

## A Surprising Journey in Translational Medicine

*Anthony Cerami*

Araim Pharmaceuticals, Tarrytown, New York, United States of America

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As I look back over the course of my work, it is easy to see how ideas, observations and discoveries are connected. At times, I must say, I was often worried about the direction of my research and experienced intense moments of discouragement. Each brief eureka moment was balanced by years of dedicated and often difficult work. However, two things propelled me forward: the idea that I could help people and the excitement of discovery. My perseverance was encouraged enormously by mentors, students, collaborative colleagues, and good luck.

I first became interested in applying science to diseases at an early age, growing up on a small chicken farm, in what was then rural New Jersey. By chance I read two books that launched me on my journey: *The Autobiography of Benjamin Franklin* and *Microbe Hunters* by Paul de Kruif. These authors inspired me to dream that although I came from a humble beginning, I might live an original life of creativity and discovery.

As I did the monotonous chores on the farm, I decided to identify what I would study when I grew up, trying to find a way to escape the tedium of farm work. The first phenomenon that interested me was the wasting (cachexia) that often occurred in sick farm animals, especially chickens. I wondered why nature would allow this to occur—it seemed counterintuitive, since the wasting brought on a rapid demise of the animals. One disease that really impressed me and particularly affected the economics of our farm was coccidiosis. When our chickens contracted this disease, they seemed to waste away to feathers in a few days, and promptly die.

The second area that I was very curious about as a child was why insulin injections did not appear to prevent the complications of diabetes. My mother's family had a number of members who suffered and died from diabetes. It seemed strange to me, since the administration of insulin should have prevented the complications.

Why didn't insulin replacement "cure" diabetes? The desire to understand these two phenomena, as well as to develop therapies that might help patients, has obsessed me my entire life.

My parents both had the equivalent of a third grade education, so higher learning was not considered a possibility. Fortunately, I had a high school teacher, Mr. Arthur White, who encouraged my academic studies and convinced my parents to let me attend college if I had a full scholarship. Naturally, I studied agriculture at Rutgers University, the State University of New Jersey, with a full scholarship, which my teacher helped me to procure. I was the first person on both sides of my family to attend and graduate from college.

After college, I was fortunate to obtain a position at a recently established graduate school, the Rockefeller Institute for Medical Research (now the Rockefeller University). I would like to take this opportunity to acknowledge the late Mable Bright, who arranged for me to be the 25th student in a normal class size of 24. The extra step that she took made a big difference in my life; as a result, I always try to help worthy students.

The Rockefeller was a unique school that encouraged the faculty to treat students as young colleagues. One of the incredible aspects of the Rockefeller was the lunch room, where everyone was expected to walk in and sit next to senior

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**Address correspondence to** Anthony Cerami, Araim Pharmaceuticals, 7580 White Plains Road, Suite 210, Tarrytown, NY 10591. Phone: 914-762-7586; E-mail: [acerami@araimpharma.com](mailto:acerami@araimpharma.com).

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and junior faculty in whatever order they came in the room and discuss science. This random seating arrangement allowed me to dine next to people such as Stanford Moore, Peyton Rouse, George Palade, Ed Tatum, Fritz Lipmann and Eugene Opie. They would actually inquire with interest about the work I was doing, and I could find out about the work they had done. A description of this dining room was accurately described in Sinclair Lewis's famous book *Arrowsmith*. Unfortunately, for financial reasons, the faculty dining room was closed in the 1970s, and a large, impersonal cafeteria was built. The Rockefeller was never the same.

My PhD thesis was carried out in the laboratory of Ed Tatum, a Nobel laureate in microbial genetics, under the direction of Edward Reich, an MD who recently had finished his PhD at the Rockefeller. The research that I carried out was in the field of nucleic acid metabolism (1,2). During this 5-year period, I became proficient in the subjects of chemistry and biochemistry.

As I neared completion of my PhD, I thought about the direction I would take in the future. I decided that although I liked basic biochemistry, my passion was to follow my original plan of trying to understand diseases and explore new ways to treat them. However, I lacked a current understanding of disease processes that would be necessary to develop new therapies.

One day, when on an interview for a postdoctoral position at Oxford University, the obvious occurred to me: I needed to obtain more information about medicine, and I should attend medical school. Through the good graces of Irving Goldberg, another Rockefeller graduate who also had an MD degree, I was able to attend Harvard Medical School as a special student.

