Serendipities of Acquired Immunity

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I am greatly honored to receive the 2016 Kyoto Prize, and sincerely thank the Inamori Foundation for giving me the opportunity to present this Commemorative Lecture. The topic of my talk today will be two kinds of serendipities: first, the many serendipities I have personally enjoyed in the course of my research, and second, the serendipities humanity has gained from our ancestors’ development of acquired immunity through the process of evolution.

Humanity has been plagued by infectious diseases since ancient times. A relief on an Egyptian stela depicting a priest believed to have lived in the latter half of the 14th century BC shows a thin, apparently paralyzed right foot (Fig. 1). This probably illustrates that humanity was afflicted by paralysis from poliovirus infection at that time. But although the affected person would remain paralyzed, they would fortunately not lose their life due to the power of immunity.

Immunity remains indispensable for human survival even today. Until very recently, infectious diseases were the leading cause of human death. Life forms that were able to avoid death from infectious diseases won the competition for existence and flourished on the earth. At the same time, this battle with pathogens is what accelerated the evolution of immune systems.

According to Thucydides, a Greek historian famous for writing “The History of the Peloponnesian War,” this long war fought between the Greek colony of Syracuse on the island of Sicily and the Phoenician city-state of Carthage in Africa around the 4th to 5th century BC was interrupted when smallpox broke out on the battlefield. He writes that when the war resumed, the Carthaginian army consisting mostly of new recruits suffered greatly, while the soldiers of Syracuse avoided infection due to having previously recovered from smallpox and won the war.

Through a succession of these kinds of experiences, humanity became familiar with the phenomenon of immunity. The word “immunity” comes from the Latin word “immunitas”. “Munitas” means social obligations of Roman society, which included taxation and conscription, and “im” means to avoid those obligations. Therefore, “immunity” essentially means avoiding something that is supposed to happen sometime in the future. The human immune system possesses acquired immunity that enables it to remember antigens, which makes it a mechanism for never getting the same disease twice.

The mechanism called acquired immunity that produces immunological memory is present in all animals from vertebrates onward, and is the reason why mammals and other vertebrates have been able to escape infectious diseases, live long lives, and flourish on the earth (Fig. 2). Innate immunity, or immunity without memory, is present in all animals.
Cells such as macrophages and neutrophils play major roles in innate immunity (Fig. 3). Innate immunity allows the body to recognize invading pathogens by differences in structural pattern from bodily components, but cannot identify and remember specific pathogens such as smallpox. The major players in antigen-remembering acquired immunity are lymphocytes called T cells and B cells. Both types of cells have receptors that can discern minute differences in antigen structure. T cells, which I will discuss later, attack cancer cells, whereas B cells produce antibodies.

The first vaccine that utilized immunological memory to prevent human disease was developed in 1796 by the British surgeon Edward Jenner. He inoculated an 8-year-old boy on the arms with cowpox virus, the cow version of smallpox, and the boy did not develop smallpox when inoculated with human smallpox virus six weeks later. Through this, Jenner proved that vaccination can prevent infectious disease.

Two people who made great contributions to our understanding of how vaccines prevent disease are Emil von Behring and Shibasaburo Kitasato. They discovered that the serum of animals injected with diphtheria toxin contains a substance that neutralizes the toxin. This was in 1890. This finding was utilized to establish serotherapies for infectious diseases such as diphtheria and anthrax. It was later discovered that the neutralizing substance in serum was antibodies. Behring was awarded the first Nobel Prize for the work.

The structure of antibodies remained a mystery for a long time until the full structure consisting of four proteins was uncovered in the mid-20th century (Fig. 4). An antibody looks like a crab’s claw, with two light (or “L”) chains and two heavy (or “H”) chains linked by disulfide bonds. It was later discovered that the L and H chains have variable regions and constant regions that depend on the amino acid sequence. The variable region is involved in antigen binding, whereas the constant region of the H chain determines the class of the antigen that binds to it, and consequently how that antigen will be processed.

