MolecularMedicine

Anthony Cerami Award in Translational Medicine

A Journey in Science: Promise, Purpose, Privilege

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Real innovations in medicine and science are historic and singular; the stories behind each occurrence are precious. At *Molecular Medicine* we have established the Anthony Cerami Award in Translational Medicine to document and preserve these histories. The monographs recount the seminal events as told in the voice of the original investigators who provided the crucial early insight. These essays capture the essence of discovery, chronicling the birth of ideas that created new fields of research; and launched trajectories that persisted and ultimately influenced how disease is prevented, diagnosed, and treated. In this volume, the first Cerami Award Monograph, by Carl Nathan, MD, chairman of the Department of Microbiology and Immunology at Weill Cornell Medical College, reflects towering genius and soaring inspiration.
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PROMISE

Mrs. C. put a lot into her last party—a lot of makeup to brighten her skin, a lot of morphine to dull her pain, and a lot of money to set bouquets about her New Haven home and freight her dining room table with drinks and delicacies. The guests were her traditional family and friends, together with her new set: her doctors and nurses.

She approached to bring me a glass of wine, put a hand on my chest and pushed me against the wall. "I hear you've done research. Is that true?" I admitted I had—five summers in high school and college, and a year in medical school. "Then promise me that's what you'll do. You doctors worked hard to help me, but you failed, didn't you? Don't just keep doing what you do. Make it better."

There are many reasons to make a promise and many ways to honor one.

When I finished ninth grade, I decided to become an artist. My parents showed me a column in the *New York Times* by the art critic, John Canaday. Some of his friends had asked him to advise their own son about his ambition to become an artist. "Do it," Canaday wrote (I'm paraphrasing from memory), "but only if you can't possibly imagine doing anything else." That summer, I began working in an experimental pathology lab at New York University (NYU) Medical Center, run by Professor Lester Grant, a college friend of my parents'. Within a month, I couldn't imagine doing anything else.

In the third summer—1962—I used the daily train ride into New York City to read Claude Bernard's *An Introduction to the Study of Experimental Medicine*, whose foundational role in medical science I would only recognize years later. In the basement where NYU housed its library, I cracked open *The Journal of Experimental Medicine* to read about discoveries Dr. Grant had got wind of, such as chambers that Stephen Boyden devised to demonstrate leukocyte chemotaxis *in vitro* (1). I took notes on index cards and reported to Dr. Grant while he operated, installing transparent chambers in the ears of rabbits so we could look through the microscope later and watch leukocytes emigrating from venules in response to focal inflammatory signals. I discharged my dual assignment—to scour the literature and the rabbits' cages—with enough diligence to be promoted to assist at surgery.

I was welcomed into a world I hadn't imagined, where doers were thinkers, thinkers were doers and membership was based on merit. Our team was diverse in our ages, colors and countries of origin. We shared science: a powerful fellowship.

Although I returned to the lab each summer through high school, I did so only one summer during college at Harvard, where I was intent on following in

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my brother's footsteps by studying East Asian history. By senior year, I decided I could admire Andy without following him. I would strike out in a direction that was new for my family by applying to medical school.

One night late in December 1966, Western Union phoned, rousing me from bed. The operator read me a telegram offering early acceptance at Harvard Medical School. Earlier that afternoon, I had watched my mother die of cancer.

By the time Mrs. C. pushed me against the wall to demand a promise, I had finished medical school, residency at Massachusetts General Hospital and Public Health Service duty at the National Cancer Institute in Bethesda. I was nearing the end of oncology fellowship at Yale and had been invited to join the faculty as an assistant professor. I had to decide whether to stay or leave. Mrs. C. would die in a few weeks, one of many to do so in my care, each of whom gave me far more than I could give them. What she demanded now didn't change my path; it made me realize that I had one.

PURPOSE

Neutrophils, macrophages and lymphocytes can destroy any tissue in the body that emits signals of both infection and damage (2). If we want to focus the destructive power of nonmalignant cells on malignant cells, we need to understand what chemistries some cells encode that kill others and by what mechanisms cells deploy, restrain or resist these chemistries. To learn these things became my goal.

