Acceptance speech by Jean-Laurent Casanova

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[Address]

German was my first foreign language at school. Between the ages of 10 and 18 years, I spent about half of my Christmas, Easter, and summer vacations in Germany, and several German penfriends came to stay with me in France. It is not without emotion that I recall my youth in Lübeck, Frankfurt am Main, Köln, Gunzenhausen, and Berlin. By the time I was 18, my German was finally becoming intelligible, but English gradually took precedence and, unfortunately, I eventually forgot much of the German I had learnt. Standing before you today, I am struck by the idea that it would do me good to spend a sabbatical year in Germany or Austria. With a bit of luck, some German would come back to me, probably, alas, just before my return to the US! All of this is a roundabout way of saying that I have my own personal reasons for feeling particularly moved and proud to receive the Koch Prize.

I would like to thank the members of the jury for this extraordinary recognition of the work of our laboratory over the last two decades. Being awarded the Koch Prize is an immense honor, given the prestige of Robert Koch himself, the members of the jury and previous laureates. I cannot help but feel like a dwarf among giants. Previous laureates include a number of role models that have actually played an important role in my scientific life, such as Gérard Orth, Carl Nathan, and Max Cooper. This makes the experience even more surprising and humbling.

I would also like to thank my scientific partner, Laurent Abel, who runs the lab with me, and is in charge of both the dry team and the Paris branch. He always brings calm and reason to the table. I want to thank our scientific associates, Emmanuelle Jouanguy, Stéphanie Boisson-Dupuis, Anne Puel, Capucine Picard, Jacinta Bustamante, Guillaume Vogt, Shen-Ying Zhang, Bertrand Boisson, Michael Ciancanelli, Aurélie Cobat, and Alexandre Alcaïs, each of whom has added his or her own specific touch of talent and diversity to this expanding and lively family. Unfortunately, it would take too long to cite all the students, post-docs, and staff, from Germany and elsewhere, who have worked with us with enthusiasm, but they nevertheless deserve a mention here. A very special thank you must go to Yelena Nemirovskaya, our lab administrator, without whom the arrival of our group in the US would have been catastrophic, much like a plane

crash with no survivors. Without these individuals, my journey would have been much less interesting and I would not have been able to do much, just generating ideas and plans, which is certainly less than they would have been able to do without me.

Finally, I would like to thank the wonderful institutions with which I am associated on both sides of the Atlantic. In Paris, the Necker Hospital for Sick Children, the Imagine Institute for research on genetic diseases, Paris Descartes University and the INSERM. In New York, the Rockefeller University, the Howard Hughes Medical Institute, the St. Giles Foundation and the NIH. These institutions have provided me with support going well beyond that usually given to scientists. They were brave enough to fund our dream of a transatlantic organization bringing together one of the oldest hospitals for sick children in Europe and one of the most prestigious research institutes in America.

So what have we done over the last 20 years? Well, we began by traveling back in time. We revisited a problem that was posed at the turn of the 20th century, when Clemens von Pirquet and Charles Nicolle discovered latent and unapparent infections, respectively. These findings made it clear that microbes were necessary, but not sufficient, for the development of infectious diseases. The key problem in the field of infectious diseases was thus posed and, paradoxically, it concerns their pathogenesis.

The work that Pasteur began on silk worms in 1865 led to the germ theory of diseases and culminated with Koch's discovery of the agent of tuberculosis in 1882. This discovery was supported by Koch's postulates, which were strict criteria that Koch considered necessary to attribute the responsibility for disease to a microbe. They include the notion that healthy individuals should not harbor the pathogen, which should be found in all patients. These rigorous criteria were necessary at the time, to convince the world that microbes cause disease, but we now know that even the most valid discoveries, or at least certain aspects of them, have a short half-life. There is no absolute truth, only causal relationships in given experimental or natural conditions, which are continually changing. As my work has followed on from a partial revision of Koch's postulates, there is a particular scientific resonance for me in being awarded the Koch Prize.

Indeed, about 30 years after Koch formulated his criteria, it became apparent from the work of von Pirquet and Nicolle that only a minority of infected individuals developed clinical disease. We

now know that, for the vast majority of microbes, including some of those responsible for the most dreadful scourges of mankind, such as *Plasmodium, Mycobacterium*, and *Influenza*, most infected people remain well or develop a self-healing disease. Ebola virus and HIV appear to be exceptions, at least for now, but they may eventually come to follow the rule as the two pandemics unfold and the human and viral populations evolve together.