When an animal is injected with an antigen, its primary response is to produce IgM class antibodies, and then produce IgG class antibodies shortly thereafter (Fig. 5). Injection of the same animal with the same antigen a few weeks later triggers a secondary response, in which the immune system quickly produces IgG antibodies that bind with high affinity to antigens. Two things change in this secondary response. The first change is that the produced antibodies bind to antigens with higher affinity. This increase in affinity is due to a somatic mutation in the variable region, which is the antigen-binding region. The second change is that the class of the first antibodies produced switches from IgM to IgG. This allows the antibodies to more efficiently process captured antigens. These two changes enable animals to remember antigens and strongly resist repeated infection. This is the principle behind vaccines. Mutations in the variable region of an antibody can produce higher or lower affinity for pathogens. However, when cells that express high-affinity antibodies are stimulated to proliferate, the body produces many high-affinity antibodies. These somatic mutations are point mutations in genes encoding the variable regions of the L and H chains. A Darwinian principle by which lymphocytes producing high-affinity antibodies are
selected from all B lymphocytes producing antibodies with various variable region mutations is at work in the body of each and every human.

In class switching, the variable regions of the L and H chains do not change. Instead, only the constant region of the H chain takes on a different structure (Fig. 6). The constant region of the H chain determines antibody class, which in turn determines how the captured antigen will be processed. Therefore, class switching does not change affinity for antigens, but rather antigen processing.

Let me give an example illustrating the utility of these memory antibodies. Memory antibodies produced by a pregnant mother after initial infection with a pathogen are transferred to her child in utero through the placenta, but these antibodies must be IgG rather than IgM. What is more, antibodies transferred from a mother’s breast milk to her newborn must be IgA. As you can see from this example, somatic mutations and class switching play critical roles in protecting children from infectious disease in utero and as newborns. Research had shown that animals exposed to antigens of any type, including artificially synthesized chemical compounds, could remember all of them, making it seem that antibodies have unlimited ability to bind to antigens. Even so, the mechanisms underlying these ingenious workings of immunity were completely unknown as late as the 1970s.

Now, I would like to share with you how I came to encounter these fantastic mysteries of immune mechanisms. When I was in elementary school, my science teacher let me come to school over the summer to look at the rings of Saturn through an astronomical telescope. I was captivated by the mysteriousness of space and wanted to study astronomy to learn what lies at the edge of space.

However, I later read books by Hideyo Noguchi and developed a great interest in the field of medicine and its amazing pursuit of saving lives. The fact that my father was a doctor may also have helped to motivate me, and I ended up deciding to pursue a career in medicine.

My father was very pleased when I later won the Hideyo Noguchi Memorial Award for Medical Sciences.

Soon after I started university, Misuzu Shobo published a book called “The Revolution in Biology” by Dr. Atsuhiro Shibatani (Fig. 7). In this book, Dr. Shibatani explained his belief that cancer is caused by gene mutations and asserted that a machine for automated analysis of nucleotide sequences in DNA must be developed. He also asserted that erroneous nucleotide sequences must be fixed as if performing molecular surgery. He showed amazing foresight by writing these things in 1960. Half a century later, we are finally discussing them as reality. These grand and lofty visions were highly attractive to my young mind and gave me the determination to become a medical researcher rather than a clinician.

After graduating from medical school, I unhesitatingly decided to continue my studies under Dr. Osamu Hayaishi in the Department of Medical Chemistry for graduate school (Fig. 8). At that time, Dr. Hayaishi was an up-and-coming researcher who had just returned from the National Institutes of Health in the United States to take up a position at the university. He taught me many things, including how a
researcher should be, not to blindly trust research papers, and the significance of an international perspective and originality in research. During the time of active student protests I became unable to conduct research and went to the United States as a research fellow to escape the university in 1971. That is where I first encountered research in molecular immunology.

It all began when I was doing my first fellowship at the Carnegie Institution of Washington in Baltimore, Maryland. In a lecture, Dr. Brown proposed a clear and simple theory that the diversity of antibodies can be explained by the existence of numerous copies of antibody genes, and that this can be verified at the molecular level.

