Nobel Laureate for Medicine Prof. Bruce Beutler's keynote speech and dialogue on "The global struggle against infectious disease" on Monday, February 2, 2015, hosted by Naresuan University in Bangkok

First I want to say how grateful I am for my reception here and also how thankful I am to Naresuan University for this tremendous honor. I am deeply touched by it, and very happy to be here today.

Let me start with a few comments about the title and why we are talking about a "global struggle against infectious disease". I think you'll see in the course of my talk, which takes a somewhat historical approach, that we've all been here before, that this is a problem solved by one part of the world after another and as communications have grown in recent times, we've come to see that it is indeed a global struggle. No one is safe from infection when it merges in one part of the world, it's rapidly transferred to another and the best of technology in certain parts of the world has to be coupled with the efforts of local populations brought to bear upon this problem. This will be necessary if we are to progress as a global civilization on earth.

I'd like to tell you also that it is my personal view that science has very much made the world that we live in today. It has shaped how civilizations have developed in some cases how they failed to develop. As you will see when I give you a few examples in the area of immunology, infectious disease, we'd really be nowhere at all, if not for scientific progress. The numbers tell the story very well, and I can begin with a few statistics for those among you who keep track of such things.

If we look at this chart, we're going to be looking at median lifespan. You see the red line, which indicates the point at which half of the population has died. It may come as no surprise to you that in the United Kingdom in the year 2000 the median lifespan was close to 80 years, and it's probably

even a little bit beyond that now. Median lifespan in this case is not so different from average lifespan. In the same year the situation was very different in the country of Mozambique. In Mozambique 50% of all people had died by the age of 39. This is a somewhat shocking statistic for someone who grew up in Southern California and considered life a dependable state of affairs from childhood on, and yet those were the numbers, and we can understand very well why they're that way. A real surprise comes when we look back at the year 1860 in the city of Liverpool, England, where vital statistics were kept and well documented. We see that the situation then, in Victorian England, was even worse than in Mozambique in the year 2000. 50% of all people born were dead by the age of 10. Maybe it's surprising also that if you go back further in European history, to Breslau in 1690 or to neolithic times based on records told by bones or even to paleolithic times, the situation never got much worse than that. In Victorian England people where living pretty much in the wild state when it came to confronting infectious disease, and you can be sure, though I won't go into detail, that most of this attrition was due to infection. Now one thing that you have to keep in mind here is that our tremendous progress from here all the way up to here (a median lifespan of 10 to a median lifespan of 80) is something that didn't evolve. It's not that we have better innate immunity or adaptive immunity now than we did then. It was intellectual progress that made the difference. It was the human brain, the collective human brain, if we talk about that, it made humans live 8 times longer in the year 2000 than in the year 1860. In other words we changed ourselves as a species, and we did it by public health measures, by immunization, by all of the methods that are probably familiar to you.

Infections have diverse effects, and they've been known since antiquity, but they have common themes as well. They are transmissible diseases, they all have inflammatory characteristics, and if we look at them all together we can say without a doubt that they have stolen more lives and more years of human life than any other type of disease, more than famine, more than war, more than lions in Africa; the real thing has been infection. Smallpox alone, before it was eradicated, is believed to have claimed about 1 billion human lives, and in the 20th century when things were well documented

the number is placed sometimes on the higher end, at about 500 million for that century alone. Imagine that it had not been eradicated!

In all, looking across the world today about one quarter of all people born will ultimately die of an infectious disease. Now infection is not the greatest killer of human beings on the planet. We can say that cardiovascular disease kills more people. But infectious disease tends to kill people at an early age, and because infection does that it stands as a tremendous selective pressure and it shaped our genome dramatically. It brought an immune system into existence long, long ago, and even in recent times it continues to influence the frequency of alleles of certain genes.

What has the selective pressure done to our species and to all species? Well, we've only known about microbes as a cause of infection for about 150 years, but the battle has certainly been going on for a billion years or more, and we have to ask ourselves how the immune system evolved. That has been a question of constant interest to me.

Most of us imagine, that immunity evolved from a simpler system in which antimicrobial peptides were elaborated by microbes in order to combat other microbes, and we still see examples of this today with antibiotics like bacitracin for example, which is a cytolytic cyclic peptide, and there are many, many other examples. But it's expensive to maintain a constitutive apparatus for fighting microbes around you, and probably for that reason sensing and signaling components had to evolve early on as well. As multi-cellular organisms developed we know that they developed specialized cells for immunity, like macrophages that could engulf microbial invaders and destroy them. That was probably the birth of innate immunity as a dedicated cellular system, and it persists to this day in many life forms including ourselves and is essential for survival. Components of a sensing- and signaling apparatus capable of detecting microbes seem to have pre-dated the split between plants and animals, and we know this because we see the remnants of common domains used for immune function in proteins that exist today. The leucine rich repeat domain of toll-like receptors, TIR

domains of toll-like receptors and other molecules and NOD-like receptors exist in both plants and animals. Here are some examples of these domains. These are molecules that I'll have much to say about later on, these are toll-like receptors, this is a plant disease resistance receptor, and they both have leucine rich repeat motifs that are excellent binding motifs for foreign molecules. They both have TIR domains, some represented free in the cytoplasm as in the plant, others attached to the toll-like receptors. There are protein kinases of a similar shape in both cases, there are adapter proteins that exist in both cases, and I could go on, but suffice it to say there is strong evidence that the split occurred early in evolution.