The two biochemical pathways I ended up exploring—production of reactive oxygen species (ROS) and reactive nitrogen species (RNS)—turned out to be universal in biology, parallel in structure and independent in evolution (3,4). At first, I was astonished that pathways whose ability to kill is necessary for our survival in a competitive biosphere also operate continually to maintain what Claude Bernard called the "milieu intérieur," or homeostasis (4). In time, I recognized that each of these seemingly opposite activities is predictable from the other (5). It was not necessary that organisms evolve different sets of molecules for killing and homeostasis; on the contrary, killing at the cellular level results from the excessive or untimely imposition of normally homeostatic signals. Host and pathogen are each skilled at forcing the other to mis-time or overexpress their own homeostatic signals.

Evidence for the broad biological relevance of ROS and RNS in signaling grew explosively, but acceptance lagged. There was a prejudice against the biological relevance of ROS and RNS in signaling because they were considered to be "nonspecific." Eventually I recognized the fallacy of the presumption that there is only one type of specificity. ROS and RNS signal with a type of specificity that is orthogonal to and complements the type that is more familiar to cell biologists (5,6).

But this gets ahead of the story. The search began with the question of regulation.

Macrophage Activation: Cell–Cell Communication

By 1968, when I took a year off from medical school courses to work in John David's lab, there was a major conceptual challenge facing immunology: a paradox posed by George Mackaness. Like Lester Grant, Mackaness had been a student of Sir Howard Florey's at Oxford, where he studied the action of antibiotics against *Mycobacterium tuberculosis* in macrophages. From the Dunn School on the edge of the Oxford University Parks, he moved to the Trudeau Institute on the shores of Saranac Lake in upstate New York. The institute had been founded by Dr. Edward Trudeau to advance the care and study of tuberculosis. Mackaness discovered that macrophages taken from mice during an infection, or after reinfection with the same organism, were induced from a state of minimal competence in the control of mycobacteria and other facultative intracellular bacterial pathogens to a state of heightened antimicrobial activity. "Macrophage activation," as he called it, was immunologically specific in its induction, but nonspecific in its expression (7–9). The importance of this discovery propelled Mackaness to the directorship of the Trudeau Institute, but the underlying mechanism remained a mystery.

This problem gripped my imagination. Since lymphocytes had recently been discovered to control immunologic specificity, I thought the explanation must lie in cell–cell communication. I could approach it by working in a lab that studied both types of cells: macrophages and lymphocytes. Then as now, there were few such labs. Fortunately, one of the best was at Harvard, in the Robert Breck Brigham Hospital atop Boston's Mission Hill. Within the hospital, though, Professor John David's lab was not on top; it was in the basement. The lab upstairs belonged to the chairman, a world-leading immunologist, Professor K. Frank Austen.

Professors David and Austen were strikingly different. John and his wife, Roberta, who was also his lab manager, invited my wife, Amy, and me to dinner at their home, where they made sure we became acquainted with Stravinsky on the hi-fi and whatever John was mastering at the grand piano. They sat through the night with us in the medical school dorm common room to watch the *Apollo 11* moon landing on TV. John even undertook to cure my naiveté by introducing me to risqué foreign films. Professor Austen, in contrast, marched through the labs with a steel-tipped wooden yardstick to swat us on the butt, so we would attend seminars on time. Time was important. He timed our practice talks with a large, ticking alarm clock, yardstick at the ready. All three—John, Roberta and Frank—became lifelong friends.

Since precisely measured time was important to Professor Austen, it would be important to me, too. I set up complex, meticulously controlled experiments that could only be carried off with operations spaced at 30-second intervals. I kept track of the steps with a stopwatch, following

flow charts I wrote out on graph paper and pasted up over my bench. First, though, I had to make myself a bench.

Cytokines: Dawn of an Era

The David lab was given over to the production of a new entity, a "factor," later dubbed a "cytokine," released by antigen-stimulated lymphocytes. The "factor" made macrophages stop migrating out of the pellets where they had been centrifuged together in the bottom of glass capillary tubes plugged with clay. Whatever persuaded the macrophages to stay put without killing them was called "migration inhibitory factor" (MIF). Enormous excitement surrounded the news of the simultaneous discovery of MIF by John David (10) and Barry Bloom (11). The excitement mounted with Peter Ward's finding 3 years later that a lymphocyte factor could have a seemingly opposite effect, promoting macrophage chemotaxis in Boyden chambers (12), and Granger's report in the same year that antigen-stimulated lymphocytes could release cytotoxic factors (13). MIF, chemotactic factor and lymphotoxin provided the first evidence that lymphocytes could release anything other than antibody (assuming these factors weren't antibodies) and thereby affect the behavior of other cells.

