A Forty-Year Odyssey in the Sea of Translational Medicine

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The beginning of this voyage began when I was still a child. The ravages of disease in my family and the deaths of animals on my family's farm had profound effects on me. My mother's family had a number of people who were afflicted with maturity onset diabetes and its complications. I observed that even though they were being treated with insulin injections, they still developed complications (e.g., blindness, amputations, kidney failure, heart attacks) that caused disability and their early deaths. I could not understand why the insulin that they were being given did not prevent their complications and deaths.

The second puzzle that struck me was why the sick animals on our farm had listlessness, loss of appetite, and weight loss regardless of whether the animals were infected with viruses, bacteria, or parasites, or had tumors. Later, I came to know the name for this phenomenon as *cachexia*. I thought if I could understand why diabetics developed complications or why cachexia occurred, then I could help people and animals that had diabetes or cachexia.

To prepare for this Odyssey, I had to develop the knowledge and skills that would be needed. I was fortunate to have had a high school teacher of agriculture, Arthur White, who encouraged me to embark on a scientific career. After studying agriculture at Rutgers University, I was admitted to one of the early Ph.D. classes at the Rockefeller Institute, where I did my thesis under Edward Reich in the laboratory of Edward Tatum. The Rockefeller was a new and unique graduate school where students were treated like young colleagues rather than bothersome students. When I finished my Ph.D., I decided that I needed to have a better understanding of medicine if I was going to have a career

in translational medicine. Fortunately, I was able to arrange, through the good graces of Irving Goldberg, to attend Harvard Medical School as a special student. My objective was not to become a practicing physician but remain a scientist who would collaborate with clinicians on medical problems. During my time at Harvard, I also read extensively the literature on diabetic complications and cachexia and looked for clues of where to begin to investigate these complex problems. Unfortunately, it was not obvious, so I kept searching for potential clues of where to begin the task of unraveling the complexities of these phenomena.

When I returned to the Rockefeller, I decided for my first independent research to purify and determine the structure of *erythropoietin*, a protein hormone that is made in the kidney and is responsible for promoting the synthesis of red blood cells in the bone marrow. I chose this area because anemia is one of the hallmarks of cachexia, and the reasoning was that if we understood this phenomenon, it might give further insight into the pathophysiology of cachexia. After 2 years of very hard work, I was faced with the realization that the tools available at the time were not sufficient to achieve the goal, and I stopped work on the project. As will be seen below, the experience gained from working on erythropoietin would serve well in ways that I would have been amazed to know at the time.

Diabetes

Fortunately, I happened to attend a cocktail party for hematologists, who were discussing a recent clinical report of the ability of high doses of urea to treat sickle cell anemia patients in crisis. The idea behind this therapy was that the urea would disrupt hydrophobic bonds between the hemoglobin S molecules and allow the trapped sickled cells to progress through the capillaries. I quickly pointed out that this was very unlikely, as molar amounts of urea were needed to disrupt hydrophobic bonds, but if the clinical results were real, then there was the possibility that cyanate, which is present in urea solutions, might be reacting with the amino groups of hemoglobin S to prevent the sickling process. Within a short period of time, we were able to show that cyanate could react with hemoglobin S and prevent sickle cells from sickling upon giving up oxygen. When we treated sickle cell patients with sodium cyanate, it was also possible to show that red cells lived longer, which led to an increase in hematocrit and, most importantly, a reduction in the number of painful crisis that these patients had.[1, 2, 3] For the first few years, we were extremely encouraged by the results. Unfortunately, after 2 years of therapy, we had two patients who developed cataracts

and peripheral neuropathy.[4] We immediately stopped the clinical program and found in animals that cyanate could react with crystalline proteins in the lens and myelin proteins in peripheral nerves. Fortunately, the patients who developed these changes reversed over the next few months after cessation of the drug. Needless to say, we were very disappointed because the drug had relieved these patients from many of the problems that they faced.