The evolution of immunity then is ancient, and we have to think what it was like in the early days of animal and plant life and what sort of immunity may have developed after that. Animals are believed to have come from dinoflagellates, which are motile organisms, while plants followed a very different path in their evolution, but perhaps 500 to 550 million years ago something extraordinary happened where animals were concerned. Everyone who is interested in evolution would have loved to be alive at that time. A tremendous proliferation and diversification of life occurred and is known as the Cambrian explosion. At that time in the shallow, oxygen-rich seas of the earth a fantastic variety of animal species developed in just a short period of time. Nobody knows why there was such an expansion in the number of different lines of animal life. Perhaps this was because there was nothing before to fill the many ecological niches that existed, waiting to be colonized. But the point was that organs developed that hadn't been present before, a segmented body plan developed, eyes developed, in some cases teeth developed, limbs developed… and everything that we know in modern animals today. Almost all modern structures can be traced to the Cambrian explosion. Out of the Cambrian explosion came all the familiar animal forms that are familiar to us. We have the vertebrates, and the vertebrates came to exist in jawed and jawless varieties. We have also echinoderms, mollusks, insects and all of these life forms. It is rather shocking for me to see that plants were a bit retarded in their development, and it wasn't until practically yesterday that flowering plants developed, the

monocots and dicots that seem to have taken over the world and with which we are so familiar today.

Where immunity was concerned, something special happened in the vertebrates, really something almost miraculous. Vertebrates developed a special means of making receptors that could recognize any molecule that existed in the exterior world or any molecule that ever could be. This depended upon a new kind of cell, born in the Cambrian explosion. That cell we call the lymphocyte. It is rather unassuming in appearance, it has a small rim of cytoplasm and a large smeared nucleus, but these cells, which existed a little bit before immunity developed, would play a huge role in immunity later on. Lymphocytes are made in diverse organs in different vertebrate lines: the typhlozole, in the jawless fish, the thymus and the bone marrow in mammals, the bursa of Fabricius in birds; and they all look quite similar, but can be distinguished into multiple types: prominently B cells and T cells, which exists in all vertebrates. Now we know that before an immune function was acquired there were already B-cells and T-cells, and we know this because both lineages were transmitted into the jawed and jawless fish. But where jawless vertebrates were concerned, a different substrate for producing receptors evolved as compared to that used by jawed vertebrates. A huge diversity of receptors was enabled by the horizontal transmission of recombinase genes from bacteria, RAG1 and RAG2, and these enzymes began to operate on different part of the genome. By recombining parts of the DNA in jawless vertebrates they made a set of highly diverse receptors that were made out of leucine rich repeat proteins, whereas in the jawed vertebrates immunoglobulin types repeats were favored, and they made what we call today antibodies and T-call receptors. These receptors, and their clonal production – just one type of receptor per cell – are the basis of adaptive immunity, and it developed 500 to 550 million years ago, give or take, we don’t know exactly when. Adaptive immunity is one legacy of our struggle against microbes. But it has a legacy all its own. The legacy of having such a sophisticated immune system is that you can develop auto-immune diseases. Rheumatoid arthritis, type one diabetes, systemic lupus erythematosus, these are all examples of autoimmunity, and it’s believed that something like 12 to 14%

of us will develop one or another auto-immune diseases in the course of our lives. This by itself is a considerable medical burden, as you can imagine. There are also inflammatory diseases that don't involve antibodies. One might call them autoinflammatory diseases, or innate autoimmune diseases. Gout would be an example, rare diseases like neonatal onset multisystem inflammatory disease (NOMID) would be another.

There are also more subtle forms of inflammations. Many today would say that arteriosclerosis has an inflammatory basis. Certainly monocytes and other inflammatory cells collect in atherosclerotic plaques, and they are probably important to the disease pathogenesis. Neurodegenerative diseases like Alzheimer's disease may have even more subtle forms of inflammation that are pathogenically important. So, depending how broadly you define inflammatory disease, it could be said to affect almost all of us at one level or another.

Let's go back to infection and to Victorian England. At that time these were the dreaded pathogens of the age: smallpox, typhoid, tuberculosis and cholera. They were known not long after 1860 to be caused by microbes, and people made attempts to deal with them even before there was that knowledge. This is a classic painting of Edward Jenner, a physician who took cowpox lesions from Sarah Nelmes, shown on the right, and inoculated them into James Phipps, a somewhat unwilling patient sitting in the chair. Jenner was able to demonstrate that this new procedure, which was called vaccination to refer to cattle as the origin of the inoculum proved effective in preventing smallpox, and much safer than variolation which had preceded it, in which people were actually inoculated with live smallpox in the hope of preventing a more serious disease later on. Then as now there were critics of vaccination. There was an anti-vaccine society that sprang up in the 19th century as people were afraid to receive this therapy, yet imagine if it had not been implemented widely, and for a long time it wasn't implemented widely. It certainly would have prevented the progress of civilization.