Bizarre though it was, the MIF bioassay was therefore the point of a very big spear, which took the form of a very pointed question: How could cells communicate with each other without using neuronal synapses or blood-borne hormones? Migration inhibition was what we saw, but what else did these messages convey, to what end, and how?

The David lab version of putting a man on the moon was to purify MIF. We slaved in teams, immunizing dozens of guinea pigs, harvesting their enlarged lymph nodes, teasing the tough nodes apart with forceps until our fingers and wrists ached, adding antigen to the cell suspensions, collecting the supernatant and turning it over to the gifted entomologist-turned-biochemist Heinz Remold. Heinz tirelessly applied what could have been called, in retrospect, low-performance column chromatography. It emerged later that cytokines work at nanomolar to picomolar concentrations, far below the capacity of the techniques of the time. It was as if we were trying to reach the moon by linking ladders together. Heinz fought off hopelessness with humor. Eventually, he achieved partial success: MIF had the properties of a moderately sized glycoprotein distinct from immunoglobulin.

After a few months on the production line, I realized my only hope of escape was to set up my own lab within the David lab. I found a barely used storeroom, cleaned it out, constructed a bench, taped my time-course protocol on the wall, ignored the laughter it provoked and began asking what the products of antigen-stimulated lymphocytes did to macrophages besides slow their migration.

I decided to look for metabolic hallmarks that might be associated with enhanced antimicrobial activity. This idea sprang from something I heard from yet another powerful individual, Professor Manfred Karnovsky, who lectured to our first-year biochemistry class. A pioneer in analyzing the biochemical basis of the "extra respiration" of phagocytosis in neutrophils, Karnovksy was just then discovering that phagocytosis triggers the hexose monophosphate shunt (HMPS) (14). It would later emerge that the HMPS generated NADPH to sustain the generation of superoxide and hydrogen peroxide (15) and the recycling of glutathione with which phagocytes protected themselves from their own ROS. Macrophages phagocytize, too, I reasoned. Might they operate a HMPS? Might "macrophage activation" enhance their capacity to do so? When I brought this question from the Harvard Medical School quadrangle back to the immunologists in the hospital on Mission Hill, it's as if I said, "Haven't you heard of intermediary metabolism?" just as a new postdoctoral fellow in my own lab, K. Heran Darwin, would say 30 years later, "Haven't you heard of genetics?"

I learned three lessons in the time I spent with John David and Manfred Karnovsky between 1968 and 1970 (after my year in the lab, I returned later for a few more months during the clinical years of medical school). First, science is a bridge. The two professors had never met, yet each gave me a key to his lab. It was my privilege to introduce them to each other. I learned how to measure cellular glucose oxidation in the biochemistry lab and applied the methods in the immunology lab. Second, I found the second example of what a soluble product of antigen-stimulated lymphocytes could do to another cell: activate intermediary metabolism in macrophages (16,17). Third, as Heinz Remold schooled me in protein biochemistry, I learned that macrophage-activating factor (MAF), like MIF, was a nonimmunoglobulin glycoprotein (18). I didn't have time to test the prediction that enhanced glucose oxidation through the HMPS was accompanied by enhanced antimicrobial activity, but this was established by a medical student who followed me in the lab, Robert Fowles (19). This body of work provided the first molecular link between lymphocytes and adaptive immunity on the one hand and macrophages and innate immunity on the other.

Did enhanced HMPS activity correspond to and help drive enhanced ROS production? Did enhanced ROS production contribute to enhanced antimicrobial activity? MIF and MAF copurified by the techniques of the day, but were they the same, or was MAF a fourth distinct cytokine after MIF, chemotactic factor and lymphotoxin? If one cytokine could activate macrophages, could another cytokine deactivate them?

Before I could address those questions, there were a few things to attend to—the rest of medical school, internal medicine residency, Public Health Service duty at the National Institutes of Health (NIH) (it was that or Vietnam, according to my draft board), oncology fellowship and a choice: whether to stay at Yale, practice medicine and do some research on the

side, or find a place to pursue research uninterrupted.

Enter ROS

Of course, there was no such choice. Nothing in life is uninterrupted. The fork in the road was whether the interruptions would include the needs of cancer patients and their families along with everything else, or just everything else. What shaped that decision, one of the most important I made, was a string of failures, leading accidentally to a life-altering experience.

