PROFILE

Profile of Bruce A. Beutler

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Late one night in October 2011, Bruce Beutler checked his e-mail and saw a tantalizing subject line: it simply said, "Nobel Prize." Beutler's first thought was that it was a general announcement about that year's Nobel Prizes, but as he read further he realized he had been awarded the 2011 Nobel Prize in Physiology or Medicine. "Usually people speak of the phone call they received, but in my case I was notified by e-mail because nobody knew my phone number," says Beutler. "There was a period of disbelief," he says, but he checked on-line and found his name showing up in news releases all over the world, "and then I was very excited."

Beutler received the prize for his discovery of Toll-like receptor (TLR) 4, the first-known mammalian receptor protein of the innate immune system. Beutler discovered the process by which mammals, including humans, detect invading microbes, a crucial initial step in mounting an immune response. He shared the prize with immunologist Jules Hoffmann, who discovered an immune function for the Toll receptor in fruit flies, and Ralph Steinman, who discovered dendritic cells and their role in adaptive immunity. "The LPS sensing role of TLR4 was a huge surprise," says Beutler, director of the Center for the Genetics of Host Defense at the University of Texas (UT) Southwestern Medical Center in Dallas. Beutler had already received several other awards for his contributions to immunology and had just returned from Hong Kong, where he shared the 2011 Shaw Prize in Life Sciences and Medicine, when he learned about the Nobel Prize. He was elected to both the National Academy of Sciences and the Institute of Medicine in 2008. As it turns out, Beutler got his first taste of scientific research thanks to another National Academy of Sciences member: his father, Ernest Beutler.

Early Introduction to Research

Beutler became interested in nature in general—and particularly biology—at an early age, and his father encouraged his interest in research. Ernest Beutler was a highly regarded hematologist. When Bruce Beutler was about 14 years old, he began working in his father's laboratory at the City of Hope Medical Center. After school, on weekends, and during holidays, he learned how to assay red blood cell enzymes and how to separate and purify proteins, skills that he says came in handy later on in his career. Beutler got his first introduction to immunology and mammalian genetics when he worked with Susumu Ohno, also at the City of Hope Medical Center. Beutler continued to learn about genetics while doing research on fruit flies as an undergraduate in the laboratory of Prof. Daniel Lindsley at the University of California, San Diego. After finishing his undergraduate degree, Beutler went to the University of Chicago School of Medicine in 1976.

Beutler says his father advised him to go to medical school to learn how entire organisms function, and gain the foundational skills he would need to do well in almost any area of science. "He also felt that if I should not turn out to be particularly good at independent research, then I would be able to fall back on my medical school training and be a clinician if I wanted to," Beutler says. Although Beutler never had any reason to leave research, he still found his father's advice worthwhile. "I am still influenced by the experiences I had in medical school," he says. "You never forget the diseases you've seen, and I definitely saw a lot of inflammatory disease and a lot of infection." Beutler says seeing these diseases influenced his decision to study the innate immune system.

After completing his residency training at UT Southwestern Medical Center in 1983, Beutler went to the laboratory of Anthony Cerami at The Rockefeller University. There he isolated a molecule known as cachectin, the mouse ortholog of human TNF, a cell-signaling molecule that can induce cell death (1). Beutler found that this molecule mediated many of the effects of LPS, a large molecule found in the outer membrane of Gram-negative bacteria that induces a strong immune response in animals. After becoming an assistant professor at The Rockefeller University in 1985, Beutler returned to the UT Southwestern Medical Center in 1986 and spent the next 14 years studying TNF biosynthesis and LPS signaling. Beutler's research led him to create a TNF neutralizing reagent, which was later used commercially to treat rheumatoid arthritis and other diseases as the drug Etanercept, also known as Enbrel (2). "That's been a great help to millions of people, and I'm proud of that," he says. However, it was Beutler's work on LPS that would lead to his Nobel Prize-winning discovery.

Bruce Beutler. Photo by Brian Coats for UT Southwestern Medical Center, Dallas, TX.

Hunt for the LPS Receptor

Beutler first heard about LPS when doing undergraduate research, well before he started working on it. During medical school he learned about the problems, including septic shock, caused by the body's intense immune reaction to LPS. When Beutler started to work on TNF, he says, he began to ask himself, "How is it that LPS is sensed? In fact, how do we sense microbes at all? Obviously we've evolved an innate immune mechanism to do that, and obviously it has nothing to do with adaptive immunity." Research into innate immunity, the body's relatively nonspecific first line of defense against infection, lagged behind adaptive immunity for a long time, Beutler says. "Adaptive immunity, and especially the ability of the adaptive immune

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system to create staggering receptor diversity, was such a spectacular intellectual challenge for so long that innate immunity, by contrast, seemed rather pedestrian to many people." However, Beutler says the innate immune system has to cope with the same problems as the adaptive immune system: "It has to be tolerant to self, and it has to be very effective at recognizing a huge variety of microbes." Beutler wanted to figure out how the innate immune system was able to detect microbes, and reasoned that there must be receptors that detect conserved molecules of microbial origin. However, he initially put aside the challenge of finding these receptors, "because it was so unapproachable in the early 1980s. Only in 1993 did I dare to approach it through genetics, and only genetics sufficed to crack the problem."