The problem was that in the time of Jenner and for some while after all approaches to infections were simply hampered by ignorance of what infection was. Microbes were known from the time of Van Leeuwenhoek and yet the link between infections and microbes hadn't been made, even though it seems second nature to us today. Until it was made, there were only vague ideas about how disease was transmitted between individuals and how they seem to produce effects akin to poisoning. The kind of arguments that went on favored either miasma as a cause of disease, somehow involving transmission through air contagion requiring direct touch. Scientists of the time did begin to look at this matter noting that infection looked a good deal like the putrefaction of organic material. They began looking at decaying meat and vegetable products, grinding them up in hopes of finding a toxic product that might convey something similar to infection. In the 18th century Albrecht von Haller and in the 19th century Francois Magendie, a great and famous physician, began doing experiments of that kind. They found that putrid materials of plant and animal origin would elicit fever and sometimes death in animals to which it was transferred. Later researchers became a bit more sophisticated, and Ernst von Bergmann, Theodor Billroth and Peter Panum began actually trying to purify the toxic principle using the tools that were available at that time. Peter Panum went quite far with this. He isolated an alcohol insoluble material, 12 milligram of which was sufficient to kill a large dog, and we know today that that material was probably what we call lipopolysaccharide or endotoxin, based on Panum's description.

But the real hey day of success against infections began with Louis Pasteur and Robert Koch. They founded, both of them independently, the germ theory of infectious disease, and from that point forward rather than starting with putrefied organic material one could actually look at pure cultures of microbes and try to understand what was toxic about them, what was recognized, what could be responded to by the immune system.

One problem with these two scientists was that they hated each other. This had a good deal to do with nationalism, and with with antagonism between France and Germany, including the Franco-Prussian war. Pasteur's son enlisted in the French army, and was stricken with typhoid. For that reason alone, Pasteur didn't liked Germans very much. There certainly was the element of professional competition that spurred him on as well, and Robert Koch reciprocated. It is interesting to read today the letters that they wrote to another. I will read aloud:

"In his Geneva lecture Pasteur bitterly complained about my having rejected his microscopic examination and inoculation techniques", wrote Koch. "However, after his inoculations with saliva and nose slime and his repeated discovery of the microbe *en huit* I am not able to change my opinion. Pasteur deserves criticism not only for his defective methods, but also for the way in which he has publicized his investigations."

It bears mention here that Pasteur had presented a lecture in which he claimed to have found a new microbe in the froth that was emitted from the nose of patients suffering from rabies, but he didn't say that this was the cause of rabies, and Koch, by design or perhaps by real misunderstanding, thought that Pasteur had said that he had found the cause of rabies.

Pasteur replied: "I find here, Monsieur, a new example of the manner of discourse that served you previously in 1881; you attribute to me some errors which I hadn't committed; you refute them and exult noisily."

It is a pity that they didn't get along, the French and the German microbiologists. I like to think that that wouldn't happen today, yet still it does from time to time. There definitely is animosity among scientists, and it certainly is to be avoided, if possible.

Despite nationalistic enmity, one after another, infectious diseases were ascribed to microbes during the 19th and 20th century, and the germ theory of infectious disease became widely accepted. Then, almost immediately, a new question arose: How is it that germs do injury to us, and how is it that we detect them and begin to mount an immune response? These were questions partly of immunology, partly of microbial pathogenesis. Among the students of Robert Koch were Paul Ehrlich, who was well known for his identification of antibodies; Emil von Behring, who worked on the solution to diphtheria and making diphtheria antitoxin, and there was Richard Pfeiffer, probably the most obscure of the three, but he is the one who founded the field that I later became involved in.

It was Richard Pfeiffer, a military surgeon in Berlin, who founded the endotoxin model of toxicity from microbial infection. It was Pfeiffer who started to work with cholera in Koch's laboratory in 1891, and noted that heat-killed cholera vibrio were lethal to guinea pigs, if injected in sufficient quantity. He attributed this to what he called "toxins in the body substance" of the bacterium. This material which he called endotoxin caused fever, inflammation, shock and sometimes death in guinea pigs.

We fast forward now a few decades. We know today that endotoxin or lipopolysaccharides are major structural component of the outer membrane of gram negative bacteria. Lipopolysaccharides have both polysaccharide and a lipid A moiety. By the 1970s the structure of several endotoxins had been solved quite rigorously, and by 1984 a lipid A molecule had been synthesized completely artificially in a laboratory in Japan and also in Germany, and these molecules could do just about everything that Pfeiffer had seen nearly 90 years before. We might draw a typical LPS molecule this way. It has what we call a KDO, (3-Deoxy-d-manno-octulosonic Acid), a polysaccharide chain trailing off into the medium and then these acyl

chains.

