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The Accidental Pathologist: A Curiosity-Driven Journey from Plant Evolution to Innate Immunity

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Abstract

I have had the singular opportunity to perform research and to participate in medical education. Not unexpectedly, people have asked me which of the two was more important to me. My answer has always been and remains that I am equally passionate about research and teaching. My research has been curiosity driven and not purposeful; hence, I was willing to take risks. That my research led to the discovery of natural killer cells and the unraveling of the molecular basis of a human disease was an unexpected reward. By contrast, my interest in medical education was purposeful, with the goal of improving healthcare by teaching pathology as the scientific foundation of medicine. It started with participation in Robbins pathology texts but progressed toward development of technology-based tools for medical education. This was driven by the belief that technology, by providing equal access to knowledge across the world, can be a powerful democratizing force.

ON THE ROAD TO MEDICAL SCHOOL: FALLING IN LOVE WITH RESEARCH

I was born on December 24, 1944, in the home of my maternal grandfather, who lived in a small town called Okara, about 100 miles from Lahore in undivided India. My grandfather was a doctor practicing medicine in this small town. He was as much a saint as a human can be. He served everyone who came to his clinic, and when asked what his fee was, he would reply, "There is a box outside; put whatever you feel like into it." Some did; others were too poor to pay. Every now and then, someone would show up at his house and offer bags of wheat or a cow to thank him for taking care of a family member who had been too poor to pay. Better times allowed these relatives to give him the fee in kind, years later. As fate would have it, when India was divided in 1947, our family migrated to India and left behind all our possessions. Not one to be deterred, my grandfather restarted his clinic in India, and even after losing all his possessions, he still told his patients to only pay whatever they could in the box outside the clinic. He was very influential as a role model for me, and I decided at a very young age that I would be a doctor like him!

My father, Bihari Lal, was the first one to be educated in his family. He joined the Indian army as a clerk and gradually rose to be an officer by the time he retired in 1973. My mother, Bimla Devi, was a quiet but strong woman who constantly reinforced my desire to be a doctor. She did not study beyond high school and was a homemaker. We lived a comfortable but not affluent life; we never owned a car or traveled by air. Because he was in the army, my father got transferred every two to three years. While this was disruptive for my parents, I loved transfers because that would mean long train journeys. These frequent transfers proved to be key to my future, as at each new school I was given an entrance test. As a result, I got two double promotions (skipped two grades) so that by the time I finished twelfth grade, I was two years too young for admission to medical school. (In India, medical schools start after completion of the twelfth grade.) We were now in the city of Poona (often called the Oxford of India), and my parents decided that in the interim I should get a bachelor of science degree at Fergusson College, a premier institution in India.

Falling in Love with Research: The Influence of Dr. Apte and Dr. Maheshwari

Attending Fergusson College was an important turning point for me. I decided to major in Botany. As part of our coursework, we were required to go on a field trip to collect plants and determine their genera and species. Others hated having to do this, but I enjoyed studying plant structure. Sometimes when I had questions relating to plant biology, I would seek out Professor V.V. Apte, head of the Botany Department, who would often direct me to the library to satisfy my curiosity. During this period, I developed a fascination for plant evolution, specifically the evolution from a flat habitat (e.g., lichens) to a vertical habitat (e.g., ferns and flowering plants). I was intrigued by the remarkable changes in structure imposed by this switch in habitat. I graduated from college with distinction and high honors in Botany and decided that I would become a researcher of plant evolution instead of a doctor! My parents were surprised and disappointed at this change of heart, but they told me that the decision was mine to make. So with my father I went to see Professor Maheshwari, an internationally renowned botanist at Delhi University (**https://doi.org/10.1038/164475c0**), to apply to be his PhD student.

Professor Maheshwari was a genial fatherly figure. After listening to me describe my decision to switch from studying medicine to studying botany, he asked, "Son, I would love to take you as my PhD student, but do you want to do botany because you like botany or because you like doing research?" I had never asked myself this question. After a pause, I answered "Sir, because I like research." He then replied, "Why don't you become a doctor and do medical research? You will be happy and your parents will also be happy." That half-hour meeting set me on the path to a career in medical research.

Having obtained my bachelor's degree, I entered medical college at Amritsar with gusto and excitement. With my parents' encouragement and support, I bought every textbook available on every subject. My routine would be to return to my room after classes, review my class notes, and read about each topic in every book. After this, I synthesized the information and made my own notes. This was laborious and time consuming, but I loved it. Little did I know that this was the best training I could have had for a future career as a textbook author. After all, writing a textbook requires the ability to synthesize information from various primary sources into a coherent and succinct message. My notes became minibooks that were copied and given to junior-year students, who then gave them to other students as "Vinay Kumar's notes."

Falling in Love with Pathology: The Influence of Dr. Chugh

Having started medical college with the intent of doing research, I still had to choose a subject. My decision was heavily influenced by one of my pathology professors, Dr. Tulsi Das Chugh, who had a unique teaching style. He would tell us not only what was known, but also what was not known about the causation of diseases. One day during a lecture on glomerulonephritis I asked him, "What causes glomerular disease?" He replied that not much was known. I decided then that I was going to be a pathologist and do research on mechanisms of diseases.

Falling in Love with Mechanisms of Disease: The Influence of Dr. Ramalingaswami

The best place to do pathology research in India was the All India Institute of Medical Sciences (AIIMS) in New Delhi, and the best person to train with was Professor Ramalingaswami (Rama), a world-renowned pathologist who was doing fundamental work on malnutrition (**https://en.wikipedia.org/wiki/Vulimiri_Ramalingaswami**). In 1968, I joined his department as a pathology resident and PhD student. Rama was an intellectual giant and a superb mentor. He was a member of the National Academy of Sciences in the United States and a Fellow of the Royal Society in the United Kingdom, and he had an honorary doctorate in science from the Karolinska Institute in Stockholm. He was always available, and always encouraging. I remember that on more than one occasion, I went to his office dejected because some experiment had failed, and I left smiling and determined to keep plugging along. Dr. M.G. Deo, one of his star trainees, was coguide for my PhD. Dr. Deo was a superb and driven scientist who refused to be stopped by any obstacle. While Rama inspired me to be a physician scientist, Deo taught me that nothing was impossible.

My research topic was to elucidate the mechanism of fatty liver in a rhesus monkey model of protein-calorie malnutrition. Rhesus monkeys fed low-protein diets develop fatty liver, as do humans with kwashiorkor. The question was whether this was due to increased fatty acid flux or a failure to secrete triglycerides into plasma because of reduced synthesis of transport proteins. I demonstrated that accumulation of triglycerides stemmed from a secretory defect. The research involved learning lipid chemistry, thin-layer chromatography, and how to place feeding tubes into the stomachs of monkeys (the most difficult part of the project). After three years, I completed my dissertation and published a paper entitled "Mechanism of Fatty Liver, in Protein Deficiency: An Experimental Study in the Rhesus Monkey" in *Gastroenterology*, a prestigious US journal (1). Although I did not have much interest in malnutrition, I had begun to learn how to think scientifically and was acquiring the discipline required for rigorous research.

After completing my residency and research, I got married to Raminder Kumar, a brilliant internist who was my classmate in medical school. It was time to move on. Rama asked me what I wanted to do next. I told him I wanted to do cancer research in Boston. He said, "I will write to a

few people." I learned later that he wrote letters to the chairs of pathology at Boston University, Harvard, and Tufts. As fate would have it, Dr. Stanley Robbins, the chair at Boston University and the author of *Textbook of Pathology*, answered first and offered me a junior faculty position sight unseen (such was the influence of Rama).

As I was leaving India, my father, who until then had never offered me any moral advice, took me aside and said, "Son, I hope you never have to choose between being good and being great, but if you are forced to, choose goodness over greatness." I have never forgotten that. Quite nervously, Raminder and I headed to the airport on June 20, 1972, to board our flight to Boston.