I became deeply interested in this theory and moved to Dr. Leder's laboratory at the NIH in 1973 looking for an institution where I could conduct research on such topics. Fortunately, the development of recombinant DNA technology around 1974 made it possible to quantify and isolate genes. This gave me the opportunity to validate Dr. Brown's model, but I ended up disproving his theory, at least in regards to the constant region of the L chain, by showing that there are only 1 or 2 genes encoding that region. I was offered to stay in the United States to continue that research. It was a difficult decision, but ultimately I chose to return to Japan in consideration of my family's future. With the great material and spiritual support I received from Dr. Brown and Dr. Leder, I joined the Department of Physiological Chemistry and Nutrition at the University of Tokyo as Assistant Professor in 1974.

I was very conflicted about what to do there. More established professors advised me not to focus on the research on antibody genes I had done in the United States because it had a low chance of success, but I decided that if I was going to research anything, it should be what I wanted to research most. I decided to tackle immunological research, thinking that if I failed, I could just move to the countryside and live a quiet life as a rural doctor. However, rather than studying the mechanisms of diversification of the variable region of the L chain that I researched in the United States, I studied the diversification of the constant region of the H chain, which nobody had worked on before.

After a long period of preparation, it became possible to quantify genes encoding the constant region of the H chain of antibodies. When I started this work by quantifying antibody genes in the DNA of myeloma cells, which make many varieties of antibodies, I was fortunate enough to discover that certain genes encoding the constant region are deleted depending on the type of antibody produced. For example, myelomas that expressed the γ2b gene and produced IgG2b were missing the μ, γ3, and γ1 genes.

After comparing many myelomas, I found that the pattern of antibody gene deletions followed a standard set of rules. Looking over the data on the train ride home, I realized how I could explain these deletions, and published my hypothesis that if the genes encoding the antibody constant region were lined up in a certain order, the portion of the chromosome between the variable region and the expressed constant region genes would be completely deleted (Fig. 9). The main person who helped me with this experiment was Dr. Tohru Kataoka, who was a graduate student at the time.
To test this model, we needed to isolate gene segments on chromosomes and determine whether there were actually deletions. In the spring of 1977, the ban on recombinant DNA technology was finally lifted and I returned to Dr. Leder’s lab for a short fellowship. There I successfully isolated cDNA clones from $\gamma_1$ mRNA I brought with me (Fig. 10). I used this cDNA as a probe to isolate the $\gamma_1$ gene from mouse DNA. With Dr. Kataoka playing a key role in the process, we purified the DNA fragments and were just about ready to insert them into a vector at the end of February 1978. However, at that point Dr. Kataoka left for his honeymoon, I took up the remaining work and completed the cloning process on March 21. This slide shows some excited words from that time that I copied from my notebook. The spot on the left is a spot of phages with $\gamma_1$ gene segments that the probe detected. On the right is a picture of $\gamma_1$ gene associating with $\gamma_1$ mRNA that was taken with an electron microscope.

After that, I was invited to join the Department of Genetics at Osaka University as a professor. Dr. Kataoka accompanied me and actually verified at the molecular level that myeloma DNA expressing the $\gamma_1$ antibody gene undergoes recombination, the constant region genes in the middle are deleted, and the variable region and the expressed constant region are pulled closely together, and that this recombination occurs in a region with nucleotide motifs that he named the S region. He published these findings in 1979 (Fig. 11).

A team led by Dr. Akira Shimizu also isolated DNA of a long region including all gene clusters encoding the constant region of the H chain on chromosomes and proved that the sequence of the constant region genes matched the proposed model (Fig. 12).