Pfeiffer, during his lifetime, was nominated to win a Nobel Prize 33 times, but he never received it. Nonetheless the reason for his nomination was quite clear. Every day hundreds of people die of endotoxic shock. This is a child with gram negative meningococcal sepsis showing many features of endotoxic shock. Such patients may bleed uncontrollably, they have a stiffening of the lungs and require intubation, the kidneys and other organs are damaged by endotoxic shock, and there is hypoxia. If it comes to this stage a 50% mortality might be expected.

It is also the case that endotoxic shock is a severe systemic form of inflammation and in those days, and in fact until very recently, the origins of all inflammation were unknown. We could guess that infectious inflammation might, on a biochemical and cellular level, be rather similar to the kind of inflammation that occurs in non-infectious diseases, yet no one knew where it started. Nearly a hundred years went by and no one was able to find a receptor for endotoxin, although the concept of receptors was well entrenched in biology from the early 20th century forward.

I personally began to be interested in this problem almost 40 years ago when I worked in the laboratory of this man, Abraham Braude, who was trying to make antibodies against LPS to protect patients against endotoxic shock. I undoubtedly first heard the term “endotoxin” then, when I was about 16 or 17 years old. I was also interested in microbiology and in the tales of the early microbe-hunters. I read the “Microbe Hunters” by Paul de Kruif. I read a book by Sinclair Lewis called “Arrowsmith”, about a young doctor and his struggle against infection in the early 20th century. Sinclair Lewis did win a Nobel Prize, I have to point out, for literature. I was inspired by these books, and they undoubtedly shaped my decision to go in the direction that I took later on. Because of those books and because of my father’s advice on the subject I decided to go to medical school and

afterward I did a residency and internship at UT Southwestern, and then I'd had enough of clinical medicine for a time, and I decided that I would go back to basic science, which was really my interest all along. I joined a laboratory in New York City where there was an interest in wasting in chronic disease. This is a cow with trypanosomiasis, and it can be imagined that there are probably only a few grams of the parasite in the entire animal, yet one sees that it's wasting away, hast lost its appetite. We see this in many chronic diseases, and the question was why.

I set up an assay system to look at the question from an immunologic standpoint. The idea was that perhaps immune cells recognized something made by the infectious organism, and made a factor which we called cachectin. That factor in turn could interact with the energy storage tissue of the body, so as to cause a failure to take up fat from the exterior medium. It was suggested, in some variants of this hypothesis that many different molecules might also trigger such a response: endotoxin, or perhaps molecules made by malignant tumors. In the end when the matter was studied closely, it seemed nothing worked as well as endotoxin to induce cachectin activity. The assay for cachectin activity was simple. I produced cachectin by stimulating large cultures of mouse macrophages with LPS, I would apply the medium of fractions thereof to a cultured line of fat cells, and I would measure suppression of lipoprotein lipase, an enzyme needed to break down fat in the form of triglycerides to release fatty acids. Using this assay I purified a single protein species. I soon discovered that this molecule was homologous to human tumor necrosis factor, recently isolated by an entirely different approach. As the name suggests, that activity had to do with the ability to lyse tumor cells in vitro. And mouse chachectin had the same ability to lysr tumor cells as did human tumor necrosis factor. In fact, chachectin was the mouse orthologue of TNF

Here was something very curious. It seemed that a single molecule, TNF, made by macrophage in response to endotoxin would destroy tumors, and

also cause the resorption of fat. These were certainly wildly different phenomena, and both were things that LPS could do.I wondered if TNF could mediate all the effects of LPS, including perhaps its lethal effect.

It didn't take long to see that TNF would cause mice to become ill and even to die if more than 20 microgram was administered to them at one time. Furthermore if mice were immunized against their own mouse TNF and then challenged with LPS, they could better survive the LPS. The LPS dose-lethality curve had shifted significantly to the right at the 50% mortality point, and this convinced me that this was at least a major mediator of endotoxic shock. So now we'd moved beyond putrefaction, caused by miasmas or contagions, we'd gone beyond bacteria, we'd gone beyond LPS, and we had a mediator of injury caused by LPS that was made by the host. It was this mediator that I hoped to follow in order to understand what the LPS receptor might be. It seemed the ideal endpoint, because it really did mediate much of the toxicity of LPS.

By the year 1990 it was known that LPS somehow required CD14, a cell surface-protein found by Wright and Ulevitch to be necessary for the LPS response, yet this couldn't be the signaling receptor, because it had no cytoplasmic domain and couldn't signal into the cell. There must be a co-receptor, most people thought, that would mediate that event, and this receptor was a complete mystery. Whatever it did, it must activate NF-KB, because the TNF gene had NF-KB motifs in its promoter region, and it had to be activated in that way to produce the TNF mRNA. That RNA existed in a locked, untranslatable form, and needed to be unlocked by a second signal from the receptor. Then it could be translated to yield processed and secreted TNF that would do what TNF does. The central mystery then was what the LPS receptor was. If we could find it, we could understand how microbes are detected by the immune system.