This was an incredible experience. Not only did I learn about medicine, but I also spent my spare time in the library reviewing the literature concerning the pathophysiology of cachexia, as well as the current understanding of the cause of diabetic complications. I formulated an

outline of how to approach each of these entities using the available knowledge. There were, of course, various theories put forward to explain these phenomena, but each lacked experimental proof. Various mediators were suggested to be responsible for cachexia, and diabetic complications were thought to be due to elevated glucose levels that stimulated the biosynthesis of glycoproteins in susceptible tissues.

In 1969, I returned to the Rockefeller as an assistant professor in my own laboratory of medical biochemistry. I decided to isolate the protein erythropoietin from the urine of anemic patients. Although erythropoietin (EPO) had been described by Carnot in 1905, it had not been isolated and characterized. After considerable effort over a 2-year period, I had to admit defeat and gave up the project. Needless to say, I was both depressed and discouraged.

A number of years later, Eugene Goldwasser, who worked in this area for many years, was finally able to isolate enough material to allow the microsequencing of the amino terminus with newly discovered microsequencing technology. EPO was finally produced by recombinant technology and is currently used with wonderful efficacy in the treatment of the anemia associated with end-stage renal disease. Many years later, I would return to work on this molecule in a way that I could have never guessed at the time.

During the time that I was working on EPO, I happened to discover that cyanate anions could react rapidly with hemoglobin S in red blood cells from sickle cell anemia patients (3). When hemoglobin S is deoxygenated, the hemoglobin molecules polymerize, leading to the sickling of erythrocytes that become entrapped in capillaries and block blood flow. Reaction of cyanate with hemoglobin S prevented the cells from sickling, reduced the anemia and prevented crises in these patients (4). However, after 2 years of exposure to cyanate, two patients unfortunately developed subcapsular cataracts and peripheral neuropathy. The long-term use of cyanate was obviously not a

viable treatment option for the treatment of sickle disease.

While we were working on the follow-up of these two patients, both the neurologist and the ophthalmologist suggested that we rule out diabetes in these patients. The obvious question to me was, why did possible carbamylation of lens or nerve proteins cause a pathology similar to that seen in diabetes? I remembered that the accumulation of glycoproteins observed in diabetic subjects was suggested to be the result of enzymatic glycosylation of proteins, including a glycosylated hemoglobin called Hb A<sub>1c</sub>. I reasoned that there could be a reactive compound in diabetic subjects that was reacting with hemoglobin and other tissue proteins that played a role in the development of diabetic complications.

Working with Ron Koneig, an MD/PhD student, we set out to evaluate this concept. We quickly found that normal mice, like humans, had Hb A<sub>1c</sub>. By administering radiolabeled iron to normal mice and taking the blood and injecting a portion into diabetic and nondiabetic mice, we observed an increase over time in the amount of Hb A<sub>1c</sub> relative to HbA in the diabetic animals (5). Because the adult red cell does not have a nucleus and does not synthesize protein, the production of Hb A<sub>1c</sub> had to come about by a nonenzymatic reaction. Further work showed that the structure of Hb A<sub>1c</sub> was an Amadori product of glucose attached to the amino terminus of hemoglobin, and this is nearly irreversible (6). With these studies, it was apparent that, by measuring Hb A<sub>1c</sub>, we could estimate the average blood sugar integrated over a number of weeks. Clinical studies confirmed the utility of accessing the degree of diabetic control of patients with Hb A<sub>1c</sub> measurements (7). Subsequent clinical trials demonstrated that the reduction of Hb A<sub>1c</sub> was associated with a decrease in diabetic complications. A more detailed analysis of the history of the nonenzymatic reactions of glucose with proteins and nucleic acid to form advanced glycation end products can be found in a recent review (8).

The Rockefeller University where I worked at that time refused to patent the Hb A<sub>1c</sub> diagnostic test because they thought that no one would use the test. It is now used all over the world as a way to diagnose diabetes and manage patients with diabetes. My determination was such that I offered to personally pay for the patenting fees, and even this they rejected. Clearly, had they patented the test, licensing fees would have supported a number of future research programs now used worldwide as the gold standard for diagnosing diabetes and evaluating diabetic control. In addition, the profit incentive would have pushed standardization of the test, which has still not been adequately addressed.