Around 1993, Beutler's main goal became to find the LPS receptor. "LPS was more than a 100 years old, and in all that time nobody had found its receptor," he says. After a number of false starts using biochemical methods, Beutler decided to use positional cloning, a technique to identify the gene corresponding to a specific phenotype based on its precise chromosomal location. Beutler's group spent five years tracking down the mutations that caused two different strains of mice to be unable to respond to LPS. "It was extremely hard to positionally clone these mutations," Beutler says. "We could never narrow the region below about five megabases, and in the beginning, at least, we mostly sequenced by hand, which made the project exceedingly difficult."

However, the hard work paid off, as Beutler found the mutations and discovered that they inactivated TLR4 function (3). "This suggested that TLR4 was the membrane-spanning component of the LPS-receptor complex, which people had searched for a very long time. It was a tremendous feeling to finally identify that mutation," Beutler says. "We felt we had likely discovered the mechanism by which most microbes were identified by the immune system of the host, and that turned out to be true in large part, as TLRs are one of the major ways we become aware of infection." Beutler says that discovering the LPS receptor was his proudest scientific achievement to date.

Moving Ahead with Forward Genetics

Buoyed by his success in finding the LPS receptor, Beutler moved to The Scripps Research Institute in La Jolla in 2000 to set up a forward genetics program. "I'd become enamored of the forward genetics approach because of the LPS positional cloning story. It just was so compelling to narrow down a phenotype and to finally find it, and I thought it would be wonderful to be proactive, creating new phenotypes to study," he says. Beutler used the potent mutagen *N*-ethyl-*N*-nitrosourea, or ENU, to mutagenize mice to create new phenotypes. He built a large-scale ENU mutagenesis laboratory at The Scripps, and says he screened more than 150,000 mice over the years, looking for immune deficiencies. "In all, we tracked down something like 260 phenotypes that cause immune deficiency, which corresponded to 95 genes that shed light on many different aspects of immunology," Beutler says. These genes revealed much about how TLRs signal, how mice resist viral infection, and how an antibody response is launched, he says. There were also other phenotypes that had nothing to do with immunology, including some that dealt with neurobehavioral control, hearing, vision, development, and iron metabolism.

The mouse phenotypes that had to do with iron metabolism led Beutler to collaborate with his father, who was the Chairman of the Department of Molecular and Experimental Medicine at The Scripps Research Institute. "That was a pleasure; it was really very enjoyable, and I was glad to be able to do that," Beutler says. He recalls that the two talked about their work nearly every day, and published a paper in *Science* in 2008 about a mouse mutation that affected iron metabolism, a subject in which Ernest Beutler was an expert (4).

Beutler returned to UT Southwestern in 2011 to become Director of the Center for the Genetics of Host Defense, and continues his ENU mutagenesis work to the present day, noting that it has been empowered by advances in technology. He says the field has changed enormously in the last 10 years or so. "It was just so hard back in the old days to track down genes and to establish cause and effect. It took us five years to find the LPS receptor mutations," he says. Beutler began focusing on a forward genetics approach when it was still a time-consuming and laborious process, in anticipation of new sequencing techniques that would drastically accelerate mutation identification. "I think everybody in the mouse genetics field was salivating at the prospect of faster sequencing for many years before it became a reality. The hard part of forward genetics used to be the positional cloning step, but now, because the mouse genome has been sequenced and annotated, and because we can sequence about a million times faster for one-millionth the cost per base pair, it's a completely different game," Beutler says.

Beutler envisions a time when sequencing will be so rapid that researchers will be limited only by the rate with which they can make phenotypes and screen them, and his current operations are set up to make that vision a reality. "It's already almost at that stage. One day we will be able to see a phenotype in the morning, and know the mutation by afternoon," he says. Beutler's group has so far archived about 13,000 mutations related to various phenotypes, and he plans to add about a million more over the next five years. The idea is to have a variety of mutations in almost every gene corresponding to various shades of phenotype. "It may then be possible to do combinatorial experiments to better understand just what it takes for the immune system to accomplish a particular task," Beutler says.

1 Beutler B, et al. (1985) Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. *Nature* 316(6028):552–554.
2 Peppel K, Crawford D, Beutler B (1991) A tumor necrosis factor (TNF) receptor-IgG heavy chain chimeric protein as a bivalent antagonist of TNF activity. *J Exp Med* 174(6):1483–1489.
3 Poltorak A, et al. (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: Mutations in Tlr4 gene. *Science* 282(5396): 2085–2088.
4 Du X, et al. (2008) The serine protease TMPRSS6 is required to sense iron deficiency. *Science* 320(5879):1088–1092.