My next goal was to uncover the molecular mechanisms that produce these major gene deletions. I made several attempts to that end, but none succeeded. In 1984, after moving from Osaka University to Kyoto University, I isolated cDNA for cytokines IL-4 and IL-5, which regulate class switching, and was able to determine their structure. I was invited to be a Fogarty Scholar-in-Residence at the NIH, and worked there for about 3 months a year for 5 years starting in 1991. During this time, I discovered the existence of CH12 cells that switch from IgM to IgA at a rate of a few percent. This was a serendipitous discovery that would lead to future findings, but was not yet useable in biochemical experiments. Instead, I was able to isolate a CH12F3 strain in which about 40% of cells switch to IgA after purification and stimulation with IL-4.

When Dr. Masamichi Muramatsu investigated differences in gene expression between CH12 cells that produce IgA on stimulation and the original IgM-expressing CH12 cells in 1999, he found that only IgA-producing cells have a molecule called AID (Fig. 13).

He then created AID knockout mice to study the role of AID. As you can see from the blue lines on the slide, mice without the AID gene could not produce IgG and produced only IgM when injected with antigens (Fig. 14). In other words, class switching did not occur.

On this slide, the vertical axis shows the frequency of somatic mutations, and the horizontal axis
shows the positions of amino acids in the variable region of the H chain. Dr. Kazuo Kinoshita and his team found that mice with AID in CDR1 and CDR2, the areas with the highest frequency of mutations, have the expected mutations in those areas, but mice without AID have almost no mutations (Fig. 15). They also discovered that somatic mutations and class switching occur even in non-lymphocyte cells made to express AID.

A French group led by Anne Durandy and Alain Fischer noticed that the chromosomal position of the AID gene is close to that of a gene for hyper-IgM syndrome type II, a genetic disease that affects 1 in 100,000 people, and discovered that this disease is caused by AID deficiency (Fig. 16). As in the knockout mice, class switching does not occur in these patients, which causes an increase in IgM, and somatic mutations do not occur either. These patients repeatedly contract infectious diseases.

Through this series of findings, it was determined that antibody memory formation, or the writing of memories of antigens into antibody genes, that is the primary mechanism of vaccines, is the work of AID.

Analysis of AID knockout mice shed light on a completely unexpected phenomenon (Fig. 17). Dr. Sidonia Fagarasan and Dr. Reiko Shinkura discovered that secretion of IgA with somatic mutations is required for symbiosis of gut bacteria populations with the human body, and that imbalance there causes serious disease. This discovery sparked the global development of a new field of research into the importance of proper symbiosis of gut bacteria with their host in the maintenance of human health.

AID works in strange ways (Fig. 18). If we think of genes like a code, somatic mutations, or gene mutations, would be a change in a letter, and class switching, or recombination of genes, would be a change in a paragraph of the code. Therefore, the genes need to be cut and switched. These two processes were previously believed to be performed by separate molecules, but the discovery of AID showed that they are performed by just one molecule. Scientists everywhere were perplexed by how this could be possible. We are still working on this puzzle today.

AID, a 198-amino acid protein with a cytidine deaminase active center in its central section, is an RNA editase that changes C to T in RNA (Fig. 19). Through many years of analysis, Dr. Hitoshi Nagaoka and his team found that the N-terminal of AID has a segment necessary for cleaving DNA and the C-terminal has a segment necessary for recombining DNA. AID is usually expressed in activated B lymphocytes, but is said to sometimes be expressed in other cells or the intestinal epithelium when inflammation or another stimulus is present.

Dr. Wenjun Hu discovered that AID uses cofactors to edit RNA (Fig. 20). AID monomers work with a molecule called hnRNP K to edit microRNA, and are involved in DNA cleavage. Once AID forms dimers, it associates with a molecule called hnRNP L to edit mRNA and produce new proteins required for DNA repair.

Dr. Maki Kobayashi found that an enzyme called Top1 is directly involved in DNA cleavage by AID (Fig. 21). Special histones gather at the transcribed antibody gene regions, and the DNA structure of these
regions unwinds with strong transcription. Top1 normally rewinds unwound DNA, so DNA takes on an abnormal shape when Top1 production is reduced by microRNA edited by AID. It is now known that when it happens, Top1 cleaves the DNA irreversibly.