The oncology fellowship program was funded in part by an outlying community hospital. I was told I must work there for 3 months. When I arrived, the lone oncologist on staff seemed overwhelmed. Perhaps he sized me up and decided it would be too hard to train me how to administer potentially lethal drugs. Perhaps it was too hard for him that so many of the people waiting outside his office for those drugs were in their teens and twenties and would not be getting older. In any event, on my fifth day in the post, he pulled up his station wagon to a side door, cleaned out his desk and drove away. I felt it would be irresponsible for me to manage the patients on my own. I petitioned the department to send a board-certified oncologist to the outlying hospital or advise the hospital to send their patients to the medical center. The department refused. In turn, I refused to continue the rotation. I was about to be expelled for insubordination when the program director made an offer. I could remain in the program, but only if I retrieved all copies of my letter of protest, tore them up in his presence and spent the rest of the rotation out of his sight.

Thus, my inglorious entry to the lab of Dr. Richard Root in the Division of Infectious Disease, a safe administrative distance from the Division of Hematology and Oncology. Under Dick's tutelage, I learned to measure the respiratory burst of neutrophils. The respiratory burst—the abrupt, large-scale, nonmitochondrial reduction of oxygen to produce ROS—was just then emerging as the first molecularly defined antimicrobial mechanism of the innate immune system (6). I saw my chance to return to unfinished business with activated macrophages. I soon found that macrophages were the second kind of cell capable of releasing large amounts of ROS. In contrast to neutrophils, however, macrophages had to be immunologically activated (20).

Almost every scientific discovery requires or affords an opportunity for self-discovery. I was devoted to medical oncology, despite certain inconveniences that may have stemmed from my status as a misfit and rebel. For example, I was the fellow most frequently on call but was never given an on-call room. If the security guards forgot to lock up the clinic, I could sleep on an examining table, using sheets I stole from linen carts on the wards. When the clinic was locked, sometimes I could cadge a key to the Heme-Onc library. No matter—I could cope with the frustrations of living in a hospital. But when I had begun assays on multiple populations of macrophages in head-to-head comparisons, and the phone rang to summon me to the emergency room, and I returned hours later, the macrophages had to be poured down the drain. That was frustration of another order. Shortly after Mrs. C.'s shove, I shelved clinical oncology. I rented an electric typewriter, sat at the kitchen table and wrote a paper for *The Journal of Experimental Medicine*, an application for a fellowship from the Leukemia Society, an RO1 grant from NIH and a request for a position on the faculty at The Rockefeller University.

Identifying Interferon-γ

Four acceptances later, I joined Zanvil Cohn's interdisciplinary lab at Rockefeller. The Cohn lab presented extraordinary opportunities. René Dubos occupied the office across the hall and would occasionally discuss articles, including mine. Ralph Steinman had just discovered dendritic cells (21). Sam Silverstein had just launched the field of cell biological studies of phagocytosis (22). Andy Luster, an MD/PhD student working with Jay Unkeless, teamed up with Jeff Ravetch to discover the first chemokine, interferon-γ–inducible protein 10 (γ-IP 10) (23). Bill Muller and Michel Nussenzweig, two more MD/PhD students, would later join me as coeditors of *The Journal of Experimental Medicine*, along with an inspiring biochemist on the faculty, Tony Cerami. Cerami's work epitomized the idea that biochemistry offered a powerful approach to understanding infection biology. His trainees included Kevin J Tracey and Bruce Beutler. As with many others at Rockefeller, his approach refuted a prevalent notion that basic scientists should ignore questions posed by disease and shun the medical application of what they learned. In this, Tony followed in the footsteps of the great Maclyn McCarty, another editor of *The Journal of Experimental Medicine*. Their approach to basic science would be counted as "molecular medicine" and "translational medicine" today.

I entered this extraordinary environment with the first biochemical assay for macrophage activation—enhanced capacity to produce ROS—and used it as a readout to return to the question, which glycoprotein is MAF? It proved to be a blessing that years of medical residency and fellowship had delayed my return to this problem. The intervening years brought advances in column chromatography and the advent of monoclonal antibody and recombinant protein technology. With these four sets of tools, I was finally able to identify the major MAF. Its identity was completely unexpected: interferon (IFN)-γ (24). Together with enhanced ROS-releasing capacity, my Cornell colleague Henry Murray showed that IFNγ induced enhanced ability of macrophages to control the protozoal pathogens *Toxoplasma gondii* (24) and *Leishmania donovani* (25).