The burden of infectious disease was colossal until the end of the 19th century, with half of all children dying of fever before the age of 15 years and a mean life expectancy at birth of only about 20 years. However, this situation principally reflected the large number of infectious agents. Individual microbes did not kill often; they killed collectively. The burden of infection was eventually controlled by the successive development of an array approaches, including hygiene, vaccines, aseptic surgery, and antibiotics, all of which were derived from the germ theory of disease.

So what accounts for interindividual variability in the clinical course of infection? In 1910, there was already a theory at hand, the immunological theory, which we now know is a somatic theory. It is based on T and B lymphocytes, corresponding to the two arms of adaptive immunity, a phenomenon of such immense biological importance that it developed twice in vertebrates, by convergent evolution, as beautifully shown by Max Cooper. The best illustration of this theory, and indeed the first evidence in its favor, was Pasteur's demonstration of vaccination by attenuated microbes in 1880-1882.

So, by 1910, one could account for interindividual clinical variability in the course of reactivation of latency or, even more easily, in the course of secondary infection, at the population level. Vaccinated individuals, or survivors previously infected with the same or a similar microbe, would have developed acquired, specific immunity. This concept is most applicable to adults, and particularly to the elderly, whose somatic immunity has had decades to diverge in response to different environmental or commensal, microbial challenges. But how about clinical heterogeneity in the course of primary infection, which typically strikes children, and was then the main public health problem?

Microbiologists and immunologists were not eager to tackle this problem, which I like to refer to as the "infection enigma", for various reasons of historical interest. The microbiologists were busy discovering new pathogens, and then new commensals and saprophytes, and studying

their metabolism and genomes, in rapid succession. They also developed the field of microbial pathogenesis and immunity to infection in animal models. Above all, they aimed to kill the microbes, an endeavor that began here in Germany, with Domagk's discovery of sulfamides, and was followed by the work of Dubos, Fleming, Chain, and Florey, with their discovery of antibiotics. This, in turn, led to the study of antibiotic resistance. Finally, microbiologists created the field of cellular microbiology, at the crossroads of cell biology and microbiology.

The immunologists were too busy to tackle the infection enigma because their attention was, understandably, drawn to another problem, equally fascinating and challenging: the antibody enigma. How could vertebrates make specific antibodies not only against all non-self structures existing in nature, but also even against structures that were not natural? Karl Landsteiner, from 1917 onward, first in Austria and then at the Rockefeller, showed that animals could make antibodies against a new type of synthesized chemical compound not found in nature. From the early days of immunochemistry, focusing on antibodies and complement, to the study of immunobiology, with the discovery of the T- and B-cell dichotomy by Jacques Miller and Max Cooper in the 1960s, and beyond, the process of antigen-specific responses has been the main focus of studies by immunologists. This diverted their attention from infectious agents and it was greatly to the credit of Stefan Kaufmann, in the 1980s, that he reminded us that hen egg ovalbumin or lysozyme, not to mention their countless haptens, were not the real antigenic threats confronting our natural defenses.

Behind these rational and understandable motives, other deep trends may have been at work, perhaps unconsciously in most scientists. Here, I will stick my neck out and offer a personal interpretation of the last 100 years of research in the fields of microbiology and immunology. Microbiologists, not only in France and Germany, but worldwide, are the legatees of the greatest medical theory ever, the germ theory of disease. This theory alone, with its direct implications, has saved billions of human lives and led to an increase in life expectancy from 20 to 80 years. The vision of microbiologists has therefore, understandably, remained microbe-centered. They guard a doctrine that has transformed the world and they are reluctant to consider other views, even if they are complementary and not contradictory.

The behavior of immunologists is a bit more difficult to decipher. My personal interpretation is that immunologists love the immune system. In particular, they have been under the spell of adaptive immunity since the discovery of vaccination. They did not really understand Metchnikoff

and most sided with Ehrlich because the macrophage did not fit into the emerging paradigm of antigen-specific responses and could not thus contribute to the understanding of acquired, specific immunity. Pulmonary physiologists love the lungs, and cardiologists love the heart, if I may be bold enough to make the comparison. So, the idea that the immune system is the weakest of all physiological systems, at the individual level, is anathema to an immunologist. The immune system in its entirety, both its hematopoietic and other components, is, however, only effective at the population level, ensuring that reproduction occurs. In natural conditions, it is not very efficient at the individual level. This makes sense and is already a remarkable achievement, given the enormous difficulty of the task – defending the host against trillions of evolving microbes. It is more challenging to deal with an infinite variety of evolving microbes than, say, with atmospheric pressure or the concentration of oxygen in the air, which vary little and certainly not at such a rapid rate. Nevertheless, immunologists are reluctant to admit that, by definition, a life-threatening infection implies an impairment of immunity, which, in the case of children and young adults, is more likely to be inherited than acquired.