DNA repair is more complex (Fig. 22). First, several previously discovered repair enzymes must work to pull the two cleaved ends of the DNA close together. A new protein produced when AID edits mRNA is also involved. Dr. Nasim Begum has led the effort to uncover DNA repair mechanisms.

In summary, mechanisms of diversification of immunity have been uncovered through a great series of serendipities. One part of the puzzle of acquired immunity was solved, and most notably, the AID enzyme that creates antibody memory was discovered and its molecular structure determined.

Up to this point, I have discussed research on antibody-producing B cells as a player in acquired immunity. Now I would like to talk about another player: T cells. Types of T cells include helper and killer T cells. Killer T cells can attack and kill virus-infected cells and cancer cells.

Antigen receptors on T cells have a structure that very closely resembles part of an antibody, and its variable region can recognize a variety of antigens (Fig. 23).

Around 1983, my team and I were also working on isolating T cell receptor genes, but were not very successful. In 1984, right before I moved from Osaka University to Kyoto University, we isolated a cytokine receptor called IL-2 receptor, and subsequently cloned cytokines IL-4 and IL-5 produced by T cells. Dr. Yasumasa Ishida proposed that we isolate genes involved in selective cell death of T cells in the thymus shortly after he entered graduate school in 1989. His proposal was a fully developed idea that included the necessary techniques. In addition, he induced very strong PD-1 expression in the cells he used and thus was only able to isolate PD-1, which allowed him to proceed to functional analysis of the molecule unwaveringly.

Dr. Ishida, Dr. Yasutoshi Agata and their team found from their analysis of PD-1 cDNA structure that this molecule is expressed in the membrane and has a characteristic structure inside of the cell (Fig. 24). The molecule has two common tyrosine residues preserved from previously known molecules that transmit positive signals to cells. However, the distance in between was very different from previously discovered molecules, leading the team to conclude that this was a new type of cell membrane receptor. Shortly thereafter, they established that PD-1 is not involved in cell death, but we continued researching its function because it was an interesting molecule.

Over a grueling period of nearly five years, Dr. Hiroyuki Nishimura created PD-1 knockout mice, and Dr. Taku Okazaki analyzed the mice with the assistance of Dr. Nagahiro Minato’s laboratory. They found that various species of mouse, including black mice and white mice, develop different diseases (Fig. 25). Nephritis, arthritis, dilated cardiomyopathy, autoimmune diabetes, and myocarditis are all autoimmune diseases that develop when the immune system is abnormally active. In other words, their finding that
mice without PD-1 have an overactive immune system showed that PD-1 acts as a brake on immune response.

Regulation of immune response is similar to cruise control of a car (Fig. 26). Initial activation of the immune system requires releasing the parking brake and slowly pressing down on the accelerator as when driving a car out of a parking lot. In the immune system, a molecule called CD28 is the accelerator and a molecule called CTLA4 is the parking brake. Like acceleration and braking when driving a car, these work by being turned “on” or “off”. There are also molecules that correspond to accelerating and braking while driving on a road. In this analogy, ICOS, which the immune system uses to attack invaders, is the accelerator, and PD-1 is the brake. These two molecules work together to dynamically regulate the strength of the immune system to ensure that it can move at any speed, even at tens of kilometers per hour.

For a very long time, surgery, radiotherapy, and chemotherapy were considered the only effective treatments for cancer. Many researchers attempted to treat cancer with the power of the immune system without success.

Earlier immunotherapies for cancer included cancer antigen vaccine therapy, which involves extracting antigens from cancer cells and injecting large doses of those antigens. Another method called cell activation therapy involves collecting a patient’s lymphocytes and reinjecting them after activating them in a test tube. Interferon therapy and other cytokine therapies designed to activate the immune system were also attempted unsuccessfully (Fig. 27). In light of these failures, my colleagues and I wondered whether our discovery that releasing the PD-1 “brake” activates the immune system could be applied to the treatment of cancer.