The first evidence was with primary human macrophages *in vitro* (24), but I was eager to test the idea *in vivo*. The Cohn lab provided yet another opportunity. Rockefeller University and its next-door neighbor, Cornell University Medical College, which was renamed Weill

Cornell Medical College in 1998, were pioneers in what is now called "global health." At Cornell, Professor Walsh McDermott was pioneering the treatment of tuberculosis and developing mouse models of the disease, whereas Professor Benjamin Kean was a leader in "tropical medicine." At Rockefeller, Dubos, whose former student Bernard Davis had gone on to teach me microbiology at Harvard Medical School, had been the world leader in studying *Mycobacterium tuberculosis* in culture. Tony Cerami and George Cross were working on African trypanosomiasis. William Trager had just discovered how to culture malaria parasites in red cells *in vitro*. No wonder that various members of the Cohn lab turned to leishmaniasis, South American trypanosomiasis and leprosy.

Cohn, Wes van Voorhis, Gilla Kaplan, Steinman and their colleagues were helping to characterize polar lepromatous leprosy as a deficiency of macrophage activation in response to *Mycobacterium leprae* (26). Where better, then, to test if IFNγ would work as MAF *in vivo*, than in patients with that disease? With the enthusiastic support of Costa Sevastopoulos at Genentech, I wrote an Investigational New Drug Application to the U.S. Food and Drug Administration (FDA), teamed up with leprologist William Levis and admitted some of New York City's hundreds of lepromatous leprosy patients to the Rockefeller University Hospital. Because the bacillus could not be cultured, progress in treating the disease is measured by scoring skin biopsies for reduction in bacterial burden. To my astonishment, intradermal jet-gun injection of tiny amounts of IFNγ led to rapid clearance of most of the bacteria from the injection site (27).

After I moved to Cornell in 1986, I found that the same treatment led to rapid clearance of most of the bacteria from distant sites as well (28). A series of studies I conducted, collaborated in or helped design soon showed that administration of IFNγ activated monocytes in the blood of cancer patients (29), led to resolution of visceral leishmaniasis (30) and reduced the incidence of serious infections in children with chronic granulomatous disease (31). The latter finding led to FDA approval of the cytokine.

The ability of IFNγ to activate macrophages *in vivo* in mice (32) was followed by evidence from others for comparable phenotypes toward mycobacterial infection in mice lacking IFNγ or its receptor (33) and in people with genetic deficiencies of IFNγ production or signaling (34). IFNγ-releasing capacity in response to mycobacterial antigens is now a clinical test for infection by *Mycobacterium tuberculosis* and a biomarker used to monitor trials of tuberculosis vaccines.

These findings contributed to a growing impression of universality involving the actions of activated macrophages in different species and perhaps in different functions. The next big fork in my path arose when that idea collided with results of our studies on tumor cells.

Initially, the studies of macrophage actions on tumor cells seemed to progress in parallel with studies of their actions on protozoa and bacteria. I saw it as evidence that macrophage activation had the potential to control tumors when we found that tumor cells went to the trouble of releasing a factor that could antagonize or even reverse IFNγ-induced macrophage activation (35). We eventually purified the macrophage deactivation factor (MDF) (36) but were never able to clone it. Descartes wrote, "Je pense, donc je suis" (I think, therefore I am); contemporary biologists are more likely to say, "It's been cloned; therefore it exists," with the corollary, "If it hasn't been cloned, it doesn't exist." Thus, it had far greater impact when we identified transforming growth factor (TGF)-β as the first cloned cytokine that could prevent or reverse macrophage activation (37) and interleukin (IL)-10 as the next (38). This work introduced a phenotype overlapping with what is now called "alternative macrophage activation" and focused attention on the tumor-supporting properties of many tumor-associated macrophages, a topic of widespread current research.

Absent MDF, TGF-β or IL-10, IFNγ-activated macrophages indeed killed mouse tumor cells, and I implicated production of ROS as one mechanism (39–47). I could even cure mice of a highly lethal lymphoma with artificial ROS-producing macrophages comprised of latex beads coated with glucose oxidase, an enzyme that produces hydrogen peroxide (44).