In this context, it is perhaps not surprising that human, animal, and plant geneticists tackled the infection enigma as a problem of host resistance or susceptibility to infection. Of course, they did so from a host genetic perspective. In the realm of human genetics, both clinical geneticists, including Archibald Garrod, and population geneticists, including Karl Pearson, proposed a germline, genetic theory of infectious diseases. A body of clinical and epidemiological evidence amassed and had become compelling by the 1940s. The beautiful twin studies on tuberculosis carried out in Germany in the 1930s are particularly worthy of mention here.

The genetic theory of infectious diseases took off, in cellular and molecular terms, in the late 1940s and early 1950s, with the description of the first primary immunodeficiencies. A key point to bear in mind here is that these conditions were revealed by the availability of antibiotics. Ogden Bruton’s first patient with X-linked recessive agammaglobulinemia, the first primary immunodeficiency identified as such, had suffered from 19 episodes of pneumococcal meningitis, all cured by antibiotics. Before the advent of antibiotics, he would have died during the first episode, like all children with any of the X-linked recessive, autosomal recessive, or autosomal dominant etiologies of agammaglobulinemia discovered by Mary Ellen Conley from 1993 onward. The pediatricians focused their attention on children with multiple, recurrent infections, and then on patients with opportunistic infections – a term coined by German hematologists to refer to infections striking patients with detectable hemato-immunological

abnormalities. They noted that such rare clinical phenotypes cosegregated as Mendelian or familial recessive traits with an immunological phenotype, such as agammaglobulinemia or neutropenia. From this moment, the field was structured, imprinted with the notion that other sick children with a single severe infection, particularly if the infection concerned was not opportunistic, were not immunodeficient but immunocompetent. This was, of course, an illusion, if not a delusion, but collective beliefs are difficult to break down, as we all know. Would we speak of respiratory failure, with normal oxygen pressure in the arteries?

From the early 1990s onward, our contribution to the field, and to the infection enigma, has been to take the human genetic studies of the 1920s and 1930s seriously and literally, leaving the immunodeficiency vs. immunocompetence paradigm defined in the 1950s to one side. We also proposed a specific architecture for the genetic determinism of infections. We proposed a monogenic architecture, which was testable and simple, but is still considered heretical by many population geneticists, who tend to study infections from a “polygenic” angle. Their reaction stems, in part, from the elegant discovery of resistance to *Plasmodium falciparum* conferred by the sickle cell trait, by Anthony Allison in 1954. However, there was never any claim that this observation explained the pathogenesis of severe malaria (even with a relative risk of 10). Instead, it provided evidence for natural selection in humans driven by infectious agents. At odds with this polygenic theory, we have suggested that life-threatening infections in the course of primary infection may result from single-gene inborn errors of immunity, displaying high, but not necessarily complete penetrance.

We proceeded in two successive steps. We began by searching for the genetic basis of Mendelian susceptibility to mycobacterial disease (MSMD). This is a group of truly Mendelian conditions — monogenic disorders displaying complete or at least very high clinical penetrance. The proportion of familial cases, as opposed to sporadic cases, is, therefore, relatively high. The patients are selectively susceptible to mycobacteria and, more rarely, *Salmonella.* Over the last 20 years, we and others have discovered, by forward genetic approaches, mutations impairing IFN-γ immunity – a group of 17 inborn errors affecting nine genes. We have also deciphered the genetic basis of other Mendelian “holes” in host defense, including chronic mucocutaneous candidiasis (due to mutations affecting IL-17 immunity), and invasive dermatophytosis (due to mutations of CARD9). In parallel, Gérard Orth has elucidated the first single-gene inborn errors underlying persistent cutaneous infections with certain human papillomaviruses in

epidermodysplasia verruciformis and other groups have discovered the genetic basis of predisposition to fulminant Epstein-Barr virus infection in X-lymphoproliferative syndrome.

The genetic dissection of MSMD was the first, and has become the most comprehensive study of Mendelian predisposition to a narrow, specific group of microbes. Mutations of genes encoding the terminal components of complement or its regulatory protein, properdin, had already been described, which was encouraging. These defects underlie recurrent disease caused by *Neisseria*. However, the cue for studies of these defects was not selective Mendelian predisposition to *Neisseria* in the absence of overt immunological abnormalities. Instead, it was a defect of complement activity that led to the discovery of specific proteins, and then to the identification of gene mutations and the study of other patients with *Neisseria* infections.