To test this hypothesis, I asked Dr. Yoshiko Iwai, a graduate student at the time, to start research on cancer treatment in early 2000 (Fig. 28). First, she grew tumors in PD-1 knockout mice and normal mice and discovered a significant difference in proliferation speed. For example, as you can see in the graph, the tumors of wild-type mice, shown on the left, proliferated in a linear fashion, whereas the tumors of PD-1 knockout mice with a very active immune system, shown on the right, did not grow. Because of these findings, we decided to do an experiment to test whether antibodies could be used to treat cancer.

Professor Nagahiro Minato, a colleague of mine who specializes in cancer immunology, created some superior antibodies, and Dr. Iwai tested whether injecting mice with these antibodies would suppress tumor proliferation. She found that tumor growth was strongly attenuated and lifespan was longer in mice injected with the antibodies (Fig. 29). PD-L1 is a molecule that binds to PD-1 and acts like a foot pressing on the brake.

Dr. Iwai used a model in which liver metastasis is produced by injecting a skin cancer called melanoma into the spleen to show that anti-PD-1 antibody inhibits metastasis to the liver. Using the same method, she also confirmed that mice without the PD-1 gene have a markedly lower rate of cancer metastasis than normal mice. After seeing these clear results in a mouse model, I was eager to try this
method in humans. Curing cancer has been a long-held dream of many medical researchers.

Spurred on by the strong belief that anti-PD-1 antibodies could help me fulfill that dream, I pitched the idea to apply this principle to humans by creating human antibodies to some companies, but ran into great difficulties. This project would require a large amount of funding, and no company wanted to take it on right away. Ono Pharmaceutical, the co-applicant on the patent, wanted to do joint research with another company inside or outside Japan because they could not do it alone, and went to meet with some potential partners. They told me that they spent a year talking to a dozen companies but none accepted, so they had decided to give up on development. Therefore, I decided to develop the antibody myself with an American venture company. When I talked with them, they agreed with zero hesitation. However, their agreement was on the condition that Ono Pharmaceutical would withdraw from the project. I relayed this to Ono Pharmaceutical, but the patent was published while they were considering withdrawal. Fortunately, Medarex, another American venture company, directly asked Ono Pharmaceutical to collaborate after seeing the patent, and development was started.

Soon after, Medarex created fully human anti-PD-1 antibody using their XenoMouse, a mouse with human immunoglobulin genes, and applied for a patent in 2005. These antibodies have high affinity and were designed to not kill T cells even when bound to PD-1. In 2006, the United States FDA approved the antibody as an investigational new drug, and clinical trials were started.

The first phase I clinical trial conducted in the United States in 2006 was a safety study in patients with terminal lung cancer, colorectal cancer, melanoma, kidney cancer, and prostate cancer. A similar clinical trial in patients with recurrent and refractory cancers was started two years later in Japan. The reason why we began with refractory cancer was that nobody believed that the immune system could cure cancer at the time. Doctors were particularly skeptical. It would be very risky to subject our own patients to this kind of trial, so we enrolled patients whom we could offer no other help. During this time, in 2009 Bristol-Myers bought Medarex.

Shortly after the trial began, I started hearing at conferences and in other places that even though the study was only supposed to be on the safety of the treatment, it was actually yielding amazing results. The results of the first phase I trial published in 2012 showed that 20% to 30% of patients with terminal cancer responded to the treatment. A total of 296 patients with terminal cancer received the treatment, and patients with non-small cell lung cancer, melanoma, and renal cell carcinoma showed a complete response or some response (Fig. 30). This was a major shock to the field of oncology at the time, and was even widely reported in business newspapers such as the Wall Street Journal and Frankfurter Allgemeine. They made the sensational proclamation that “cancer can be cured”. This is because nobody could have imagined before that a treatment so effective in terminal cancer patients could exist.