Boston University, like many older medical centers, was located in a poor part of the city called Roxbury. As I walked to the university on the first day of work, I saw litter, garbage, run-down homes, and alcoholics staggering along the sidewalks. Was this the land of milk and honey that I had imagined America to be? No, I thought, this could not be America; this must be a bad dream.

After a few days of living with friends, we rented a place in the Jamaica Plain neighborhood where many Indian doctors were rumored to live. We took the Orange transit line to Green Street station and walked about 15 minutes to the Forest Hills apartments. Indeed, we saw many Indians there and our homesickness was relieved in some measure. Our apartment was just above that of Deepak Chopra (now a New Age guru, then a resident) in what could fairly be called an Indian ghetto.

RESEARCH: TAKING THE ROAD LESS TRAVELED

While a resident at AIIMS, I had been impressed by the work on chemical carcinogenesis being carried out at the University of Wisconsin by Henry Pitot and Charles Miller (2). Around the same time, G. Barry Pierce (at Denver) had demonstrated that teratocarcinoma cells could give rise to a normal mouse, implying that cancer was a disease of differentiation that was reversible. At the heart of the issue was whether cancer was caused by genetic or epigenetic changes. I decided to tackle this problem head on using nuclear transplantation. I wrote a detailed proposal involving exposure of primary fibroblast cultures to carcinogenic chemicals and then performance of reciprocal nuclear transplantation between exposed cells and normal cells. At that time (1970–1972), nuclear transplantation had been performed in frogs but not in human cells. With these experiments in mind, I came to Boston armed with a research proposal. Robbins introduced me to two recently hired faculty members—Drs. Hugh J.-P. Ryser and Michael Bennett. As luck would have it, Ryser worked on chemical carcinogenesis and had published a review on the subject in the *New England Journal of Medicine* in 1971 (3). With much excitement I asked Ryser to read my proposal, hoping he would invite me to do nuclear transplantation work in his lab. A few days later, Ryser told me that while my experiments had merit, there was no evidence that nuclear transplantation could be done in mammalian cells.

Somewhat disappointed, I decided to meet with Mike Bennett, and that meeting changed my life. He told me about his work on bone marrow transplantation in mice, which I did not much understand, but what really impressed me was his childlike joy in talking about research. He described a rather obscure phenomenon called hybrid resistance, which is the rapid rejection of parental marrow grafts by lethally irradiated F1 hybrid mice. He also gave me two papers to read that were published in 1971 by Bennett and his mentor Gustavo Cudkowicz in the *Journal of Experimental Medicine* on peculiar immunobiology of bone marrow allograft rejection (4, 5). By 1970, the laws governing the rejection of solid tissue grafts were established, as was the dominant role of T cells in graft rejection. Thus, an inbred strain of mice A would reject a major histocompatibility complex (MHC) disparate skin graft from inbred strain B, and vice versa. (A × B) F1 hybrid mice would accept solid tissue grafts from either parent because MHC molecules are codominantly

inherited. Thus, (A × B) F1 hybrid mice would express MHC molecules of strain A as well as strain B, and so a solid tissue graft from parent A or B would be recognized as self and not evoke rejection. In light of this, rejection of parental marrow grafts by irradiated F1 hybrid mice broke the laws of transplantation. As if this was not peculiar enough, Bennett and Cudkowicz further demonstrated that hybrid resistance occurred rapidly in lethally irradiated mice, in which T and B cell functions are suppressed. Thus, both the genetics and the effector system that mediated hybrid resistance were perplexing. But for me, peculiar and perplexing were challenging and exciting, so throwing all caution to the wind, I decided to work with Mike and not with Ryser.

Before I get into the details of our work on natural killer (NK) cells and hybrid resistance, I would like to quote from David Raulet's commentary on Bennett & Cudkowicz's 1971 papers on peculiar immunobiology that were selected for the Pillars of Immunology series (6, p. 2923). This commentary accurately describes our journey into the wonderland of hybrid resistance:

> Much modern research is quite focused, attempting to understand aspects of systems that are already revealed in broad outline. Researching a problem at its birth is an opportunity that many will never experience, a lonely exercise that many may avoid. In these situations, pre-existing findings from other scientists offer only limited help. Years or even decades may be necessary to make real progress, and most discouragingly, the initial surprising findings may be of limited interest to scientists focused on more established systems or problems. Working on such a novel problem has its perks: witnessing an unexpected new biological system come to light is uniquely exciting, even if the emergence is gradual. The Pillars papers by Cudkowicz and Bennett reviewed here represent a good example of such emergent research. They focused on a phenomenon mediated by cells (NK cells) that had not yet been discovered and that use a recognition strategy that had not yet been conceived.

In a later part of his article, in referring to the molecular basis of hybrid resistance, Raulet writes that, "A series of studies, some carried out by Bennett in cooperation with his long-time collaborator Vinay Kumar, helped reveal the answer" (6, p. 2924).

Genetic Resistance to Leukemia: Discovery of M Cells

In Mike's lab, I chose to focus on a new line of investigation—genetic control of leukemogenesis—which had its roots in his work on the peculiar immunobiology of marrow graft rejection. There were several parallels in the genetic control of acute erythroleukemia caused by Friend virus (FV) leukemia: First, there was a correlation between the ability of various inbred mouse strains to reject allogeneic marrow grafts and resist leukemia induced by FV; next, both hybrid resistance and genetic resistance to FV leukemia were unaffected by suppression of T cells, B cells, and macrophages; and, finally, resistance to allogeneic marrow grafts and to FV could be transferred by marrow cells from resistant mice to susceptible mice. Thus, it appeared that genetic resistance to leukemia and rejection of marrow allografts either were mediated through a hemopoietic cell in the graft or depended on the marrow microenvironment of the host for their development.

It should be recalled that thymectomy and bursectomy established the role of the thymus and the bursa as central lymphoid organs for the development of T and B cells. How would one determine whether bone marrow could act as a central lymphoid organ for the development of cells with antileukemia activity? Because marrow diffusely occupied the space in bones, surgical removal of the marrow could not be done. But what about ablation of marrow by selective irradiation? Previous work at Argonne National Labs and the University of Chicago had shown that injection of mice with the bone-seeking radioisotope strontium 89 (Sr89) ablated the bone marrow but permitted survival because the spleen takes over hematopoiesis (7). Sr89 is a weak beta emitter that exchanges with calcium and thus selectively irradiates the bone marrow cavity without any effect on other lymphoid tissues. Indeed, T cell, B cell, and macrophage functions

remain intact in Sr89-treated mice. We reasoned that any cell that depended on the bone marrow microenvironment would be depleted in Sr89-treated mice. Therefore, we tested the ability of Sr89-treated mice to reject allogeneic marrow transplants and to develop FV leukemia. I was responsible for the leukemia experiment, while Mike and his technician tested rejection of allogeneic marrow transplants. To our utter delight and amazement, we found that Sr89-treated mice lost resistance to allogeneic bone marrow (8) and became exquisitely susceptible to leukemia (9). I still remember how, with baited breath, I sacrificed mice to look for leukemia (leukemic mice develop massive splenomegaly) and jumped up and down with joy and excitement when I saw the enlarged spleens.

Because the cells responsible for resistance to leukemia were marrow dependent, we called them M cells. The paper describing these experiments was submitted to the *Journal of Experimental Medicine* in December 1973 and published in May 1974, less than two years after my arrival in Boston. This paper, entitled "Mechanisms of Genetic Resistance to Friend Virus Leukemia in Mice: I. Role of ^{89}Sr-Sensitive Effector Cells Responsible for Rejection of Bone Marrow Allografts," is not the most cited of my publications (9), but in my view it is the most important paper that I have published. The work was noticed and invitations to major meetings started to come. Mike refused to go and told me, "This is your work; you should go." I learned later that such willingness to give up glory is exceedingly uncommon in the competitive work of science.