There were several ways to go after the receptor, and I would say we tried most of them. The one that worked was the genetic approach. The answer to the question lay in two sub-strains of mice that wouldn't respond to LPS because of spontaneous mutations. The existence of these mice suggested that LPS was sensed in a very specific way, dependent on a certain protein. But which protein, and how to find it? The first of these two strains was the C3H/HeJ mouse, which had been known to be LPS resistant since 1965. It was known, in our hands to fail to make a cytokine response to LPS and only to LPS. In 1978 the C3H/HeJ mutation was mapped rather broadly to a point between two visible phenotypic markers by Watson and Riblet, and these markers were on chromosome 4. Their physical positions aren't known, even today. In 1978 another strain of mouse, C57BL/10ScCr, was found to be resistant to LPS by Antonio Coutinho. He crossed these mice to C3H/HeJ animals and found that the same gene was affected: the offspring were all strongly LPS resistant.

Now, how to track down such a gene, required for the LPS response? It was a difficult matter in those days. One knows that in the mouse there are 20 chromosomes (including the X chromosome), and each one is packed with chromatin. In chromatin structure you have DNA that's wrapped tightly around nucleosomes, and the DNA itself is a long string of base pairs, and if you hope to find a mutation that may be caused by a single base pair change, you may have to search through 2.7 billion bases of DNA to do it. That was the challenge, and yet we were quite passionate about finding what we considered the "holy grail" of innate immunity.

Fortunately there is a way to exclude much of the genome. This is achieved by a process called genetic mapping, and I won't go into it in detail, but one has to come to a point where one proves by geometric reasoning that the mutation of interest is between two flanking mutations on that chromosome. And then one has to clone all of the DNA spanning those two markers, and then one had in those days to search it for its content of genes. This was a

backbreaking task, it involved sequencing largely by hand, then searching the sequences with a computer program called BLAST to look for expressed sequence tags that might match the genomic DNA. Day-in and day-out we kept doing that for a period of about 3 years. By August of 1998 about 90% of the critical region had been thoroughly explored for genes, and we'd found only pseudogenes. Of course they didn't come labeled with a note that said: "I am a pseudogene", and so we had to prove in every case that they really weren't the gene we were after by comparing the mutant and wild-type control strains.

All at once, in September of 1998, I was blasting as usual in my study one night, and I found a powerful hit, much better than ever before. Then another hit, a few minutes later, and I felt, surely we have found the gene this time, because these were the best quality hits I'd had ever seen, and also we had explored almost all of the region, and there just wasn't much space for anymore genes to exist. There was still another reason for excitement as I looked at the gene and saw what it encoded. I mentioned to you that CD14 was one component of the LPS receptor complex, and it was a leucine rich repeat protein. So was this new item that I had found, TLR4. But this protein had a membrane spanning domain and a cytoplasmic protein domain similar to that of the Interleukin-1 receptor (IL-1R), a protein with known inflammatory potential. It made sense to think that LPS might be transferred from one molecule to the other and that the signal would be elicited by the TIR domain, as it was called, that might activate NF-KB.

There was another reason to believe that this was the right gene. Earlier work by Jules Hoffmann had focused on the Toll- pathway in Drosophila, formerly known for its develop mental effects. He had shown that in the fruit fly, Toll and its ligand spaetzle were necessary for an effective response to a fungal infection. If inoculated, flies with aspergillus fumigates lack either of these two genes, they are overgrown by the fungus and killed, because

they are unable to make antimicrobial peptides needed to fight the infection. This situation was similar to that in the LPS-resistant mice, which were intolerant of Gram negative infection. So I thought we'd probably identified an evolutionarily homologous pathway.

In the LPS-resistant mice there were mutations that destroyed TLR4: a point mutation in the C3H/HeJ strain and a complete deletion of the locus in C57BL/10ScCr strain, and finding that was probably the high point of my career. It was something that we hardly could believe, when we found the mutations, and we knew we solved a problem that had lasted almost a hundred years. We also were able to deduce that TLR4 was a physical receptor for LPS, because we had a genetic means of determining this. Our conclusion was later supported by X-ray crystallographic data. And in the meanwhile, other workers established that there was still another protein component to the receptor, a small molecule called MD2.

10 years went by before the complex was crystallized, and today we can see the structure in three dimensions.. We can visualize the backbone of TLR4, this leucine rich repeat domain in cyan, attached to a small magenta basket, MD2. The lipid A of LPS fits into that basket, and a conformational change results, which triggers endotoxic reactions. I've estimated that something like 0.1 nano-gram of this complex is all it takes to mediate the lethal effect of LPS in a mouse.

Time went by, and several other toll like receptor structures were solved by X-ray crystallographers. They all directly bind relatively conserved molecules of microbial origin. There are in humans 10 toll like receptors, in mice 12 and in both species together 13. This is where recognition of microbes largely begins, though it is not the only system by which it occurs.