One day in the mid-1970s, Ken Warren, a friend of Jim Hirsch, dean at the Rockefeller at that time, appeared in my lab to make an unusual offer. He had recently been appointed a vice president at the Rockefeller Foundation to set up the Great Neglected Disease Network (GND). This network would be composed primarily of scientists who had an interest in the field of parasitology but were not currently working in the field. He offered funding of US\$100,000 a year for a 10-year period—a remarkable offer. Although I had not studied parasites, I always had an interest in the area since I had first read about Paul Ehrlich in *Microbe Hunters*. Over the next 10 years, my laboratory worked on trypanosomiasis, malaria, hookworm and the cachexia associated with infections.

In the early part of the 20th century, Ehrlich studied the trypanosomes that caused severe wasting of cattle in Africa. He synthesized a number of organic arsenicals that could kill the parasites in infected animals. Later, when the organism responsible for syphilis was found, Ehrlich thought that the organism was a small trypanosome and started treating with the same arsenic compounds that he previously studied. One of these compounds (# 606) was used to treat syphilis for a number of years. Ehrlich proposed that the mechanism of arsenical killing of trypanosomes was by binding to impor-

tant adjacent sulfhydryl groups that were critical for the parasite. We discovered that trypanosomes do not have the enzyme glutathione reductase, an enzyme that reduces oxidized glutathione to reduced glutathione, but rather had a unique cofactor that we named trypanothione (9). This cofactor was comprised of two glutathiones attached to spermidine. Eighty years after Ehrlich proposed this mechanism, we were able to confirm it. Alan Fairlamb, who was a postdoctoral fellow in my laboratory, has continued the research of looking for new trypanocidal drugs based on this unique pathway.

The second area that we investigated was malaria pigment. In 1717, the Italian pathologist Lancisi noted that people who died of what we now identify as malaria had a dark brown material in their livers, spleens and brain. The chemical nature of this pigment (called hemozoin) was not known. Various structures had been proposed for this material, but these structures could not account for the physical properties of hemozoin. Using chemical synthesis, infrared spectroscopy, electron spin resonance spectroscopy and x-ray absorption spectroscopy, we found that hemozoin consists of heme moieties linked by a bond between the ferric iron of one heme and a carboxylate side group oxygen of the other (10). The aggregation of this insoluble product represents a novel way for the parasite to avoid the toxicity associated with soluble hemozoin. Subsequent work showed that the quinolone-containing antimalarial drugs chloroquine and quinine inhibit this polymerization process, leaving the free heme to promote free radical formation and death of the parasite.

At one point in our GND work, we discovered a novel compound that could eradicate trypanosomes in experimental animals. We were very excited about these results and decided to evaluate the compound in trypanosome-infected cows in Kenya. For reasons that we have never understood, this compound caused the rapid demise of *Trypanosoma brucei*-infected cows and goats. Non-

infected animals did not show any untoward effects when given the compound. During the investigation of these deaths, I was surprised to find that, in contrast to rodents, the infected cows that were extremely cachectic had very few parasites (certainly not enough to cause the extreme wasting). In fact, a similar parasitemia occurred with infected antelopes that did not exhibit any signs of cachexia. The question that I asked was: how did so few parasites cause the wasting and death of cows, whereas antelopes with a similar parasite load did not develop a cachectic catabolic state? One possible explanation was that the European cows, in contrast to the African antelope, were overreacting to the small number of parasites by producing a factor that was causing the cachexia. This idea prompted the search for that mediator.

Over the next few years, Masanobu Kawakami and I were able to identify a protein with an apparent molecular weight of 70 kDa that was made by macrophages in response to endotoxin, malaria pigment, trypanosomes and a number of other agents that signaled a pathological invasion of the mammalian body. See Cerami (11) for a more detailed summary of this work. We reasoned that, if we neutralized this protein mediator with antibodies, we could treat the consequences of a number of infectious, chronic and immunological diseases. In 1981, we wrote a patent that has become the cornerstone patent of anti-tumor necrosis factor (TNF) therapies. Anti-TNF treatment of rheumatoid arthritis, Crohn's disease and psoriasis has helped millions and has resulted in over 100 billion dollars in sales of the various different monoclonal antibodies.

In the 1990s, I worked on several compounds that I thought might help treat diabetes and its complications. All of these compounds failed in clinical trials. However, I learned a number of important lessons from these disappointments. Of paramount importance is the critical need to select clear objective endpoints that can quickly identify efficacy. This

has been an important element in the clinical development strategies for all the compounds on which I have subsequently worked.