A year later, in 1975, two groups led by Hans Wigzell at the Karolinska Institute and Ron Herberman at the National Institutes of Health published papers describing cells in spleens of unimmunized mice that killed leukemia and lymphoma cells in vitro (10, 11). Of note, these cells were not T cells, B cells, or macrophages. Because these antitumor cells were present in spleens of nonimmunized mice, they were called NK or natural cytotoxic cells. In November 1975, I was invited to present a short talk at the meeting of the New York Academy of Sciences where Dr. George Klein (from the Karolinska Institute) and Dr. Herberman presented their work—announcing the discovery of NK cells. As I listened to them, it became very clear to me that the antileukemia M cells we had discovered were identical or very similar to the NK cells, so much so that I took to the microphone and braved to say so during the discussion period following their talks. In those days, the New York Academy of Sciences recorded the discussions and published them in the proceedings. Below is the discussion as published in the proceedings (12, p. 39):

> Dr. Kumar: "I have some comments and questions about the nonspecific or nonimmunity reactions which Dr. Herberman and Dr. Klein mentioned. It seems to me this is in many ways extremely similar to something which our laboratory has studied for years, that system responsible for bone marrow rejections. This is a non-T cell, non-B cell reaction; as far as we know, macrophages are not required. We have recently found it to be important not only in marrow graft rejection but also in two other systems: genetic resistance against leukemia viruses, and in early resistance against *Listeria monocytogenes*. The important point I wish to make is that the functions of this particular effector system can be specifically abrogated by treating mice with strontium89, a bone-seeking isotope that destroys only the bone marrow, not the thymus, spleen, nor other lymphoid organs. Mice treated with strontium89 have perfectly normal T- and B-cell, as well as macrophage functions; yet they lose the ability to reject bone marrow grafts and genetic resistance to leukemia. So we feel there are some cells that probably require a bone marrow microenvironment for maturation which is destroyed by treatment with radioactive strontium. I would be interested to know if a similar cell operates in natural 'immunity' to tumors."
>
> George Klein replied: "We have seen your paper, there are some similarities but there are differences in genetics."

After this discussion it occurred to me that if NK cells were the same as M cells, then NK function would also be lost in Sr89-treated mice. This was an experiment that needed to be done. As fate would have it, soon after the meeting was over, Wigzell, then a professor at the Karolinska

Institute, came over to me and started discussing NK cells and M cells. I was somewhat taken aback because he was a well-known senior immunologist and I was an unknown researcher with a single publication! Eventually, he asked me to provide him, in great detail, the method for treating mice with Sr89. I did not hesitate for a moment. Wigzell took notes as I spoke. On my return to Boston, I shared with others in the lab details about my meeting with Wigzell. Most of my colleagues, except Mike, castigated me for having divulged everything to Wigzell because with a big lab he could test NK function in Sr89-treated mice more quickly than we could. To me, science is for sharing, with the goal of finding the truth and not being the first one who makes a discovery. It is not a game in which there are winners and losers. Everyone wins with each new discovery. I have followed this rule in my entire career, and not once have I been scooped because I shared unpublished information. As I had expected, Wigzell tested NK cell function in Sr89-treated mice, and, as predicted, NK cell function was abrogated (13). Thus, formally, M cells became NK cells. This initial discussion with Wigzell benefitted me tremendously both by continued scientific dialog with the Karolinska group and, in some cases, by early access to unique transgenic mice created by them.

Subsequent to publication of my first paper in 1974, I continued to work on the FV model. In addition to causing leukemia, FV causes immunosuppression. In two papers published back to back in one issue of the *Journal of Experimental Medicine* in April 1976, we reported that immunosuppressive effects of FV were also under genetic control, that the resistance to immunosuppression was also abrogated in Sr89-treated mice, and that FV caused immunosuppression by activating suppressor T cells in genetically susceptible mice and in resistant mice rendered susceptible by Sr89 treatment (14, 15). I was also curious about genetic control of immunosuppression and wondered whether *Fv-2*, the gene that controlled resistance to FV leukemia, was also responsible for its immunosuppressive effects. By using segregation analysis, we found that susceptibility to leukemia and immunosuppression were under separate genetic control. We named this new gene that controlled susceptibility to immunosuppression *Fv-3* (16). Subsequently, we demonstrated that *Fv-3* controlled immunosuppression both in vivo and in vitro (17). The paper describing this was the fifth in a series of papers with the title "Mechanism of Genetic Resistance to Friend Virus Leukemia in Mice" published between 1974 and 1978, the first four in the *Journal of Experimental Medicine*.

It was a period of remarkable productivity that I ascribe fully to the intellectual environment created by Mike Bennett. He not only was brilliant but also was an extraordinary person—the most gracious man I have ever known. He gave me the freedom to design experiments, make mistakes, correct them, and keep moving. We engaged in intense daily discussions, had lunch together, and became very good friends. He was from Texas, so people jokingly called us the Cowboy and the Indian. Mike taught me more than research. From him I learned selflessness, support for junior trainees, open-mindedness, and rigorous pursuit of truth. I followed these lessons when I had my own graduate students and postdocs. Mike showed me that fame was not important, but substance was. Finally, he taught me that nothing was impossible, to dream big, and not to be stifled by the limitations others posed. His attitude was infectious and invigorating. In 2015, I endowed what would become the Michael Bennett Lecture in Immunopathology at the University of Texas Southwestern Medical Center (UTSW), so that Mike's name will live on.

NK Cell Development: Closing the Loop on M Cells

Around 1978, I decided against continuing to work on the FV leukemia model because I wanted to pursue the original studies that led us to believe that NK cells were a distinct lineage of lymphoid cells. I felt that this was a fundamental question of developmental biology that needed to be tackled.

In 1981, Mike and I got an offer to move our labs to UTSW in Dallas. UTSW is now the home of three current and three past Nobel laureates, but in 1981 it was relatively unknown. Both Mike and I knew that its Immunology program was strong. I had also begun to feel that I had become the go-to person for advice on immunology research at Boston University, and I did not want to be at a place where I was the smartest immunologist. Although I was being promoted to professor in Boston, UTSW offered me a position as an associate professor. My friends thought it would be foolish to take a rank cut anywhere, never mind Texas! But to me, rank and salary were far less important than the environment. So we packed our bags and moved to Texas in 1982. In 1983, I got promoted to full professor with tenure. The very next year (1984), an endowed chair was established to honor Charles Ashworth, a past chair of the department. Everybody assumed that the most senior professor would get the chair. Instead, the dean summoned me to his office and said he had polled all 15 professors in the department and they had unanimously recommended that I hold the Ashworth Chair in Pathology. I was not only the most junior of all the professors but also, at 40, the youngest.

The Immunology graduate program at UTSW was excellent, and one of the biggest benefits of moving to Dallas was the ability to attract top-notch PhD students. There is no doubt in my mind that a major reason for my success in research was the quality of graduate students who came to work with me and Mike. John Hackett, a tall Midwesterner (from Rockford, Illinois), was the first PhD student to join the lab. He was assigned the task of defining NK cell precursors in the bone marrow and their relationship to myeloid and lymphoid progenitors and exploring the requirement of the bone marrow microenvironment for NK cell development. Several technical advances contributed to John's success in research: (*a*) the development of an assay for transplantable progenitors of NK cells in bone marrow; (*b*) the discovery by Seaman and Talal that osteopetrosis induced by β-estradiol treatment replicated the loss of NK cell function in Sr89-treated mice, thus providing a nonradioactive method of ablating the bone marrow (18); and (*c*) the development by Gloria Koo (in New York) of a specific antibody against the NK1.1 molecule expressed on mature NK cells (19). Using these tools, John was able to show that NK progenitors were present in mutant mice severely deficient in myeloid progenitors, and that when normal bone marrow cells were transplanted into osteopetrotic mice, they failed to give rise to mature NK cells. These experiments further strengthened the hypothesis that because of their unique maturational requirements, NK cells belonged to an independent lineage.