On that very first night when we found TLR4 in our critical region we began to do some evolutionary studies, and we found that the TIR domain was the most conserved part of the molecule. We made trees to look at the inheritance over evolutionary time and what surprised me the most was not that not only humans and flies had TIR domains and Toll like receptors, but even plants had TIR domains, and wherever they were seen in plants, those domains conferred resistance to infections. In this case we're looking at a flax plant and infected by a fungal rust. This truly this was an ancient system that evolved to give protection against microbes.

In the long run we'd like to understand how toll like receptors signal. We'd like to understand the whole immune system, in mechanistic terms. Of course, we ourselves are biological machines, and the immune system ultimately is a machine, in its own right. We've used genetics as our tool to achieve such understanding. We've induced mutations in mice at random, and then we've tracked them down, just as we tracked down the Lps mutation in C3H/HeJ mice. However, we can do so much faster nowadays. At present, in our laboratory, it doesn't take five years anymore. Instead, tracking a mutation down is a real time process. If one observes a family of mice with an immune deficiency, within perhaps one hour one may know the genetic cause of that immune deficiency.

I haven't time to discuss that, but I'll just say that while I was at Scripps Research Institute, over a period of about 10 years we mutagenized mice and looked at Toll-like receptor responses, measureing TNF production as readout. Everywhere you see a box with red lettering, you see that we have found one or more mutations in a particular gene important for a response to occur. We were able to build quite an elaborate picture of how signaling works. It's complicated, and I won't go through it in detail, but I'll just show you that again, the structural biologists have been at work. We can see in 3D illustrations many of the molecules that participate in signaling, many of which are labeled by our mutations. We can begin to put together quite a strong mechanistic picture of how signaling operates. Though I have to

admit that some of the interactions here are real and some are still just fanciful. I have no doubt that in the next decade or so, we'll understand this far better than we do today. The ultimate goal might be to understand it about as well as we do a pocket-watch. I think it's a realistic goal.

What does all this knowledge do for us in the struggle against infection? First of all we now know how we "see" infectious microbes during the first minutes and seconds after they are inoculated. We might think we could mitigate the intense inflammatory response that actually leads to tissue injury and death when we have serious infections, if we're able to keep the infection itself under control with antibiotics, and we often are able to. Many of you know that there are also many immune deficiency diseases that are caused by failure of immune sensing.

This is the famous "bubble-boy", who had a problem with adaptive immunity, but there are people no less badly immune compressed than the bubble boy who have innate immunity defects. At last we have a hope of diagnosing such patients, because we know which genes are involved in innate immune reponses. I'd like to think that we can make better vaccines, having the knowledge that we do about how the innate system is activated and how that transfers to adaptive immune activation: vaccines that are more efficacious have lower toxicity and are better for specific purposes. And remember what I told you before about the legacy of our immune system being autoimmunity. Now we can understand how some of it comes about. Most particularly in the case of systemic lupus erythematosus (SLE) we've developed some insight into Toll like receptors involvement. This is largely from the work of Ann Marshak-Rothstein. In very broad terms we believe that in SLE, there is inappropriate cell death, and as a consequence nucleoproteins, RNA and DNA protein complexes, are released. We all have B cells that recognize such complexes, and yet, usually our B cells remain quite quiescent. To these receptors on B cells the nucleoproteins bind, and they become internalized. In the endocytic environment there is a acidification, then recognition by Toll like receptors,

either by the TLR7 or the TLR9 complexes. That gives a signal for cell proliferation, and where there may have been just a few such B cells to begin with, soon there are many of them. They may differentiate to make plasma cells and memory B cells. The plasma cells will make more antibodies against nuclear proteins and immune complexes will feed back to stimulate the memory B population and cause even more clonal expansion. This goes on indefinitely, with the end consequence of immune complex deposition in the tissues and an autoimmune disease. We can see how this model operates very clearly in the mouse. Whether it's possible to interrupt it in humans at the level of TLRs is questionable, but it is certainly worth trying.

I am going to conclude now with some thanks to my staff. The work that led to the Nobel Prize for me was done by a pretty small group of dedicated workers. I am not showing all of them but the most critical ones would be Betsy Layton, Alexander Poltorak, Christoph Van Huffel and Irina Smirnova. Things change after you get the Nobel Prize, and now there are 62 people in our group. This picture is already a bit outdated. The most exciting thing we're pursuing these days is the automated positional cloning of newly induced mutations, which may let us find all the components of the innate and the adaptive immune systems before long.

I want to thank you again for this tremendous honor, and I'll be glad to have a dialogue with you and to answer any questions you might have.

Question:

Could you please give us your opinions and thoughts on new trends and strategies about cancer immunology, especially vaccinology and immunotherapy?