We assessed the broader significance of these encouraging results, by studying children with invasive pneumococcal disease and children with tuberculosis. We found mutations of TLR and IL-1R genes in children with pneumococcal disease. We also discovered the first cases of Mendelian tuberculosis, in rare families with IL-12Rβ1 deficiency manifesting solely as severe tuberculosis. We also discovered the genetic basis of isolated congenital asplenia and rare cases of classic Kaposi sarcoma of childhood. These studies, which we are still pursuing in the laboratory, served as a platform for a much more difficult study, that of herpes simplex virus encephalitis.

We selected herpes simplex virus encephalitis for study because there was no obvious reason to think that it would have a genetic basis – only an intuition, and the lack of an alternative hypothesis, at least in our view. This life-threatening disease strikes otherwise healthy children once, in the course of primary infection with the herpes simplex virus, which is ubiquitous. The arguments against a genetic hypothesis, and against a monogenic hypothesis in particular, included the sporadic nature of the disease, with only four multiplex families having been reported since its first description in 1941. This disease is the most common sporadic viral encephalitis in the Western world. Moreover, none of the known primary immunodeficiencies, including severe combined immunodeficiency, and none of the known acquired immunodeficiencies of childhood, including AIDS, were risk factors. Finally, this infection is strictly limited to the central nervous system, at odds with the disseminated infections constituting the hallmarks of immunodeficiency. We felt that there could be no better infection on

which to test our non-Mendelian monogenic model, i.e. the idea that single-gene lesions with incomplete penetrance may underlie severe infections.

Two initial observations guided the entire project in the right direction. First we found that 14% of French children with herpes simplex encephalitis were born to consanguineous parents. This was surprising, because all the cases in the nationwide epidemiological study were sporadic. There was not a single multiplex family. This simple clinical survey therefore suggested that herpes encephalitis was probably caused by monogenic inborn errors, including autosomal recessive traits, albeit with incomplete clinical penetrance, and therefore not Mendelian.

We also serendipitously discovered two children with a unique phenotype of herpes encephalitis and mycobacterial disease. These children played a key role in subsequent studies, as their mycobacterial susceptibility phenotype enabled us to identify their genetic lesions. We found that they had mutations of the *STAT1* and *NEMO* genes, leading us to hypothesize that herpes encephalitis in otherwise healthy children might be caused by inborn errors of interferon immunity. We analyzed genome-wide linkage data and both blood and fibroblastic responses to herpes virus and various viral intermediates from this interferon-based angle, and discovered mutations affecting the TLR3-interferon pathway in children with isolated herpes encephalitis. With the aid of iPSC technology, we went on to show that the mutations in these patients impaired intrinsic antiviral immunity in neurons and oligodendrocytes, but not in leukocytes and epithelial cells, accounting for the brain-tropic nature of herpes encephalitis. Of course, these findings have opened up a whole new can of worms: what is the clinical penetrance of these genetic lesions? What governs incomplete penetrance? What is the proportion of cases caused by variants of the TLR3-interferon pathway?

At any rate, the discovery of single-gene inborn errors of interferon-dependent brain-intrinsic immunity in children with herpes encephalitis is important from both clinical and biological perspectives. First, these findings provide proof-of-principle for our monogenic (but not Mendelian) theory of life-threatening primary infectious diseases. They pave the way for the prevention or treatment of herpes encephalitis with interferon, in addition to acyclovir. Second, the immunological implications, which can be seen as a happy by-product of these studies, are that intrinsic immunity in non-hematopoietic cells is life-saving. Our other studies, dealing with molecules as diverse as IFN-γ, IL-17, TLRs and IL-1Rs, have also turned up a number of immunological surprises, revealing, in particular, a much greater degree of redundancy in

outbred humans in natural conditions of infection than in inbred mice in experimental conditions of infection.

Beyond the pathogenesis of infectious diseases and the analysis of immunity to infection, the field of primary immunodeficiency has expanded in many other directions over the last two decades. Our model of a monogenic architecture of inborn errors of immunity to infection may turn out to apply to various types of phenotypes other than infections. In areas such as allergy, autoimmunity, autoinflammation, and malignancies, this model of rare monogenic lesions underlying heritable phenotypes with incomplete penetrance may be valid and drive greater progress than the common variant-common disease and polygenic models. Various areas of pediatrics and internal medicine may increasingly be connected with primary immunodeficiencies. The field of primary immunodeficiencies, once considered a very minor subfield of hematology, is pervading an increasing number of areas of pediatrics and internal medicine. We may soon see a paradigm shift, with a monogenic (but not Mendelian) theory of diverse life-threatening conditions of mankind.

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