Dankesrede
von
Prof. Dr. Charles Dinarello

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Es gilt das gesprochene Wort.

Ladies and Gentlemen, distinguished guests, colleagues and friends. It is with gratitude that the field of cytokines will be part of the heritage of Paul Ehrlich and Ludwig Darmstaedter Prize. I am grateful to the committee of scientists as well as to the Paul Ehrlich Foundation. Needless to say, this is an honor for me personally but I accept this prestigious prize in the name of the many who dedicated their scientific careers to unravel the world of cytokines.

We know today what cytokines are – small proteins that are very active at both causing inflammation but also at regulating the immune system. In many ways, it has been an adventure of unexpected surprises. Some will say it began 25 years ago, but actually it goes back to over 3,000 years ago. Since recorded history, philosophers and physicians wrote about fever. Without doubt they were impressed with the cold shaking chills, the rapid increases in body temperature, the burning heat of the skin, only to be followed by flushing and profuse sweating. Within hours, the whole process would repeat itself. The history of cytokines actually begins with a desire to understand fever, and specifically what causes fever. What happens during an infection or an injury or a cancer that results in fever? Ancient cultures recorded fevers with pictographs placed over different parts of the body. There were Sumerian and Egyptian hieroglyphics to indicate fever. The Greek physicians had their theories based on the fevers of typhus and malaria. Roman military physicians made the connection between "pus" and fever. During the Middle Ages, fever was thought to be due to possession by devils inside the body. When William Harvey discovered the circulation of the blood, fever was thought to be due to the heat generated by the friction of blood in the vessels. It was not until the Renaissance that glass blowers developed the first thermometers to measure fever in humans. The great German pathologist Rudolf Virchow and the British experimentalist John Hunter theorized about fever. The interest in fever seems to be present at each phase in the development of science. But what does fever have to do with cytokines?

As a medical student at Yale University, I was assigned to care for a young woman, with a terrible disease called Lupus. She was admitted to the Hospital with high fevers. We had a great difficulty controlling her disease and I remember the violent shaking chills that started the process as her body temperature rose to 41 and ever higher. We searched for an infection as a cause of her fevers but found none. We knew then that fever was due to a small protein produced by white blood cells. The small protein was called "endogenous pyrogen" from "pyros" – the Greek word for fire. Endogenous pyrogen was produced during an infection or an inflammatory disease, entered the blood stream and reached the brain, where body temperature is controlled. There, the brain would send signals back to the body to increase body temperature. I was determined to work on this fever protein, endogenous pyrogen, and wrote my medical school thesis in 1969 on the production of endogenous pyrogen by the liver.

But very little was known about the physical characteristics of the “endogenous pyrogen” protein. What did the fever molecule looked like, what were the sequences of its amino acids, what was its structure like and how did it work? We could make endogenous pyrogen in the laboratory from white blood cells in fresh human blood. But the real problem was to separate this small protein – “endogenous pyrogen” – from the tens of thousands of other proteins produced by white blood cells. When we started the project in 1971, I had no experience in purifying proteins. There were so many problems that we had to overcome. We constructed glass columns over 180 cm long and containing over 3,000 mL of filtration beads in order the separate the different proteins. We had to use a small ladder to reach the top. We had to devise our own concentrating methods. And all of this had to done in a cold room of 4 degrees.

In 1973 I came here to Frankfurt to learn a new technique called flat-bed iso-electric focusing to separate the proteins in an electrical field. Because our losses in fever-producing activity were so great, we changed the purification steps, I think as often as the seasons changed. We measured fever by injecting rabbits using rectal thermometers, and I came home many a night with rabbit feces under my fingernails. As we came closer and closer to purifying human endogenous pyrogen to a single protein, we could not see anything using standard protein tests. We had to make the proteins radioactive to see something. It took nearly 7 years with many failures, broken dialysis bags, jammed fraction collectors ending up with months of work on the cold room floor. But in 1977 we published our findings and wrote that only a few nanograms per kilogram of this protein were needed to cause a fever. A few nanograms is one-milliard of a gram.