NK Cells and T Cells: It Is Better to Be Right than to Be First!

Our ongoing work to define the nature of the marrow microenvironment had to take a back seat because a couple of papers published in influential journals—*Nature* and *Science*—claimed that T cell receptor genes were rearranged in clones of mouse and human NK cells (20, 21). These results firmly placed NK cells in the T cell lineage and were discussed in a News & Views article written by Miranda Robinson in 1985 (22). Around this time, Lewis Lanier and I met at an international NK cell workshop in Hawaii and shared unpublished data at variance with the T cell hypothesis. Lanier had probed freshly isolated NK cells from human blood and discovered that NK cells do not contain rearranged T cell receptor (TCR) beta (23). We had discovered that severe combined immunodeficient (scid) mutant mice (provided by Mel Bosma, Fox Chase Cancer Center) had perfectly normal NK cells despite the fact that the scid mutation prevented rearrangement of T and B cell receptor genes (24). Both Lanier and I concluded that the NK cell clones used to show TCR rearrangements were actually T cell clones that had acquired the ability to kill tumor cells nonspecifically after prolonged culture and hence appeared to be like NK cells.

Upon return from the meeting, I realized that to prove that NK cells were an independent lineage of lymphoid cells, we would have to show that mouse NK cells do not rearrange TCRs. This

was not trivial because normal mouse spleen has only about 1% NK cells and to purify them to homogeneity would be a Herculean task. John Hackett and Michelle Tutt (another graduate student) joined hands to purify NK cells by cell sorting. The FACS II machine that was available at that time was painfully slow, and John would typically sort from 8 p.m. to 2 a.m. to get about 10,000 purified NK cells, which Michelle would put in culture with interleukin-2 (IL-2) to expand them to about 100,000 cells (99% pure) over the next several days. Since we had no molecular expertise in our lab, I approached Phil Tucker, an excellent molecular immunologist at UTSW in Dallas, for help. Phil directed his best graduate student, Bill Kuziel, to assist us in looking for expression of TCR-alpha, TCR-beta, and TCR-gamma transcripts by Northern blots. As we had predicted, there were no TCR transcripts. We quickly wrote up the results and sent them to *Nature*, since the paper describing TCR rearrangements in mouse NK clones was published there (20). To my dismay, *Nature* sent it back without review. I called London and spoke to Miranda Robertson, the editor (who had written the News & Views article), making the point that since *Nature* had published the original article, it was only proper that they review our paper and hopefully publish it. After a couple of such phone calls, I was told by the editor that I was wasting my time. I then submitted the paper to *Science*, but it was not accepted because Lanier's paper on human NK cells had just been published as a brief definitive report in the *Journal of Experimental Medicine* (23). Eventually, our paper was published in the *Journal of Immunology* about a year after the *Nature* paper (25). I have used this example many times to tell my graduate students that it is more important to be right than to be first.

One of the problems in the field of NK cell research was that many different cell types—promonocytes, basophils, and T cells—when cultured (particularly in IL-2-containing medium) develop the ability to kill tumor cells in vitro and were hence called NK cells. Thus, the question arose, What are NK cells? To address this issue, I approached Joe Feldman, then the editor-in-chief of the *Journal of Immunology*, to allow me and Lanier to submit an Opinion paper to the same issue of the journal in which our paper on lack of TCR expressions in murine NK cells was to be published. Given the confusion in the field, Feldman readily agreed, and our paper, entitled "Natural Killer Cells: Definition of a Cell Type Rather than a Function," was published (26).

Having settled the issue of T cell relatedness, we proceeded to develop experimental approaches to delineate the differentiation of NK cells and, in particular, define the cellular and molecular bases of the requirement for the bone marrow microenvironment. Because the bone marrow contains progenitors that can develop into NK cells by adoptive transfer into NK cell–depleted hosts, Tom Moore (a graduate student) attempted to isolate committed NK progenitors by flow cytometric methods. While he could greatly enrich the NK progenitor population, we could never prove that they gave rise only to NK cells. In effect we were enriching for multipotential progenitors. We concluded that fate determination occurred when pluripotent stem cells receive lineage-specific signals (cytokines/stromal cells) at the sites of their maturation, such as the thymus. This made us change our approach from trying to find committed NK progenitors to defining the signals required for NK development from stem cells. Eventually, Noelle Williams (a postdoctoral fellow) succeeded in creating a culture system in which highly enriched multipotent progenitor cells could be made to develop into a pure culture of NK cells. Crucial to the success was the discovery of IL-15, by others, as a growth factor for NK cells and the availability of marrow stromal cell lines. With these new tools, we were able to show that multipotent hematopoietic progenitor cells isolated from the bone marrow, when cultured in the cytokine Flt3 ligand, acquired IL-15 receptors, which then under the influence of marrow stromal cells and IL-15 developed into mature functional NK cells (27). Both IL-15 and marrow stromal cells were an absolute requirement for NK cell development. Thus the M cell hypothesis was proven to be correct! It took almost 30 years from inception to proof.

Hybrid Resistance: No Longer Peculiar

After we established that both hybrid resistance and NK cell function were abrogated by Sr89 treatment, NK cells became the prime suspects for rejection of parental marrow transplants by F1 hybrid mice. Two additional sets of experiments supported this hypothesis. First, we found that scid mice, genetically deficient in T and B cells, could mediate hybrid resistance (28). Second, purified NK cells could adoptively transfer the ability to reject parental marrow grafts (hybrid resistance) (29). But what do the NK cells of F1 hybrid mice (H2d × H2b) recognize on parental (H2d or H2b) marrow cells?

The solution to this problem had to await a paradigm-shifting hypothesis put forth by Klas Karre at the Karolinska Institute. He noted that NK cells could kill MHC class I–deficient tumor cells much more effectively than class I–positive cells. It appeared then that presence of class I expression on the target cells inhibited NK cell killing (30). In 1986, these and other experiments led Klas to propose the missing-self hypothesis of NK cell recognition. According to this hypothesis, NK cells have two sets of receptors—inhibitory and activating. The inhibitory receptors recognize self–MHC class I molecules and silence NK cells. Normal cells, expressing MHC class I molecules, evade NK cell–mediated killing by engaging the inhibitory receptors, thus maintaining self-tolerance. When class I molecules are lost or downregulated by either viral infections or malignant transformation, inhibitory signals are lost and the activating receptors take over and kill the target. Thus, T cells and NK cells use different and complementary strategies to recognize their targets. T cells recognize nonself in the form of antigens presented by MHC molecules, whereas NK cells recognize missing self in the form of loss of self-MHC molecules. Powerful proof of this hypothesis in the setting of marrow transplantation came from David Raulet's lab in 1991, when they showed that marrow cells from gene-targeted mice lacking all class I molecules were strongly rejected by NK cells of normal mice (31). Over the next several years, the cellular basis of the missing-self hypothesis was amply confirmed by the discovery of MHC class I–recognizing inhibitory receptors in mice (the Ly49 family) as well as humans (the KIR family).

Our laboratory contributed to these studies by discovering the Ly49C/I receptor that inhibited NK cell killing by recognizing mouse H2b class I molecules and by developing antibodies against Ly49 molecules (32). We demonstrated that Ly49C/I-expressing NK cells from H2b mice failed to kill H2b class I–expressing target cells (self-tolerance) and that this could be reversed by blocking the interaction of Ly49C/I with H2b by anti-Ly49C/I antibodies, thus supporting the missing-self hypothesis.