When we were investigating the reasons for the cataracts and peripheral neuropathy, we were advised by the ophthalmologists and neurologists to rule out diabetes as the potential cause. We found that none of the patients had diabetes; this appeared as an important clue to be examined. I remembered that a minor hemoglobin, hemoglobin A1c (HbA1c), had been found to be elevated in patients with diabetes. The idea existed that as a result of the diabetic state, there might be a reactive molecule like cyanate that could react with the amino terminus of hemoglobin, as well as amino groups on other proteins, that could account for the pathogenesis of diabetic complications. Working with Ronald Koenig, an M.D./Ph.D. student in my laboratory, we pursued this idea. The first experiment was to determine whether diabetic mice had the mouse equivalent HbA1c in their red cells. We found that diabetic mice had about 2.8 times the amount of HbA1c than non-diabetic mice. The *red cell* is a unique cell in the body that does not carry out protein synthesis once formed. Thus, we could ask the question: Was the increased amount of HbA1c in the diabetic animal made at the time of synthesis of hemoglobin when the cell resided in the bone marrow, or was it made as a post-synthetic modification of the hemoglobin during the time that the cell was circulating in the blood? When radiolabelled red blood cells were injected into diabetic and normal mice, it was clear that HbA1c was made as a post-synthetic modification of hemoglobin and that the rate of production of HbA1c was nearly three times faster in the diabetic animals compared to the non-diabetic mice.[5] Subsequent structural studies showed that glucose was chemically reacting with the same amino group on hemoglobin that we had seen with cyanate.[6]

In the early 1970s, there was a raging debate among clinicians over the role of glucose in causing the complications of diabetes. Thus, some physicians encouraged their patients to keep to their diets and maintain their blood glucose levels as close to normal as possible, whereas others did not see the advantage of this and believed that the complications would arise anyway. The measurement of HbA1c offered an independent objective measure of glucose control for the previous months. When we, in fact, measured HbA1c levels in diabetic patients from these two different schools of thought, we were astonished to see that

they were both the same (about two to three times) normal values. To show that diabetic control as measured by glucose in the urine was related to HbA1c in the blood, we admitted diabetic patients into the Rockefeller Hospital for several months and brought them into good metabolic control. Over time, the HbA1c values fell to values that are seen in normal individuals.[7]

The obstetricians were the first medical group to document that good glucose control was clinically important. At this time, the incidence of birth defects in children born to Type 1 diabetic patients was greater than 20%. By encouraging strict diabetic control and monitoring HbA1c, the incidence of congenital malformations dropped to 1% incidence observed in non-diabetics.[8] The importance of diabetic control in the pathogenesis of the other complications of diabetes took considerably longer to achieve. The Diabetes Control and Complications Trial with Type 1 diabetics[9] carried out by the National Institutes of Health in the United States and the United Kingdom Prospective Diabetes study with Type 2 diabetic complications in the United Kingdom[10] clearly showed that improvement in diabetic control, as measured by HbA1c, was associated with a lowering in the incidence of many of the complications of diabetes. In the subsequent years, HbA1c has become a mainstay in the management of diabetic patients throughout the world. Despite the improvement in diabetic control by the monitoring of HbA1c, patients with diabetes still constitute the main population affected by blindness, amputations, kidney loss, and neuropathy, as well as an increased risk of heart attacks and strokes. There continues to be a great need for developing new therapies to prevent and treat diabetes, as the incidence is continually increasing in our society as a result of obesity. More about this need will be discussed below.

Parasitology and Cachexia

One day in 1976, Kenneth S. Warren, who had just been hired by the Rockefeller Foundation, walked into my office and offered me a deal that I could not resist. He began by telling me how impressed he was with our work on diabetes, and he wanted us to do the same thing in the field of parasitology. It was his belief that the field of parasitology needed new people with modern scientific methods to re-invigorate the field. It was his goal to comb the world looking for 10 individuals who could join him in a new enterprise—The Great Neglected Disease Network (GND)—that would be sponsored by the Foundation. At first I was hesitant in signing up, until I heard that each group would receive $100,000 a year for 10 years. Even in those days, research money was

difficult to obtain, and I was also enthralled with his enthusiasm to help people in the Developing World. So we started on an adventure together that continued on for a number of years beyond the 10-year period. During this period, my laboratory ended up working on a number of parasitic diseases, including malaria, hookworms, Leishmaniasis, Chagas disease, and schistosomiasis. I have never met an individual who had such enthusiasm and commitment to the field of parasitology. The young people who were trained in the GND are now the leaders in the field of tropical medicine. I am sure Ken would be proud of them and the success of the program.