Prof. Bruce Beutler:

Yes, thank you for the question. Cancer immunotherapy used to be a distant dream. Many tried to realize Paul Ehrlich's passion to make magic bullets by tagging antibodies with toxins or with highly radioactive molecules, and I must say that didn't succeed very well when it came to targeting tumors. In any case one would like to have active immunity against a tumor, and there is a feeling that only with such active immunity can one truly eradicate a malignant disease. It has been shown in recent years by investigators in several laboratories that there are inhibitory molecules like CTLA-4 and PD1 that tend to put the brakes on immune responses, and if one can inhibit those molecules to the right degree, the mutations that are present in cancer cells can be recognized sufficiently by the adaptive immune system to create a powerful response.

It's still early days in the investigation of this strategy. There have been patients who have shown complete remissions. Remissions have been observed, in large part in adenocarcinoma of the lung, renal cell carcinoma and malignant melanoma; and those latter two malignancies have shown occasional rare spontaneous remissions. Adenocarcinoma of the lung is a real surprise, and if that holds up for a period of time, then I would say there is a bright future to immunotherapy in cancer. It's a tall order when you think about it. Cancer has always been hard to combat with chemotherapy or with immunotherapy, because malignant cells are so similar to our normal cells, yet maybe the distinction is great enough for

immune recognition. That would be an enormous advance, and perhaps it would push the longevity curve out a few years more by itself.

Question:

In Thailand we are experiencing problems with superbugs or antibiotics resistance of bacteria. A part of this problem is due to overuse of antibiotics among our health professionals. What are your professional suggestions to relieve this serious problem?

Prof. Bruce Beutler:

Thank you for this question. This is a serious problem, and it is a global problem: the kind of problem that should concern everybody on the planet. We've been protected for about 70 years with antibiotics, and before that we had nothing to rely upon but our immune system. It's not clear that antibiotics are going to be good forever, and it may be that the bugs will eventually win, or it may be that we humans, with our ingenuity can chemically engineer antibiotics and always keep a step ahead of them. It will be an interesting few decades to see which way it goes, but for the moment the best approach to superbugs is education of physicians about what antibiotics to give. There is too little reliance on experts in infectious disease medicine. Doctors in the United States and perhaps here as well tend to simply prescribe medicines without expert consultation, often inappropriate ones for the disease. Either the diagnosis or the choice of antibiotics may be incorrect, culture susceptibility may never be tested, and so one doesn't know what the best antibiotic would be. This is what leads to disease resistance and to multidrug resistance. That's the problem which we have to concentrate, and where we can make some progress right now.

Question:

Thank you very much for your wonderful lecture. I was amazed to learn about TLR, that among plants and animals TLR receptor and system are highly conserved, and my question is why is that so, and number two, both of them are worlds apart, yet they can detect or sense bacteria, which is such a different kind of species. How is that possible?

Prof. Bruce Beutler:

I believe the answer to that question is going to involve some speculation, as evolutionary reasoning always does. All I can say is that the binding function of leucine rich repeat proteins proved to be very good. Probably this is the reason such proteins evolved as innate immune receptors. We see that the leucine rich repeat was used later for binding almost any kind of molecule in the jawless vertebrates. I didn't mentioned it, but also proteins like ribonuclease inhibitor, a cytoplasmic protein that we all have and that binds ribonuclease very tightly, approximately a 10^{16} affinity constant. This was the substrate that was available in the primeval soup, before plants and animals differentiated a billion years ago, and it became so important to defense that neither the plants nor the animals let go of it. They did develop other means of detecting microbes and defending themselves, but those core mechanisms have stayed the same, probably all of those billion years, gradually evolving to recognize different molecules. LPS for example isn't recognized at all by insects, so far as we know, not by the fruit fly anyway. But it is recognized by vertebrates and especially by mammals. Evolution has changed the targets, but we've kept those same molecules all this time.

Question:

My question is about the dilemma of the innate immunity. Having it is essential to us, but if we have it too much or too strong we get autoimmunity. Nowadays we have a immunotherapy, but I am wondering on how we are able to find the balance on having it too much or too little, could you elaborate on that?

Prof. Bruce Beutler:

I have to fall back on evolutionary reasoning again and to say that, quite clearly, TLR4 didn't evolve to give us endotoxic shock. That wouldn't make very good sense. One might say that it may have evolved to weed out infected members of the herd and to prevent the transmission of disease, but I doubt that as well. I think without TLR4 an individual has diminished fitness. This is a system that evolved to work in the microenvironment. If you should happen to stick yourself with a thorn and introduce a few gram-negative bacteria, that's when the system performs particularly well. It will create local inflammation, it will attract the attention of the adaptive immune system, and it will contain the infection before it becomes widespread: before you develop sepsis. That's the balance that evolution seems to have struck, between local action and systemic action. Innate immunity has to be strong enough to marshal responses beyond the immediate wound, but not so strong that it kills the host. That's where the balance lies, and in thinking about therapeutic effects we have to be mindful of that balance and probably arrange for drug delivery that's consistent with it.

Question:

Looking at the smallpox epidemic or the Spanish flu that happened in the last century and which caused a huge mortality in the population, do you

think it would be possible to occur again in the near future, and how can we prepare for it?

