into mice denuded of gut microbes reinforced the link. "The gut microbes seemed to be required for making Th17 cells," Littman explains. But the identity of these microbes remained unknown.

Until, that is, Littman's team singled out a group of microbes teeming in the small intestines, called segmented filamentous bacteria, distant cousins of the human intestinal bacterium *Clostridium*, which helps keep inflammation in check. When introduced into the intestines of mice lacking Th17 cells, segmented filamentous bacteria induced the development of Th17 cells, triggered a protective immune response, and boosted resistance to the intestinal pathogen *Citrobacter rodentium*. Published in *Cell* in 2009, the findings pointed to a potential therapeutic target for enhancing gut mucosal immunity and treating autoimmune disease (7). Working with his Harvard Medical School collaborators Diane Mathis and Christophe Benoist, Littman also found that segmented filamentous bacteria trigger inflammation in mice prone to autoimmune disease.

Pursuing the therapeutic implications of the link between gut microbes and autoimmunity, Littman soon showed that people with untreated rheumatoid arthritis, an autoimmune disease marked by painful and debilitating inflammation of the joints of the hands and feet, among other symptoms, had higher levels of an intestinal bacterium called *Prevotella copri* than healthy people (8). By dominating the gut flora and crowding out beneficial bacteria, the thinking goes, *Prevotella* might influence inflammation and the risk of rheumatoid arthritis, a hypothesis that Littman's team is testing. "Together, these findings suggest that it may be possible to alter the immune response in people by altering the microbes in the lumen of the intestine," says Littman.

Over the past three decades, Littman has loped freely across the vast field of immunology, seeding its byways with insights so deep they may lead to a bounty of clinical benefits in the foreseeable future. Gazing into the crystal ball, Littman says his goal is to illuminate the countless ways by which gut microbes marshal groups of immune cells to guard against pathogens and shape human susceptibility to inflammation and autoimmune disease. "Ultimately, we would like to be able to manipulate immune responses with microbial products or their mimics to restore the immune system to homeostasis from disease. Understanding how microbes influence the T-cell immune response can also lead to ways to improve cancer immunotherapy and even vaccines," he adds with genial but well-founded optimism.

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