What a surprise, then, when human tumor cells proved to be about two orders of magnitude more resistant to hydrogen peroxide than mouse cells (48). Investigation into the basis for this led to two important findings. Many human tumor cells produce as much ROS as activated macrophages (49), and in some cases, this contributes to their autonomous growth (6). They are protected from ROS by up to six peroxiredoxins, among other antioxidant defenses (50). It was becoming clear that not all the microbicidal or tumoricidal actions of activated macrophages could be attributed to ROS. The next big question is "what other chemistries were involved?"

Beyond ROS: Inducible Nitric Oxide Synthase

By 1987, John Hibbs had discovered a macrophage-mediated tumoricidal process dependent on L-arginine and associated with production of nitrite, although nitrite was neither tumoricidal nor microbicidal (51). The burning questions were the identity of the cytotoxic substance and the enzymatic process by which mammalian cells could generate it. In 1989–1991, Dennis Stuehr, Hearn Cho, Nyoun-Soo Kwon and I identified a cytokine-induced enzyme that produced nitric oxide via incorporation of molecular oxygen into the guanidino group of arginine via an *N*-hydroxy-L-arginine intermediate and showed that this accounted for much of the antitumor activity of activated mouse macrophages (52–60). What allowed purification of all three mammalian nitric oxide synthases was our identification of NADPH as a cosubstrate, leading to an affinity chromatographic technique (58), and our discovery (52), simultaneously with Michael Marletta's lab (61), that tetrahydrobiopterin is a cofactor. Both findings allowed us to complement protein fractions with the cosubstrates

and cofactor so we could monitor enzyme activity during purification.

When we purified (54) and cloned (62) the enzyme from macrophages, we could formally demonstrate its synergistic transcriptional induction by cytokines and microbial products (62–64) as well as its calcium independence. Both features stood in contrast to the NO synthases cloned earlier (neuronal NOS) and later (endothelial NOS) (4,65). Accordingly, I named the new enzyme "iNOS" for NOS *i*nduced in response to *i*mmunologic and *i*nflammatory stimuli and *i*ndependent of elevated *i*ntracellular calcium (62). This was followed a few months later by the enshrinement of nitric oxide as "molecule of the year" by *Science*. There ensued a torrent of iMacs, iPods, iPads, iPhones, iPS cells, iNKT cells and iTregs. Whether "iNOS" had anything to do with this can be left to linguistic historians.

Biochemically, it was a surprise that there is a family of mammalian enzymes that contain both flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). More baffling, the other two NOSs were catalytically inactive without Ca^{2+}-activated calmodulin, an observation first made by David Bredt and Solomon Snyder en route to purifying nNOS (NOS1) (66). How could the highly homologous iNOS dispense with this requirement and thereby acquire the capacity to produce orders of magnitude more product per response episode? With the help of colleagues at Merck Research Laboratories, we discovered that the iNOS complex included calmodulin as a tightly bound subunit without requiring that calmodulin bind calcium (67). Using chimeric proteins, we demonstrated that specific regions of iNOS conferred this unprecedented property (68), endowing iNOS with the high output phenotype that gives it antimicrobial potential. Thirteen years later, the second example of this phenomenon emerged in a study of *Bacillus anthracis'* adenylyl cyclase, a type of enzyme that is normally calcium-dependent but in this case had acquired cytotoxic potential—this time, against the host—by attaching calmodulin without calcium (69).

Pharma Power

iNOS afforded another instance where discoveries about the question under study went arm-in-arm with self-discovery. The self-discovery was that I found myself powerfully attracted to the effectiveness of working with a diverse team of outstanding scientists in a well-equipped and well-resourced drug company on an academic, collaborative basis. That experience profoundly affected my thoughts and actions beginning about 15 years later. The collaboration was extraordinary. Philip Davies, Executive Director of Immunology and Rheumatology Research at Merck, invited my student, Hearn Cho, to stay with him in his home near the Rahway campus, where he gave Hearn access to a 250-liter mammalian cell fermentor. Hearn returned to the lab at Cornell with grapefruit-sized cell pellets of IFNγ- and lipopolysaccharide (LPS)-activated RAW 264.7 macrophages as starting material for purification. Richard Mumford, an ace biochemist at Merck, encouraged me to rent a pickup truck and drive up to a side entrance of the Rahway facility. Richard distracted the guards while we borrowed several chromatography workstations to supplement the single unit we had in our lab. At Cornell, we worked in shifts to purify the protein. I then drove a truck back to Merck. Richard distracted the guards again while we returned the equipment.