With the available tools and the conceptual framework of the missing-self hypothesis, we returned to address the cellular and molecular bases of hybrid resistance. In a typical experiment, NK cells from the spleens of (A × B) F1 hybrid mice killed target cells from both parental strains, A and B, just as (A × B) F1 hybrid mice could reject parental marrow cell grafts in vivo. Our work and that of others demonstrated that there are several distinct MHC class I–specific Ly49 inhibitory receptors that can discriminate between allelic variants of MHC class I molecules. Importantly, these receptors are expressed on distinct subsets of NK cells in (A × B) F1 hybrid mice. Thus, (A × B) F1 hybrid mice possess an NK cell subset that expresses the Ly49 receptor inhibited by MHC^{a} but not by MHC^{b}. This subset would kill cells from the parental strain B. Conversely, the NK cell subset that expresses the inhibitory receptor for MHC^{b} but not MHC^{a} would kill cells from parental strain A. Neither subset would kill self ($MHC^{a/b}$) because both NK cell subsets would be silenced. The paper describing these findings was published in 1996 (33), 24 years after I entered the field of NK cell biology and at a time when NK cells had not yet been discovered.

Discovery of a Novel NK Cell Receptor: Evidence for Another Layer of Self-Tolerance

As discussed above, NK cells express Ly49 family molecules that recognize self–MHC class I molecules and thus ensure self-tolerance. Quite unexpectedly, we discovered a non-MHC-recognizing receptor that provides another layer of self-tolerance.

Early in our research on NK cells (around 1990), we decided to make monoclonal antibodies against highly purified mouse NK cells in hope of making antibodies against cell surface receptors that may be involved in NK cell–mediated killing. We hypothesized that NK cells of mouse strains that exhibited high NK activity possess cell surface receptors that NK cells from strains with weak NK activity did not have. We chose two inbred strains of mice, C57Bl/6 mice with high NK activity and MHC-identical 129/J mice that had weak NK activity. Purified NK cells from C57Bl/6 mice were used to immunize 129/J mice, and we screened the hybridomas for antibodies that stained purified NK cells (and not T or B cells). Each antibody was then tested for its ability to activate or inhibit NK cell function in vitro. We assumed that antibody-mediated cross-linking of a putative triggering structure would activate NK cells. Using this strategy, we developed an antibody called 2B4 that activated killing by NK cells (34). To understand the molecular basis of activation of NK cells, we decided to clone the gene identified by the 2B4 antibody. The structure of 2B4 revealed that it belonged to the CD150 (signaling lymphocyte activation molecule) subfamily of the CD2 family of receptors (35). It is formally called CD244 or SLAMF4. We did not realize the significance of the molecular structure until much later.

As we began to extend our earlier work, we noted that, in some cases, treating NK cells with the 2B4 antibody enhanced the killing of tumor cells (as noted above), but in others it suppressed killing, and in yet others it had no effect. Around the same time, human 2B4 was cloned, and antibodies against human 2B4 routinely activated NK killing, supporting our initial published results. I was very concerned about the variability and feared we were chasing artifacts. A breakthrough occurred when, in collaboration with Marion Brown at Oxford, we discovered that the ligand for 2B4 is CD48, another member of the CD2 family (36). When we stained various tumor cells used in our previous experiments, a clear pattern emerged: The killing of tumor cells that expressed high levels of CD48 was activated by anti-2B4, while the killing of those that expressed low levels of CD48 was unaffected or inhibited. These results suggested that 2B4 was acting as an inhibitory receptor, and the anti-2B4 antibody activated the killing of CD48-expressing tumors by blocking the off signal delivered by the CD48 molecule. To confirm this hypothesis, we generated 2B4 knockout mice and proved that 2B4-CD48 interactions inhibited NK cells (37). Thus, we had discovered an inhibitory receptor that, unlike the previously characterized Ly49 family of receptors, did not receive the off signal from MHC molecules (38). Using 2B4 and MHC class I knockout mice, we were able to demonstrate that NK cell tolerance is mediated by multiple layers of self-protective systems (39): those dependent on recognition of self-MHC molecules (Ly49 receptor mediated) and those silenced by non-MHC molecules (2B4 receptor–CD48 mediated).

From Bench to Bedside: Molecular Pathogenesis of a Fatal Human Disease

To understand how 2B4 transmitted a negative signal upon interaction with CD48, we investigated the molecular basis of 2B4 signaling. The cytoplasmic tail of 2B4 contains four immunoreceptor tyrosine-based switch motifs (ITSMs; TxYxxV/I). Others had shown that ITSMs bind SH2 domain–containing proteins including SH2D1A (SH2 domain–containing protein 1A), which could recruit Fyn tyrosine kinases and deliver an activating signal or recruit phosphatases and deliver an inhibitory signal. We confirmed that when 2B4 was ligated by CD48 it could recruit SH2D1A and that the 2B4 signal delivered through SH2D1A varied on the basis of the density of

CD48 on the target cells (40). With high density of CD48 on target cells, and consequent heavy cross-linking of 2B4, an inhibitory signal was delivered. Conversely, with lesser cross-linking of 2B4, an activating signal was generated, an elegant example of how nature repurposed one receptor to perform two different tasks. This also solved the puzzle of 2B4-mediated activation or inhibition based on the density of CD48 on tumor cells.

The centrality of SH2D1A in regulating signal transduction from 2B4 came into sharp focus in 1998, when Cox Terhorst's laboratory discovered that X-linked lymphoproliferative disease (XLP) is caused by a mutation in the *SH2D1A* gene that maps to the X-chromosome (41). XLP is a fatal immunodeficiency disease caused by Epstein-Barr virus (EBV) infection. An important feature of XLP is a cytokine storm that activates hemophagocytosis by macrophages. Since 2B4 signals through SH2D1A, we became interested in the possible role of 2B4 in the pathogenesis of XLP. Could loss of 2B4 signaling impair NK cell–mediated killing of CD48-expressing B cells, macrophages, and EBV-specific $CD8^+$ T cells, thus causing their expansion and secretion of cytokines such as tumor necrosis factor and interferon gamma? While the idea seemed attractive, we realized that since three other members of the CD2 subfamily also bind to SH2D1A, it would be very difficult to prove which member of the family is involved in causing XLP. This realization piqued our curiosity about the possible role of 2B4 in other XLP-like syndromes. Was there a human knockout of 2B4? On reviewing the literature, we found another hemophagocytic syndrome called familial hemophagocytic lymphohistiocytosis (FHL) (42). Was FHL caused by a mutation that disabled 2B4? We reasoned that since 2B4 signaled through SH2D1A, a mutation in the 2B4 receptor might give rise to a syndrome that mimics XLP caused by a mutation of SH2D1A.

FHL is a rare autosomal recessive disease with an incidence of 1 in 50,000 live births. The affected infants die of normally trivial viral infections that evoke massive proliferation of $CD8^+$ T cells and macrophage activation. Death is caused by an uncontrolled antiviral immune response. On reviewing the literature, we noticed a paper from de Saint Basile's group in France in which they had described a linkage analysis of the FHL trait (43). They found FHL to be genetically heterogeneous. In two-thirds of the patients, the trait mapped to chromosome 10q22. With this genetic data, we realized that FHL could not be caused by mutation of the 2B4 gene since it mapped to chromosome 1q23.3. But out of curiosity we started to look for other genes in the 10q22 region and found that the human perforin gene maps to this location. Susan, the graduate student, exclaimed "Eureka!," and both of us knew why. All cytotoxic cells have essentially similar wiring. The signal starts from receptor cross-linking (e.g., 2B4) and it is transduced by molecules recruited to the cytoplasmic tail of the receptor (e.g., SH2D1A), leading to a series of biochemical reactions that release the effectors of cytotoxicity (e.g., perforin).