The question before me was: What could we do? As a child, I, like many scientists of my age, had read *Microbe Hunters* by Paul de Kruiff. One of the scientists described in this book was Paul Ehrlich, who had discovered 606, the "magic bullet" for syphilis. I remembered that Ehrlich had discovered this compound while trying to find new drugs for the treatment of typanosomiasis. This parasite is carried by the tsetse fly and causes African sleeping sickness in humans and nagana in cattle. European derived cows, sheep, and goats are very susceptible to this disease; large areas of grazing land cannot be used for cattle, which severely limits the agricultural productivity of sub-Saharan African land.

With Steven Meshnick and Peter Ulrich, we initiated a search for new drugs to selectively kill the parasite. One of the compounds that we developed could cure mice infected with trypanosomes with a single injection. We were excited about these results and decided to try this compound with infected cattle in Kenya. When we injected the drug into the infected cows, they promptly went into shock and died. We quickly determined that the drug only caused death in infected cows and had no effect on non-infected animals. This prompted the veterinarians to pronounce that this compound was a great diagnostic tool; however, it was a pity that the animal died in the process. As we were trying to better understand what was going on, I was struck by the fact that infected cattle had very few parasites but still developed a severe cachexia. The question was: How could so few parasites cause this severe wasting, while antelopes infected with the same parasite did not? One possible explanation was that the cows, which were recently imported from Europe, were producing a mediator that was inducing cachexia, whereas the African animals did not. It was obvious that if we could understand more about this mediator, we might be able to understand the basis of cachexia and possibly treat it.

Further work with various infections in experimental animals led to the discovery that *macrophages*, scavenger white cells in the mammalian body, could recognize signaling compounds from invaders such as

bacteria, malaria, trypanosomes, and viruses, as well as tumors that prompted the production of a mediator which we called *cachectin*.[11, 12] We found that cachectin, when injected into experimental animals could elicit all aspects of cachexia, the phenomenon that I had always wanted to understand. We were able to show that the molecule was a protein with an apparent molecular weight of about 70,00 daltons. Armed with this information, Masonobu Kawakami and I wrote a patent[13] describing this molecule and its activities and proposed that monoclonal antibodies to cachectin could be used to prevent cachexia, shock, and the sequelae of a number of other inflammatory diseases, including rheumatoid arthritis.

At this time, the cloning of proteins was in its infancy, and we were only able to sequence and identify the protein several years later. To our great surprise, cachectin turned out to be one and the same as tumor necrosis factor (TNF).[14] The name for TNF had its origins in the hope that the body could elicit a protein that could selectively kill tumor cells.[15] A number of attempts were made to use recombinant TNF as an anti-tumor agent, but they failed because of its innate ability to cause cachexia, shock, and death. It is of interest that the potential utility of using anti-TNF monoclonal antibodies to overcome the ravages of cancer cachexia has never been evaluated in cancer patients even though animal studies would suggest that they might be useful. I fear that the name implies some potential promotion of tumor growth if there was neutralization of in situ-produced TNF that might allow the tumor to grow more rapidly. Names are powerful! On the other hand, the use of anti-TNF monoclonal antibodies has been found to be very useful in treating a number of clinical entities, including rheumatoid arthritis, Crohn's disease, psoriasis, and a number of other inflammatory diseases.[16, 17]

In the late '90s, my research efforts took what appeared to be a sudden new turn, but in fact, it turned out it was an integration of all the work that I had previously done in diabetes and cachexia. The story began with Loretta Itri coming to my office and offering a grant. At that time, Loretta worked for Johnson & Johnson, where she had been developing the drug erythropoietin (EPO) for the treatment of the anemia of cancer. Clinical studies suggested that administration of EPO was associated with an improvement of the anemia and a rapid reduction of the malaise that these patients suffer. Because we had done considerable work on cachexia, she thought that we could help provide animal data that would substantiate the clinical observations. My daughter, Carla Cerami, and I proposed a series of potential experiments, and literally the next day, we had a check to start the work. Our

hypothesis was that EPO might be interfering with TNF, which we knew could induce malaise in animals and people.