We used the protein to raise antibody. Qiao-wen Xie used the antibody to isolate phages expressing the cDNA. We sequenced the cDNA by hand and took turns reading the gels. When we had electrospray ionization mass spectroscopic evidence of the masses of individual tryptic peptides, we sat at Richard's kitchen table trying to match them to the masses predicted from the cDNA, taking natural isotope abundance into account. What we labored at was one of the earliest examples of a technique that is now a computerized routine.

Soon another student, John MacMicking, moved in with Phil Davies and worked with another star at Merck, mouse geneticist John Mudgett. By 1995, they had produced iNOS knockout mice. We tested the mice first in models of endotoxic shock and listeriosis. Gary Hom, a physiologist at Merck, used carotid artery catheterization of wild-type and knockout mice to demonstrate that iNOS mediates the hypotension of endotoxic shock. Shortly after that, I developed septic shock myself. When I was moved out of the intensive care unit to a regular hospital bed, my lab mates brought me a laptop and a stack of reprints and I finished writing our manuscript on iNOS knockout mice (70). This contributed to the impression that it takes a near-death experience to write papers that are readily accepted at *Cell*.

John Mudgett and our lab shared the iNOS knockout mice with over 100 labs, while Jackson Labs distributed to others iNOS knockout mice that had been generated independently by Victor Laubach and Oliver Smithies (71). The ensuing worldwide effort revealed that iNOS plays an important, complex role in host defense and inflammation. Our own contributions focused on tuberculosis (72), viral infections (73) and Alzheimer's disease (74), while others addressed conditions ranging from obesity-dependent insulin resistance (75) to chronic obstructive pulmonary disease (76,77), to name just a few. Thus, high output production of RNS took its place alongside high output production of ROS as a cardinal feature of "classical" macrophage activation and a principle effector of both the enhanced antimicrobial activity and host toxicity of macrophages, but both sets of signaling molecules have diverse roles in numerous other cells (4–6,78).

Continuing John MacMicking's work, Michael Shiloh then studied mice lacking both iNOS and phagocyte oxidase ("phox"), the enzyme that generates most of the ROS in activated macrophages. Surprisingly, the two enzymes were mutually partly redundant and collectively indispensable for host defense (79). The singly deficient mice were susceptible to separate sets of specific pathogens, as long as the mice were inoculated with them. In contrast, the doubly deficient mice all died from spontaneous infections

by their own microbiota. We learned how to keep most of them alive by continuous administration of antibiotics and antifungals, but even with this treatment, many of the mice developed massive abscesses filled with neutrophils and monocytes. To my knowledge, this is the most severe infectious phenotype reported for a mouse that has normal numbers of leukocytes and can mobilize them to infectious sites. Apparently, nothing else in the immune system's armamentarium suffices to protect mice routinely from their own microbiota if mice lack both iNOS and phox.

Microbes, Medicines, Society

For all its power, the immune system is the one organ system in the body that most often fails before old age. After all, we all suffer from infectious diseases from time to time, and though we usually recover, each such episode represents a failure of the immune system. Moreover, many do not recover. Even in the era of sanitation, nutrition, immunization and antibiotics, infectious diseases remain one of the major causes of death. With this in mind, now that we had a partly defined enzymology and chemistry of host defense, it was time to focus on the failures, or put differently, the success of the microbes we call "pathogens" in suppressing, evading or withstanding our immune defenses.

This search launched me on the current leg of my journey, opened my eyes to enormous challenges facing science and society in antibiotic research and development, and gave me reason to draw on the extraordinary collaborative experience with Merck in the early days of characterizing iNOS to ponder how we might reorganize to improve our chances of coping (80–84).

Antibiotics rescued me from septic shock. Not so fortunate are hundreds of thousands of people with tuberculosis that has become drug resistant. In fact, for numerous infections, the spread of antibiotic resistance is outpacing our dwindling success in finding new antibiotics. One solution on the scientific side is to seek new antibiotics as tools with which to intervene in the host–pathogen interaction, rather than as compounds that kill bacteria in axenic culture under conditions that are largely irrelevant to those in the host. Potential solutions on the organizational and societal side are to set up "Open Labs" for academic-industrial cooperation (80,81) and to realign incentives, innovation and access (81). I'll say no more about those ideas here, because they are contemporary and this essay is only meant to explain how I came to the present point in an ongoing journey.