Another aspect of this report that caught even more attention was that even though the treatment
was discontinued after 6 months, data showed that 20 of the 31 patients whose tumors did not grow during that time did not relapse for at least a year and a half after discontinuing treatment (Fig. 31). On this graph, “0” indicates the size of the patient’s tumor at the start of treatment. Tumors that grew are plotted by percentage above that size, and tumors that shrank are plotted by percentage below that size. While it should be noted that this graph does not include data from patients who did not respond at all, you can see on the graph, with the scale marked in weeks, that tumors that did not grow for at least a year and a half after the patient discontinued the antibody treatment at the 6-month mark continued that trend, and some tumors even disappeared completely. This kind of long-lasting effect was unprecedented with conventional cancer treatments and caused a big sensation.

I also collaborated with Professors Ikuo Konishi and Junzo Hamanishi of the Department of Gynecology and Obstetrics at Kyoto University to conduct a clinical trial investigating the efficacy of the anti-PD-1 antibody Opdivo in terminal ovarian cancer refractory to conventional anticancer drugs.

We used two different doses in groups of 10 patients each for a period of one year. We measured efficacy by the disease control rate, defined as the group of patients whose tumors disappeared, shrank, or remained the same size, and found that 40% to 50% of patients showed some response.

To give an example, in one patient who developed a large peritoneal tumor from recurrent ovarian cancer, the tumor disappear completely and tumor markers decreased to nearly 0 after 4 months of treatment. She is still doing very well today, 3 years after the trial.

In the ovarian cancer trial, treatment was provided for one year and then discontinued, but two patients are still doing well more than 2 years later (Fig. 32). I think we may have also been able to save the other seven patients who responded if we had re-treated them after recurrence.

After this trial, universities and hospitals from all over the world began publishing results of anti-PD-1 antibody therapy for various types of cancer. I would like to present a few examples. In one study, 418 patients with melanoma who were treatment-naive, meaning that they had been diagnosed with melanoma for the first time, were randomized into two groups and received either Opdivo or the anticancer drug dacarbazine, which was the established best option (Fig. 33). Efficacy was evaluated objectively without the patients knowing which treatment they were receiving. After a year and a half, the survival rate in the Opdivo group was 70%, compared with less than 20% in the dacarbazine group, causing the ethics committee to order the termination of the study because the difference in efficacy was so great.

Another study of Opdivo in patients with Hodgkin’s lymphoma refractory to conventional treatment also showed signs of efficacy: all 23 patients had their tumors either shrink or stop growing (Fig. 34).

There are three reasons why cancer immunotherapy by inhibition of PD-1 is such a breakthrough. The first is that it could potentially be effective in all types of cancer, the second is that the effects of treatment persist for several years after discontinuation with low rates of recurrence, and the third is that adverse reactions are relatively mild because the treatment activates the immune system rather than
directly attacking cancer cells.

The reason why the power of the immune system can be used to treat cancer is that cancer is a foreign invader (Fig. 35). Analysis of DNA of various cancer cells has shown that all of them have several hundred to several thousand times as many mutations as normal cells. Therefore, even though cancer cells used to be the body’s own cells, they accumulate mutations through repeated proliferation and ultimately come to be recognized by the immune system as foreign. This is why it is hoped that anti-PD-1 antibody therapy will in principle be effective against any type of cancer.

Conventional anticancer drugs also kill normal cells, and thus cannot be administered at the doses required to kill 100% of cancer cells. Though only few cancer cells remain after treatment, they proliferate and acquire even more mutations, which produces recurrent cancer with cells resistant to anticancer drugs. Therefore, even if the cancer is then treated with a different anticancer drug, the same process will repeat: the few cancer cells that remain will gain additional mutations and produce new resistant cancer cells. In contrast, the immune system is equipped with cells called lymphocytes that can distinguish countless types of antigens and make it possible to find and kill all mutated cells, which produces a long-lasting effect with a low rate of recurrence.