We hired Michael Brines to help in this new endeavor. Previous studies had shown that the administration of TNF to normal rats caused these animals to take longer to learn a water maze. Before we began, we thought we should do a control of administering EPO to the animals. To our amazement, animals given EPO learned the maze faster than animals given saline. We repeated this many times and confirmed the original observations. In addition, we could measure EPO, a large protein in the cerebral spinal fluid in animals injected intravenously with EPO. Because we knew that TNF played a large role in promoting the damage of tissues due to stroke, head trauma, and eye and kidney damage to name a few, we decided to see if EPO given intravenously could reduce the amount of damage in these tissues.[18] We observed substantial protection in all of the tissues that we studied. We also observed that we could wait considerable periods of time before we gave the EPO. For example, we could wait up to 9 hours with animals that had received head trauma.

We were very excited about these animal results, as well as a clinical study with patients who had strokes.[19] Unfortunately, the amounts of EPO that were needed for tissue protection could also induce a pro-coagulant state that led to thrombosis. A large clinical study revealed that trauma patients admitted to the intensive care unit had a lower death rate, but the incidence of thrombotic episodes was significantly increased.[20] A similar increased incidence of thrombosis was also seen in bicycle riders, who used EPO to increase their performance. This led to the subsequent placement of a black box warning for EPO by the U.S. Food and Drug Administration. It was readily apparent that EPO itself could not be used as a tissue protective molecule in clinical medicine.

The question that we asked ourselves at this time was whether we could engineer the EPO molecule so that it would not induce coagulation but retain the tissue protective activity. Over a several year period, we were able to modify the EPO molecule so that it no longer interacted with the homo-dimer EPO receptor but still retained the tissue protective activity. We next identified a second receptor for the tissue protective receptor that was composed of the EPO receptor disulfide linked to the beta common receptor.[21] We named this receptor the *innate repair receptor*. By studying the interaction of EPO derivatives with this receptor, we were able to define which amino acids were responsible for the tissue protective activity. Eventually, we were able to synthesize an 11 amino acid peptide, ARA290, which reflected the

amino acids in space of the B helix of EPO that only interacted with the innate repair receptor and not the homo-dimer EPO receptor.[22] This peptide on a molar basis is as active in vitro and in vivo as the whole EPO protein at shutting off inflammation and turning on repair of damaged tissue. Although this peptide has a half-life in the blood of only two minutes in experimental animals and humans, the biological activity remains in place for a number of days after the peptide is no longer present.[23, 24, 25]

In a number of animal models, we were able to show that ARA290 had similar activities as EPO but without the side effects that prohibited its use. The question was: What clinical indications should we pursue? After considerable discussion and debate with advisors, we decided to develop ARA290 for patients suffering from neuropathy, but specifically the neuropathy associated with sarcoidosis and diabetes because of the lack of useful therapeutic agents. These patients suffer from loss of sensory and autonomic nerves that can lead to pain, numbness, and autonomic dysfunction. We chose the neuropathy of sarcoidosis because it represented an *orphan disease*, which will usually have an easier path to regulatory approval—an important point for a small biotech company. Diabetes, on the other hand, represented a potential large market for a major pharmaceutical company who could afford much larger clinical trials.

In a double blind, placebo-controlled trial, the daily subcutaneous injection of ARA290 for 28 days in sarcoidosis patients significantly improved the neuropathic symptoms, whereas the placebo group of patients did not change.[26] Of considerable importance was the observation that the patients receiving ARA290 had an increase in the number of small nerve fibers in the cornea of the eye after only 28 days of drug. This result was very rewarding to us because we had seen similar repair of peripheral nerves in experimental animals.

An exploratory phase II trial in Type 2 diabetic patients has also shown an improvement in neuropathic symptoms by ARA290. In this trial, we also saw an improvement in HbA1c values in the ARA290 group but not in the placebo group (manuscript in preparation). Further clinical studies are planned to determine whether ARA290 will be able to improve the ability of the beta cells in the islets to produce insulin and possibly prevent diabetes from occurring in the first place. This possibility I find extremely exciting.

As I look back at the Odyssey that I have been pursuing for a lifetime, I am struck by the relationships between diabetes and cachexia. Who would have thought that? Certainly not me when I first began this exciting trip.

Coda

As a young child growing up in a poor farming area in New Jersey, I happened to select *The Autobiography of Benjamin Franklin* from the traveling county library (a former bread truck). This book has given me the courage to escape from the bondage of financial and intellectual poverty. I would hope that other American children will discover this wonderful book.

Endnotes

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