Prof. Bruce Beutler:

Thank you. Yes, I do think there can be great pandemics again. We witnessed ourselves the start of the HIV epidemic, and there was a time early in the epidemic when no one had any inkling about what caused that disease. Fortunately it occurred late in the 20th century, so there were good molecular biology tools available. The virus was isolated in a fairly short period of time and amazingly, pharma was up to the task of making drugs that were at least able to control the infection, even as monotherapy for a while. It is just remarkable how now we have highly active antiretroviral therapy that's really effective against HIV. Not that it isn't a problem! It certainly is still a huge problem, but it's not as it would have been if it had happened in the 18th or 19th century.

Pandemic flu could happen again like 1918 and could even be much worse. That is a matter of great concern for everyone. It is something that would obviously spread much faster, it could cause a terrible rate of mortality, and certainly we should try to be prepared for it as best we can. Now in a way we do prepare for it by such devices as predicting the epitope specificity of each year's seasonal flu, and repeated seasonal flu immunization may offer some protection against a pandemic flu. But we could be surprised and should at least have the capacity to make a protective antigen and antibody quickly, if the need arises. Also there is a long way to go in making pharmacologic therapy for infectious flu. We have tamiflu and some other agents, but this is not to say that we couldn't do much better than that.

Question:

Last year Bill Gates said that he would like to see Malaria eradication during his lifetime, and given all the technology we have at the moment, would you think that it is possible to achieve this with Malaria or another of the bigger infectious diseases say in the next 50 years?

Prof. Bruce Beutler:

I am not prepared to say it's impossible, that's always a foolish thing to do, but I think it would be very tough because Malaria seems to have very good mechanisms to beat the adaptive immune system. To make a fully efficacious vaccine has always been out of reach, but I won't be too dogmatic about it.

Question:

It has been mentioned that nutrition might have a part to play in the hosts response to inflammations and infections, so I'd like to ask what are the important factors you consider that may substantially influence the response of the host to microbial entries into the body or its toxins?

Prof. Bruce Beutler:

I think the question might be posed in reverse as well. We know of a few things like zinc that seems to be important for some innate immune functions, and of course iron may have a counterproductive effect on some infections. We don't know much more about trace nutritional factors that

are influential. Patients with marginal nutritional status, may not do as well surviving an infection.

Question:

As you have been exposed to research since a young age, I wanted to ask what and who inspired you to become the very accomplished scientist that you are today?

Prof. Bruce Beutler:

A big contributor was my father, beyond a doubt. He was a biomedical scientist, a hematologist by clinical training, and quite eclectic in what he worked on: iron deficiency anemia, red cell enzyme deficiencies, some leukemias and glycolytic storage diseases. One would ordinarily think such a person is a dilettante, but he went to considerable depths in all of those areas and was a renowned figure in science. From the time I was quite young, I remember having conversations with him about evolution, about various issues in medicine, about areas of science that neither of us knew very much about as well, but it was inspiring to talk to him, and certainly he must have influenced me a great deal.

I had other great teachers as well. A great genetic teacher, Dan Lindsley, who taught me the value of drosophila and the forward genetic approach, or at least he tried to; I am not sure I absorbed it all from him when I was in college. But being exposed sometimes makes a big difference, even if you don’t go to work in that area right away. I had other teachers in medical school who were valuable to me also, Patricia Spear, Susumu Ohno and many others. So, teachers and my father certainly influenced me. None of us grows up in a vacuum and everybody needs some kind of role model to look to, usually a living person, but not always even that.

Question:

I am intrigued by the reasons of the last question and would like to ask you, when you were a neurology resident why did you change course to take up fundamental research in molecular biology, because many years ago neurology was regarded as the prince of medical specialty? In fact, Macfarlane Burnet, I was one of his last students before he retired, he wanted to go into neurology, but as he was not the top student he could not get the post, so he went into chemical pathology and did research in influenza and later on immunology. So I am interested to learn what made you not pursue further neurology and to go into immunology?

Prof. Bruce Beutler:

I went into neurology probably for all the good reasons you can imagine. I was fascinated by what I read about strokes that caused alteration of higher cortical function, I wanted to know how we think, I wanted to know about the great mystery of consciousness, and I went in that direction thinking that there would be perhaps technologies available in the future that would let us answer these questions. But clinical neurology was a bit disappointing for me in certain ways. I found that those syndromes where not quite as pure as they were represented in the textbooks, I found that for the care of strokes, epilepsy, all of the neurological problems I had to treat, there was a rote solution. I found myself following A, B, C, D to diagnosis. It wasn't so much that one couldn't treat all of those diseases because there was therapy, and at least in the case of epilepsy, it was quite satisfying to see the results, but I didn't see an opportunity to move much beyond that and be creative and do experimentation. That's what I missed. I decided that would be a good stopping point for me to find a fellowship in a lab once again, possibly working with immunology once again as I'd had some

exposure to that before. That’s why I drew the line after one year of neurology and moved on.