But I can't resist a concise characterization of this new chapter in the story: It is the most exciting yet. There is nothing we can imagine about bacterial resistance to host immunity for which we can't find examples—and in many cases enzymes, and in some cases inhibitors of the enzymes (85–91). We are working with multiple drug companies on an "open access" basis, and some of them are working with each other, under an innovative organizational scheme set up by the Bill and Melinda Gates Foundation. We are moving toward clinical trials of small molecules that inhibit the ability of *M. tuberculosis* to withstand what IFNγ-activated macrophages do to the bacilli.

Promoting macrophage activation (27,30,31,92) and interfering with bacterial defenses against activated macrophages amounts to a pincer movement, a classical tactic in a war with one of our oldest enemies. It has taken 45 years and counting to develop this strategy. It's not yet ready for cancer, but that was the plan.

PRIVILEGE

There are many privileges in research. The most obvious is to choose your own path. The most important is to communicate, potentially with anyone, in terms that allow verifiable understanding. Communication in music, art, literature and dance is limitless in intensity, but indeterminate in accuracy. Van Gogh's paintings move me, but there is no way to tell if I understand what he wanted to say. In science, we can repeat the experiment. If we get the same result, we understood, and we can build on that.

The person who introduced me to the privileges of science when I was in high school was the late Professor Lester Grant. Orphaned young, his parents left him little but his father's tuberculosis. Tuberculosis destroyed his knee but steeled his ambition. After years as a newspaperman writing about medicine, Grant decided to do what he wrote about. He graduated from Harvard Medical School and went to Oxford to study with Sir Howard Florey. The 1945 Nobel Prize in Physiology or Medicine recognized Florey's role in the purification of penicillin, work done with Chain and Heatley, but Florey's postwar work involved pioneering studies on the role of leukocytes in inflammation. My own career has crossed the same bridge in the other direction—from leukocytes to antibacterial chemotherapy. It was a great honor to be invited to Oxford in 2006 to give the Norman Heatley lecture and a moving privilege to discover on the Dunn School wall a photograph of Lester Grant.

Yet another privilege in science is to be helped, mentored and taught, and to pass it forward. My first helper was the late Judy Cohen, MD, a member of the Sulzberger family whose newspaper, *The New York Times*, published the article by John Canaday that encouraged me to find a career that I couldn't imagine not pursuing. Judy helped drive my mother to her radiotherapy sessions. Later, as a memorial to my mother, she offered to lend me the money I needed to attend medical school, on one condition: that I not follow through on what she considered a self-defeating plan to marry just as that arduous journey was to begin. I refused Judy's offer and married the amazing woman without whom I would have had nothing to write about. Judy relented and made the loan. Amy and I ate beans and franks in a freezing Boston apartment furnished with fruit crates from the Faneuil Hall farmers' market and bricks and boards for shelves. Every time we put together $100, I sent it to Judy. Finally, she couldn't stand it any more and forgave the loan.

Decades later, along came Kevin Brine, an Overseer at Weill Cornell. His gift allowed me to recruit a chemist to my lab,

Gang Lin, fueling a transformation in the lab's outlook. Then the transformative generosity of Abby and Howard Milstein enabled our search for a new chemotherapeutic approach to infectious disease. Others gave guidance and advice with similar spirit and impact, among them Seymour Klebanoff, Richard Johnston, Tony Cerami, Ralph Nachman, the late Ralph Steinman and the late Lloyd Old. Lloyd understood that my commitment to cancer research underlay everything I did, whether I was working on cancer or not. He invited me to join him on the scientific advisory board of the Cancer Research Institute, where I have served for 33 years, most of them under the leadership of Executive Director Jill O'Donnell-Tormey, my former postdoctoral fellow. Cancer Research Institute has helped hundreds of scientists get their start in tumor immunology.

Telling a story centered on one person is as misleading as trying to understand a tapestry by tracing one fiber. The story I would have told if I had the skill and the space is woven from the efforts of extraordinary students, fellows, research assistants, collaborators and colleagues who shared their knowledge, enthusiasm and creativity. I'll let one represent all: Aihao Ding. She arrived as a postdoc and retired as an independent professor with whom I shared a lab. Her special gifts were the example of her personal fearlessness and the benefit of her unstinting criticism—two things a scientist needs if he or she is to have the greatest privilege of all: to see something for the first time, and then to see it again.

DISCLOSURE

The author declares that he has no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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