So endogenous pyrogen appeared to be an incredibly potent molecule, far more potent than any hormone. Of course, typical of scientists, many were skeptical and some criticized our purification as bacterial contamination. But we had taken extraordinary measures to prevent bacterial growth. Keeping bacteria contamination away from our purification steps was an absolute necessity but it also became an obsession. After 1977, we continued to improve the purification methods but still ended up with precious little.

At about the same time, there was another small protein being studied by immunologists called “lymphocyte activating factor”. We realized that “lymphocyte activating factor” had many physical similarities to those of endogenous pyrogen. So in 1977, we tested our best endogenous pyrogen on mouse lymphocytes and it activated them; it activated them amazingly well! We repeated and repeated by testing endogenous pyrogen on mouse lymphocytes. We wanted to be absolutely sure. Why would a human protein, which induced fever in rabbits, also activate the lymphocytes of a mouse? Finally, after two years of seemingly countless experiments, we published in 1979 that human endogenous pyrogen was the exact same molecule as lymphocyte activating factor. For immunologists, such a conclusion broke with the specificity of the immune system. Understandably, there was a lot of criticism. At scientific meetings, our endogenous pyrogen was called “lymphodreck” and “schmutzie stuff”. A famous scientist at the Institut Pasteur in Paris even published an entire paper on why endogenous pyrogen was not the same molecule as lymphocyte activating factor and also gave talks against our data at scientific meetings. These were challenging years for us.

We gave our purified endogenous pyrogen to collaborators and it became increasingly clear that the molecule did more than cause fever: endogenous pyrogen killed the insulin-producing cells, broke down cartilage in joints, induced sleep, depressed appetite but stimulated the liver and increased antibody production. It caused the loss of muscle mass, blood pressure to fall, lowered the pain threshold but stimulated the bone marrow. The molecule seemed to re-create the entire scenario of an infection or immunization. As a result, the names endogenous pyrogen and lymphocyte activating factor changed and the term Interleukin-1 was adopted for both. The year was 1979 and the first cytokines were termed interleukins from the name "leukos" meaning white – for white blood cells. Interleukins were made by white blood cells and activated other white blood cells. Later we learned that these molecules were produced by all cells and acted on all cells, hence the name cytokine, meaning acting on all cells. Yet, in those early years, no one could produce enough Interleukn-1 to analyze the protein.

We really had to settle the issue whether a single small protein could have so many different properties on nearly all cells, and so it was necessary to isolate the gene for Interleukin-1. On February 1, 1982, I was relieved from patient care duties and started the cloning project with Phil Auron, Drew Webb and Alex Rich. Like the purification project, isolating a gene in 1982 was a challenging task, primarily because molecular biology was in its infancy. Only 3 human genes had been isolated by 1982. There were few reagents available and no kits. We also had heavy competition from big pharma and new biotechs. It was like a David against a family of Goliaths. We had a low budget from the United States National Institutes of Health and a very small laboratory. I remember walking across the Longfellow Bridge and looking down on the Charles River and saying to myself: "this gene project is not working, it is just impossible". We had used the white blood cells from over 60 liters of fresh human blood and still had nothing. But slowly, very slowly, we had some successes. Back in 1977, we had made antibodies to human endogenous pyrogen and these were very helpful in the effort. And after two years, some 25 years ago, in the summer of 1984, we isolated the gene for Interleukin-1.