We were excited about the idea that mutations in perforin, which lies downstream of 2B4 and SH2D1A, might cause FHL, but how could we test this hypothesis? FHL is rare fatal disease and only someone actively studying the disease would have samples from patients. Who better to ask than Genevieve de Saint Basile, the senior author on the genetic linkage study! I sent her an email in which I wrote, "We think we know the cause of FHL. If you send us DNA samples from four patients and four controls (blinded to us) we will attempt to identify the DNA from patients and controls and inform you. To her credit, without seeking additional information, she promptly sent us the samples by FedEx. Susan worked around the clock, and within a week we had sequenced the perforin genes. Four DNA samples showed nonsense and frameshift mutations and the other four did not. I called Genevieve, who confirmed that our identification was correct. We then decided to sequence DNA from additional cases and to perform functional and phenotypic studies. Frozen lymphocytes from the patients we had sequenced were thawed and cultured in IL-2. These cells were tested for killing activity against target cells known to utilize perforin (and not Fas). Those derived from patients with perforin mutations failed to kill. In addition, confocal

microscopy showed absence of perforin protein in the granules of cells from patients. Two months after I had contacted Genevieve, all our experiments were completed. Four months later the paper was published in *Science* (44). Our discovery of perforin mutations as the cause of FHL was completely serendipitous. We did not plan to study FHL. We were studying signaling through an NK receptor and ended up discovering the cause of a human disease—an example of bench-to-bedside research. This also exemplifies how basic science can unexpectedly shed light on the pathogenesis of diseases.

We predicted that other cases of FHL and other hemophagocytic lymphohistiocytosis syndromes would be caused by molecular lesions that impair the cytolytic machinery of lymphocytes. This has turned out to be correct (45). Equally importantly, this work showed that perforin mediates cytolytic effector functions as well as immune homeostasis. Because of these insights, our paper has been quoted more than 1,000 times and was selected as a Pillar of Immunology by the *Journal of Immunology* in June 2015 (45). It is most satisfying that two papers on hybrid resistance (4, 5) were also selected as Pillars of Immunology in October 2015 (6). Sadly, by then Mike Bennett had passed away.

MEDICAL EDUCATION

Teaching has been an integral part of my academic career, and it started around the same time as did my research in Boston. My teaching efforts have occurred in two separate settings: as coauthor/coeditor of Robbins's pathology texts and more broadly as a medical educator in the United States and in India. I will start with my role in Robbins pathology and follow with the latter, less well-known, but (in my view) very important, facet of my academic career.

A couple of months after my arrival in Boston, in 1972, Robbins asked me what I wanted to do in addition to research. There were two options: to do clinical (diagnostic) work or to do teaching. The latter appealed to me since I had enjoyed teaching medical students during my residency at AIIMS. Robbins asked me to start by giving lectures, and he assigned pathology of fungal diseases as my very first lecture (a complex topic that almost no one wanted to teach). After I finished my maiden lecture in the United States, to my delight I received thunderous applause. Later that afternoon, Robbins called me to his office and told me that the students who did not attend the lecture asked him to have the lecture repeated or tape-recorded, which I did! Soon thereafter, Robbins put me in charge of teaching in the pathology labs. When I came to the United States in 1972, I had a three-year exchange visitor visa, so I had to return to India in 1975. When the medical students learned of this, the entire class submitted a signed petition to the dean to sponsor me for a permanent residence visa. I was most touched by this validation of my teaching efforts.

Robbins Pathology: Inception and Evolution

Born in 1915, Robbins was a Boston Brahmin. He attended Brookline High School, the Massachusetts Institute of Technology (MIT), and then Tufts University School of Medicine. After residency at the storied Mallory Institute of Pathology, he joined the faculty of Boston University School of Medicine in 1944 (the year I was born). Since he was dissatisfied with the pathology texts available at that time, he decided to write his own book of pathology, which was published in 1957 and entitled *Textbook of Pathology with Clinical Applications*. It had 1,350 pages and 933 illustrations—a Herculean task that he undertook as the sole author. In the preface of his book (46), he wrote, "The pathologist is interested not only in the recognition of structural alterations, but also in their significance, i.e., the effects of these changes on cellular and tissue function and ultimately the effects of these changes on the patient. It is a discipline not isolated from the living patient, but rather a basic approach to better understanding of disease and therefore a foundation

of sound clinical medicine." This approach was a departure from the usual morphology-heavy teaching of pathology.

The book was sent for prereview to two senior academic pathology giants, whose comments were strongly negative, since in their view there was "not enough descriptive morphology and too much emphasis on clinical details"; however; a Temple University medical student wrote to the publishers, "I have begun reading my new Robbins textbook. I know someone up there loves me" (47). Robbins presented pathology as the scientific basis of the practice of medicine along with clinical applications, as evident from the title of the book. Another factor that endeared Robbins's *Pathology* to the students was its conversational style and the jokes peppered throughout the book, such as, "The tattoo pigment has the distressing property of persisting in situ throughout life in dermal macrophages, creating difficulties if one wishes to marry 'Alice' when the adornment is seductively titled 'Mary.'"

When I met Robbins in 1972, three editions of his textbook had already been published (in 1957, 1962, and 1967). It was immensely popular and the fourth edition was due in 1972, but Robbins decided not to revise the book. Instead, he wrote a brand-new book entitled *Pathologic Basis of Disease*. He explained to me that he wanted the textbook to be even more heavily focused on mechanisms of disease and hence decided to write the book from scratch with a title that reflected the content of the book. The first edition of *Pathologic Basis of Disease*, published in 1974, became as popular as its predecessor, *Textbook of Pathology*.

For the second edition of *Pathologic Basis of Disease*, Robbins deviated from his past practice of being the sole author and asked Ramzi Cotran to be his coauthor. Ramzi was born in Palestine in 1932 and educated in Beirut. He was well known to Robbins since he was a resident at the Mallory Institute from 1956 to 1959 and then a faculty member of the Harvard Medical Services of Boston City Hospital from 1960 to 1974. Robbins felt that for the book to be true to its mission of emphasizing mechanisms of disease, the authors must be practicing experimental pathologists. While Robbins in his early years was a credible clinical investigator, with some landmark papers (48, 49), by the early 1970s he had become distant from research. Ramzi, on the other hand, was an active investigator who had made seminal discoveries in renal pathology as well as endothelial biology (50, 51). The second edition of *Pathologic Basis of Disease* by Robbins and Cotran was published in 1979. Ramzi's stamp on the book was obvious. For example, there was expanded coverage of free radical injury and of mediators of acute inflammation (a topic on which Ramzi had performed some pioneering studies with Guido Majno). There was also more in-depth coverage of carcinogenesis. With the blending of Robbins's style and Ramzi's science, a new more invigorating Robbins emerged.

In 1971, Robbins, along with Marcia Angell, wrote another textbook called *Basic Pathology* (often called baby Robbins by the students). According to Robbins, "It arose from an appreciation of the modern medical student's dilemma. As the curriculum has become restructured to place greater emphasis on clinical experience, the time for reading is correspondingly curtailed." Accordingly, *Basic Pathology* had about one-half of the pages of the big Robbins and it covered primarily the major disease entities.