During the 1990s, my laboratory spent considerable time attempting to identify the body's natural mechanism to shut off the production of proinflammatory cytokines, but we never identified one. In 1999, Michael Brines came to work with me in a collaborative effort that has been both exciting and productive for 15 years. We happened, by chance, to discover that recombinant human erythropoietin could completely turn off inflammation and initiate repair in a number of animal models (12). After all those years, I was back working on EPO. Further work revealed that in response to damage, when the blood supply was compromised, hypoxia occurs, which leads to the turning on of HIF1 $\alpha$  (hypoxia inducing factor 1 $\alpha$ ), which turns on the synthesis of a number of genes, including VEGF and EPO. This EPO is hypoglycosylated and has a very short half-life *in vivo* (2 min), compared with the EPO that is made in the kidney (5 h), which is responsible for the production of red cells in the bone marrow.

However, a clinical study of recombinant erythropoietin (rhEPO) in patients admitted to the intensive care unit showed a 50% reduction in deaths, but a 40% increase in the incidence of thrombotic events. Additionally, although clinical studies of treatment of stroke by rhEPO appeared to show a benefit in neurological outcome, patients on thrombolytic therapy also suffered an increase in cerebral hemorrhage. Additionally, a concern was also raised that rhEPO could support tumor growth. Ultimately, rhEPO was given a block box warning by the U.S. Food and Drug Administration for these potential side effects.

The question arose whether we could separate the hematopoietic and thrombopoietic activities from the antiinflammatory activities of EPO. Subsequent work (13) showed that rhEPO interacted with two receptors: the homodimer receptor, important for hematopoietic and

thrombopoietic activities, and the heteromer receptor of the erythropoietin receptor (EPO-R) disulfide linked to CD131 ( $\beta$  common receptor), which we have named the innate repair receptor. CD131 is also the signaling receptor for a number of cytokines including interleukin (IL)-3, IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF). The innate repair receptor is normally not expressed on cells, but can be rapidly externalized in response to inflammation or damage. Further work allowed us to discover that the B helix of EPO bound to this receptor and that we could synthesize an 11-amino acid peptide (ARA290), which represented the amino acids in the region of the B helix that interacts with the receptor (14). ARA290 is as active as EPO on a molar basis, but completely lacks the hematopoietic and procoagulant activities of EPO. This peptide activates the body's natural repair system and has been tested in many animal models, including head trauma, myocardial infarction, burns, kidney damage, retinal damage and nerve damage, showing efficacy in stopping inflammation and initiating repair in every tissue evaluated (15).

Having had failure with several agents in the past going from animal models to the clinic, we decided it was important to select a chronic disease as a primary endpoint, where tissue damage was an important aspect and methods were available to objectively measure an effect in a 30-day period of drug administration allowed at that time by the preclinical toxicology. In collaboration with Albert Dahan and his colleagues at Leiden University Medical Center, where we had moved to proceed into clinical work, we began studies to test the efficacy of ARA290 to treat neuropathic pain in two chronic diseases: the orphan disease sarcoidosis and type 2 diabetes.

Our clinical development strategy was to include both patient-reported outcomes (PROs) as well as several objective endpoints for the most rigorous test of efficacy. Whereas PROs are important

indicators of what the patient is suffering from, more quantitative measures are critical to allow the evaluation and substantiation of the possible drug effect. The two objective tests we have used in these studies are corneal confocal microscopy (a method to visualize and count the numbers of small nerve fibers in the cornea) and quantitative sensory testing of the face, hand and foot.

The results of the sarcoidosis trial of daily subcutaneous administration of 4 mg/d for 28 d have been published (16), and the results of the diabetes trial are in preparation. In both trials, we have seen an improvement in the ARA290 group versus the placebo group in the PROs as well as an increase in the number of small nerve fibers in the cornea in the active group. This finding substantiates the results of our animal trials, where we have seen inflammation attenuated, followed by the repair and regeneration of damaged tissue activated. With these encouraging clinical results we will now initiate longer trials in these two groups of patients.

I have to admit, I am humored by the fact that in the studies of ARA290 in diabetic patients, we have seen an improvement in Hb A<sub>1c</sub>—a test that I developed many years ago. How great is that?

## CODA

As one can see by this review, the path of translational medicine is not easy; in fact, some days (more honestly, years) were very discouraging. There are surprises, both good and bad, but the work is deeply rewarding when something is discovered that helps patients. The basic biological questions I asked as a youth have been the framework for most of my scientific work. It has taken courage as well as perseverance to explore ideas that are new and original. There is no map for the unknown, but that is the fun of it.

## DISCLOSURE

A Cerami is the CEO of Araim Pharmaceuticals, which is developing ARA290 for the treatment of diabetes.



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