We submitted our manuscript to the most prestigious scientific journal at the time but it was rejected. Then, only a few months after rejecting ours, the same journal published the Interleukin-1 gene manuscript of a biotech company. 10 years later, we found out that the same biotech company that rejected our manuscript had taken our gene during the manuscript review process. In the following years, it was bitter for us to see scientists quoting the paper of the biotech company and not ours. But then there was good news in all this darkness. Each of the different and varied properties of endogenous pyrogen was confirmed by Interleukin-1 made from the Interleukin-1 gene. Things started coming together and the new field of "cytokines" was now validated. As a result, no one doubted anymore that a single molecule could be so active in so many different parts of the body. And yes, for me personally, Interleukin-1 made from the Interleukin-1 gene was the most potent fever-producing molecule, as we had predicted in 1977. To this day, I cannot fully describe to you my internal satisfaction when humans developed fever following the injection of only a few nanograms.

What happened next? More cytokines were found to induce inflammation and arthritis such as tumor necrosis factor and genes for new cytokines that stimulated the immune system were given names such as Interleukin-3 and 4 and 5, 6 and 7. Today there are over 50 cytokines with the name interleukin and over 100 different

molecules that fall under the classification of cytokines. And true to their character, many cause inflammation and stimulate the immune system. Why did nature duplicate so many different cytokines? One explanation is that nature could not trust these important properties such as fever, inflammation and regulation of the immune system to just a few molecules. Through millions of years of evolution, nature had developed the ultimate back-up system.

If all of this was not exciting enough, there was another surprise: the working part of the interleukin-1 receptor was found in the fruit fly and also in fish! What was the working part of the interleukin-1 receptor doing in these primitive animals?

The working part of the interleukin-1 receptor is called the Toll-Interleukin-1 protein. The word "Toll" comes from the discovery of the Toll protein in the fruit fly and the exclamation of the German scientist who said "aber das ist toll". With the discovery of the Toll-Interleukin-1 protein, another field started called the "innate immune response". This field is based on the presence of the same Toll-Interleukin-1 protein in receptors that recognized invading bacteria and viruses. What is the significance of the Toll-Interleukin-1 protein? For over 300 million years, nature made certain that infections induced inflammation and that inflammation helped survival. Yes, when the fruit flies are infected, the Toll-Interleukin-1 protein triggers inflammation. But without the Toll-Interleukin-1 protein, they do not survive the infection.

Today we know that cytokines affect nearly every biological process; these include embryonic development, most, if not all diseases, cognitive functions and degenerative processes. But cytokines also help regenerating cells and are used to improve vaccines. Even what we eat affects cytokines. Whether we eat salmon or salami influences cytokines. In 1979, no one could have imagined that the field would expand so much from brain diseases to kidney diseases and from rheumatology to cardiology. Scientists in academia and in the pharmaceutical industry have contributed equally to the field and this would please the two great scientists that bear the name of this prize.

Today new anti-cytokine drugs are being developed to reduce inflammation in joints, to treat diabetes, to save transplanted organs from rejection, prevent a heart attack and to stop the loss of cognitive functions. I return to Interleukin-1, the cytokine that started this adventure and first studied because it caused fever. I remember reception for my medical school class. With a cocktail in her hand, the wife of the Dean was busy asking the students about their research theses.When she approached me, I said that my thesis was on fever. She replied with all seriousness, "well…. doesn't aspirin work anymore?"

Looking back, we see how obvious it all was that fever is only one manifestation of disease. We have used what we learned well – because drugs that block inflammatory cytokines are very effective in reducing pain and misery and return function and quality of life to so many patients. For those who contributed to the field, some of whom are in this great space this morning, there is no equal to the satisfaction that your efforts have reduced the burden of disease.

In closing, I think of the giants who did not live long enough to see how their spark of inquiry provided the basis for this new branch of medicine and biology. I owe much to my teachers: the late Phyllis Bodel and Elisha Atkins and particularly to my

mentor, the late Sheldon Wolff. None of this was not possible without our students, our post-docs and our collaborators, some in front of me, and I recognize them for your dedication, productivity, creativity and hard work. And I cannot leave this platform without thanking my companion, my partner and my friend for the past 30 years, Professor Edward Kinney, a gifted scientist and international leader in his own field of nuclear physics, for his sacrifices, encouragement and support, without which I could not stand before you today and accept this prize.