Call from Robbins: An Uncharted New Journey

Between 1974 and 1979, my research on genetic control of leukemia was moving along exceedingly well; I had my first MD-PhD student; our son Rohit was born in 1974 and our daughter Ambika was born in 1979; we bought a car and moved to a rental house in a nice neighborhood; and life was, as they say, beautiful. But one day in 1979, Robbins called me to his office and sprang a surprise on me. He said, "I am going to retire in a couple of years and I am wondering if you

would be willing to be a coauthor of *Basic Pathology*." I almost blanked out and started to stammer, thinking, "Me, coauthor of the world's most widely used textbook of medicine? Me, who is an assistant professor with no training in pathology in the United States?" Surely, many senior people would salivate at this offer. Seeing my hesitation, Robbins said, "I understand your hesitation. Your research is going very well and you must be concerned that it will suffer. Think about it and let me know." I came out in a daze and walked into the office of Mike Bennett and told him what happened. He told me that I would be a fool to turn down the offer, and that it was an opportunity of a lifetime. I asked, But what about my research? He told me that he was confident that I could do both. That was Mike's lesson to me: Nothing is impossible. The next day, I accepted the offer of becoming a coauthor of *Basic Pathology*. Marcia Angell, who had coauthored the first two editions, was moving to become deputy editor of the *New England Journal of Medicine*. Because of her substantial contributions to the first two editions of *Basic Pathology*, we retained her name on the cover at her request. Robbins and I divided the chapters equally, and we each wrote one-half of the book. I made extensive revisions of the chapters assigned to me (which included all general pathology chapters) and in particular added several schematics and the latest information on disease mechanisms. Both of us exchanged drafts of the chapters we were revising and returned them with comments. I started with revision of the chapter on male genital system (because it was the shortest!) and after revising it extensively, I gave it to Robbins for his comments. Not surprisingly, I got back a heavily marked copy. I sent him a second revised copy and then a third one, only to find that the red ink still flowed from his pen. At the bottom of the last page was a note that said, "Vin, there is no good writing, only good rewriting." I saw firsthand Robbins's relentless pursuit of excellence. I have on many an occasion used this quote from Robbins when sending edited chapters to contributors. After two years of rewriting, the third edition of *Basic Pathology*, by Robbins, Angell, and Kumar, was published in 1981. As I held the copy of the book in my hands, I could not believe what I was seeing. I had never aspired to be a textbook author, but in some strange way it felt right. Forty-one years later, the magical feeling of holding a new edition still remains.

In 1982, it was time to start revising *Pathologic Basis of Disease*. Ramzi Cotran asked me to join him and Robbins as the third author. Initially, I hesitated because I felt that this would take away additional time from research. I spent several days thinking about this and finally agreed because I felt that to teach a vast audience gave me not only joy but also a sense of purpose. I knew that I would have to make compromises in research, but I felt ready to do so. Thus, the third (1984) edition of *Pathologic Basis of Disease* carried on its cover three names: Robbins, Cotran, and Kumar. The three of us remained editors/authors for the next three editions (the fourth, fifth, and sixth) of the big Robbins. The fourth edition of *Basic Pathology* (1987) was revised by Robbins and me. For the fifth edition of *Basic Pathology* (1992), Ramzi joined the team. We three remained editors for the next two editions (the sixth and seventh).

In March of 2000, I moved to the University of Chicago as the fifth chair of the Department of Pathology. I had never in my life managed anything other than my lab and did not hold a license to practice medicine in the United States. To say that I was somewhat nervous would be an understatement. Furthermore, soon we had to start working on the next edition of the big Robbins. Ramzi, who was quite instrumental in my recruitment to Chicago, had assured me that since I would be busy starting as a new chair, he would step up and do the lion's share of the revision.

As luck would have it, my safety net came crashing down when Ramzi died very prematurely in October 2000 at the age of 67. His death hit me hard because over the years he had become my elder brother to whom I turned for advice, knowing that it was never colored by his affection for me. His advice to me was always honest and objective, even if at times it may have appeared to be harsh. I am forever grateful to him for this.

As the reality of Ramzi's departure began to sink in, I realized that out of the trio of Robbins, Cotran, and Kumar, only one was left, since Robbins (who died three years later) had stopped writing in 1999. On the flight back from Ramzi's memorial service, I thought hard about whom I would ask to carry forward the legacy of Robbins and Cotran. I wondered whether it would be wise to select someone much younger than I (for succession planning) or pick more than two people and put them through a trial run in the next edition and then pick the winner. What did Robbins, Cotran, and I share that made our books so widely used? We all had passion for excellence in teaching, we were obsessive and compulsive to a fault when it came to good writing, and we had very high academic standards. But what made our 23-year partnership last? While I could list many factors, by far the most important was to not allow egos to get in the way of resolving points of difference. Although I was the most junior in the group, when it came to making critical decisions about the books, we were equals. My viewpoint received the same consideration as that of Robbins, the most senior. We argued, sometimes vociferously, and critiqued each other's writing mercilessly, but we remained friends. I recall that once I had a three-dimensional structure of the glomerulus drawn by our graphic artist for the kidney chapter, which Ramzi wrote. On seeing the illustration, Ramzi called me and said, "This is a terrible picture." We argued back and forth, and eventually Ramzi said, "OK, you can have it." Several months later, after the book (*Basic Pathology*) had been published, Ramzi called to say that the figure was good and we should use it in the next edition of the big Robbins. He then told me that the residents had liked the figure very much. Neither seniority nor ego came in the way of settling a disagreement.

By the time I landed in Chicago, the churning in my mind was over. I knew exactly whom I would ask—Abul Abbas and Nelson Fausto. Abul was an excellent immunologist with an international reputation as a superb teacher and was the senior author of two of the best-selling books in immunology. I had known Abul from the time we overlapped at AIIMS. Nelson was known for excellence in his work on liver carcinogenesis and as the editor-in-chief of the *American Journal of Pathology*. I got to know him better when I was vice president and later president of the American Society of Investigative Pathology. The next day, I called both of them and they readily agreed. The author list on the cover of the seventh edition of the big Robbins read Kumar, Abbas, and Fausto. With the sad demise of Nelson in 2012, we have been extremely lucky to have recruited Jon Aster from Brigham and Women's Hospital to our team. A world-renowned scientist and hematopathologist, Jon shares the same values, obsessions, and compulsions, and he is certainly the best editor of the three of us. Robbins is in very good hands.

It has been my privilege to have worked for 40 years on 16 editions of Robbins's texts: 8 for *Basic Pathology* and 8 for *Pathologic Basis of Disease*. This number exceeds the revisions in which Ramzi participated, but only because of his untimely death. To honor Ramzi, I decided to incorporate his name in the title of the big Robbins.

People often think revisions must be simple because all that is needed is to update the text. In reality, it takes about two years to revise a text because we review each chapter as if we were starting from scratch. Each sentence is carefully considered—is it the best way to convey the information? Each revised chapter is read by all three of us. We live by Robbins's adage, "There is no good writing, only good rewriting." Since we add the most up-to-date information on disease mechanisms, space must be created for it by deleting some parts. In fact, deciding what to delete is as challenging as (if not more challenging than) deciding what to add. In addition, we ask ourselves, Has the new finding been repeated? Does it provide a better understanding of human disease? We call these filters the Abbas rule.

Textbooks have to evolve with the learning styles of the students (or risk being dinosaurs). Two major changes have had an impact on how the discipline is taught now and how students learn it. First, in most medical schools, the basic and clinical sciences are taught in an integrated,

organ system–based curriculum. Second, because of early clinical exposure, the time devoted to pathology (and other basic sciences) has progressively decreased. In view of these changes in the curriculum, we have written a new book—*Robbins Essential Pathology*. It is intended to satisfy the needs of today's medical students by distilling basic concepts of pathogenesis and morphology and providing clinical vignettes to highlight the relevance of pathology to the understanding of disease.

Medical Education: Initial Efforts in India

Despite my focus on research in the United States, I constantly thought about medical education and research in India. After moving to the US, I used to give lectures on my family trips back to India, mostly at AIIMS. After some time, I started doubting the utility of such lectures that seemed more like boutique efforts. I decided to spend a little more time and effort in India. In 1989, I came across a United Nations program that supported expatriates to come to India for no less than two months and get involved in a significant teaching effort. I thought hard about what I could do on such an assignment. Graduate education in India at that time did not have structured courses that were required in addition to the lab-based research. I decided that I would create a graduate-level course in immunology for PhD students at AIIMS, with the hope that eventually the faculty might create their own courses for the PhD program. Thus, I thought that I would teach them how to fish rather than serve them fish on a platter.

I created a comprehensive syllabus that involved daily reading assignments, discussed during the forenoon followed by bench research in the afternoon. To do real experiments, I shipped inbred mice to India (to the consternation of Lufthansa cargo people in Dallas). I also sent a refrigerated package containing close to $100,000 worth of reagents.

The day after I reached Delhi, I convened a meeting of the senior PhD students and the faculty to discuss how we would run the course. I asked the head of the department to encourage faculty members to be observers so that they could critique the program and then create their own courses. The students really immersed themselves in the routine but the faculty gradually stopped coming. On the flight back, I wondered what I had accomplished and felt it was very little, except for having made an impact on the training of seven students, out of thousands. I had not changed the culture, and there was little likelihood that my efforts would be scalable. In the absence of scalability, the impact would be at best trivial. I knew that I had to find another way. But that had to wait.

Developing a Competency-Based Curriculum in the United States

In the early 1990s, I began to feel that one of the problems in medical education in general was that we trained students to acquire knowledge but did not do enough to make them competent to use knowledge. We needed to move from knowledge-based to competency-based medical education, especially because medical knowledge was expanding rapidly and could be easily accessed via the internet.

Thus began a big experiment in teaching that I initiated at UTSW in Dallas between 1993 and 1995. We replaced lectures with clearly defined competencies and objectives, provided electronic resources, and freed up lecture time for self-study. One hundred cases were constructed for the entire pathology course. We then met the students in small groups to discuss the cases, emphasizing application, not memorization. The clinical cases were provided electronically along with other learning tools. This model is now called the flipped classroom. When I initiated this, most of my faculty colleagues thought that the students deprived of spoon-feeding through lectures would not learn and would rebel. I persisted, and to everyone's utter surprise the students loved it because they were being treated like responsible adults and were learning the skills needed to practice

medicine. As I shared this method with colleagues across the country, they requested that I make the modules available to others. I did so by creating a website linked to the Robbins's textbooks. I had realized two of my goals in education, impact and scale. Following the success of this program, the dean appointed me as associate dean for medical education to extend such teaching to other departments. In 2006, the American Association of Medical Colleges and the Howard Hughes Medical Institute created a task force for writing down competencies and objectives for basic science courses for the MD curriculum. Thanks to David Korn and Bob Alpern, both of whom knew of my interest in education, I was one of 10 medical educators invited to participate. The report entitled "Scientific Foundations for Future Physicians" was published in 2009 and is available at **https://www.hhmi.org/sites/default/files/Programs/aamc-hhmi-2009-report.pdf**.

Health Sciences Technology Institute in India: A Ray of Hope

In 2007, I got a call from Professor Martha Gray, director of the Harvard–MIT Health Science Technology program. She informed me that India had decided to set up a new research institute in New Delhi that would develop multidisciplinary teams of medical doctors, chemists, physicists, and engineers. She asked me to join the team that would write the white paper on this. That was like music to my ears. Shortly thereafter we went to Delhi, where Dr. Mashelkar, then director of the Council of Scientific and Industrial Research, gave us our charge. He was inspirational. He exhorted us to think big, not to limit the scope of the project because India is a poor country, and not to develop it just for India but for the entire world. I had never heard anyone in India give such a bold speech. We returned with a mandate to write up a plan for the new institute to be called the Translational Health Science Technology Institute (THSTI).

Back in the United States, Martha and I teamed up with Shiladitya Sengupta, a brilliant young scientist who had just started his research lab at MIT. Within a few weeks, Dr. M.K. Bhan, a physician scientist who was secretary of the Department of Biotechnology, came to Boston for discussions. At that meeting, we made the following key recommendations: Scientists would have a five-year contract, not a lifetime appointment; they would be peer-reviewed every five years; and all recruits would be new and would be specifically recruited for THSTI. We wanted the institutional culture to develop organically. These points were debated vigorously; compulsions of government rules were described, but we remained firm. I also convinced others that an MD-PhD program based at THSTI should be developed.

A few days after our last meeting in India, I got a call from Martha informing me that she and the group in India wanted me to become the founding director with a minimum three-year term to establish THSTI. I was very surprised since that had never been my intention. Nevertheless, I thanked her and told her that I needed to think about it. Frankly, I found the idea very appealing and felt deeply inclined to accept it. Dr. Robert Zimmer, president of the University of Chicago, was supportive and assured me that I could get a leave of absence and maintain tenure. I held discussions with members of my family, my friends, and those who had worked in India. I agonized for weeks, and then with a sad heart and a sense of defeat I wrote an email to Bhan, Martha, and Shiladitya expressing my inability to take the position (as well as my associated anguish). THSTI has been established. I am happy to have participated in its birth but I am still not sure whether I made the right decision.

A Simulation Platform for Competency-Based Medical Education: Some Success and More Hope

One day in 2012, I got an excited call from my friend and colleague Dr. Scott Stern, professor of medicine, with whom I had often discussed the idea of developing an integrated competency-based curriculum that could be delivered electronically all over the world. He exclaimed, "I have

something that is exactly what you have been dreaming of—a web-based simulation platform that is designed to teach medicine in an integrated fashion." The prototype of the software had been developed by Craig Knoche, a Silicon Valley techie. The University of Chicago had signed an agreement with his company, i-Human, Inc., to create simulation cases that would form the basis of a medical curriculum. The program presented computerized human patients, and the student could take a history, perform a physical exam, order tests, and make a treatment plan. Attached to the case were relevant basic science exercises that related to the disease of the patient. I immediately thought of India, where there was a pressing need to upgrade and standardize the teaching of medicine. On the basis of projections by the Medical Council of India, India needed to train about 1.6 million additional doctors by 2025 to meet the healthcare needs. This would require enormous capital and human resources, and i-Human could partly help solve the problem by disseminating this curriculum via broadband to virtually every part of India without having to train an enormous number of medical educators. Technology, by reaching the rich and the poor, could become a democratizing force, particularly in India, where the huge wealth disparity among its people limits access to equal opportunities for quality education.

Although Scott and I had developed the roadmap to create cases, we could not secure funding for the project. One day I happened to run into my friend Dipak Jain, the former dean of the Kellogg School of Business and a well-known educator, and asked him to look at the prototype i-Human case. He realized the potential of this tool and asked me to accompany him to Mumbai to meet Mr. Mukesh Ambani, a billionaire businessman who is the chair of Reliance Industries. Dipak serves on the Reliance Board of Directors. When I made the presentation to Mr. Ambani, he immediately liked it and asked us to work with the nonprofit Reliance Foundation to develop a comprehensive program for India. I was clear in my mind that a program for use in India must have the input and participation of Indian medical educators. I recruited 14 Indian doctors and Scott recruited 14 US-based educators. We completed the program on time and under the $10 million budget! There are 150 cases covering the most common diseases that would be required for core competency of a medical graduate. This has turned out to be very timely as India now embarks upon the shift to a competency-based curriculum for graduate medical education.

LESSONS LEARNED

Following are the lessons I have learned from my journey in science and academic pathology. Hopefully, they are reflected in this essay.

1. Be fearless: Take the road less traveled, and do not be afraid to take risks.
2. Remove the word impossible from your lexicon, since impossible is just a limitation of your imagination.
3. Share knowledge openly; holding on to it will not make you smarter.
4. Be rigorous; do not look for shortcuts.
5. Being right is far more important than being first.
6. Be respectful to junior colleagues; often they are the source of new ideas.
7. Compete with yourself, not others. Ask yourself, "Am I better today than I was one year ago?"
8. Do not set anyone else as your benchmark, for then you will be only as good as they.
9. And, finally, there is no good writing, only good rewriting.

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