If the immune system is on full alert from the onset of cancer, it should be able to eliminate all cancer cells in the early stages. Though the reason is currently unknown, it is believed that cancer cells proliferate because they promote excessive “braking” of the immune system and reduce immune activity. Therefore, removing the brake on the immune system and permanently changing its stable balance to where it can fight cancer may be what produces the long-lasting effects of immunotherapy. Weakening the braking function is effective in treating infectious diseases and cancer, but causes autoimmune diseases as adverse reactions.

With these outcomes from treating cancer with anti-PD-1 antibody, at present the treatment has been approved in Japan, the United States, and Europe for indications such as refractory non-small cell lung cancer and refractory renal cancer, making it possible to save the lives of many cancer patients (Fig. 36). It is a great joy for me as a medical researcher to see this day come.

I believe that anti-PD-1 antibody therapy will become the first-line option for treating all cancers in the near future (Fig. 37). This is because clinical trials in melanoma have shown that the treatment is more effective in the early stages of disease. Besides, chemotherapy, radiotherapy, and surgery all weaken the body’s immune activity. It is also known that immunotherapy produces fewer adverse reactions than any other treatment and yields long-term effects with only about 6 months of treatment.

This treatment does present some challenges at present. The first is that, in principle, the anti-PD-1 antibody Opdivo does not cure all types of cancer in all patients. There are clear successes and failures. Further research will be necessary to determine whether it is possible to predict response before treatment or assess response immediately after treatment, as well as whether response rates can be
improved (Fig. 38). Another challenge is that many practicing oncologists have insufficient knowledge of immunotherapy or experience using it and will need to be trained. In particular, they will need to be thoroughly versed in protocols for promptly detecting and responding to adverse reactions.

To that end, my colleagues and I are continuing our research to strengthen anti-PD-1 antibody therapy (Fig. 39). We recently discovered a more potent method in mice. When T cells proliferate and attack cancer cells, mitochondria grow larger and core enzymes involved in energy metabolism such as AMPK and mTOR are activated. Dr. Kenji Chamoto and his team found that treatment with the low molecular weight compound bezafibrate, which activates the downstream transcription factor PGC-1α, produces a tumor-suppressing effect many times more potent than treatment with anti-PD-L1 antibody alone.

This effect occurs because frequent division of activated T cells once every 6 hours requires a large amount of energy. In other words, activation of mitochondria becomes the rate-limiting step. The action of activated oxygen, energy sensors, AMPK, mTOR, and PGC-1α accelerates T cell proliferation.

This new development brought several things to light. First, inhibition of PD-1 activates the mitochondria of tumor-responsive T cells. Next, the therapeutic effect against cancer increases remarkably when this effect is enhanced. Therefore, it will be necessary to select the best compounds and attempt combination therapy in humans as well. Once that happens, I think that cancer immunotherapy will advance much further, as methods become more effective and the decreased antibody dose makes treatment more economical.

The author of an article in the British scientific journal New Scientist published in March 2016 states that “we’re at the point where we’ve discovered the cancer equivalent of penicillin” (Fig. 40). He uses this analogy because although penicillin did not cure all infectious diseases, the series of discoveries of antibiotics that followed after that brought about a major revolution in medicine, almost eliminating once-fatal infectious diseases.

I have four predictions about the future of cancer treatment. The first is that immunotherapy centered on inhibition of PD-1 will become more effective. The second is that it will be basically possible to treat all cancers with the power of the immune system (in the United States, there is a very large-scale program called Cancer MoonShot 2020 aimed to enhance cancer immunotherapy). The third is that we may continue to see the current trend of tumors not disappearing completely but still not growing larger. Finally, the fourth is that a time will come when cancer will be considered a single chronic disease and will be able to be controlled as such.

Vertebrates’ development of acquired immunity in the evolution of their fight with pathogens yielded a dramatic increase in lifespan. It has been proved that infinitely proliferating cancer cells become
recognized as foreign invaders as they accumulate mutations, and then become a target of acquired immunity. This has brought unforeseen serendipities to humanity in the course of evolution (Fig. 41).

Acquired immunity helped humanity to overcome infectious diseases, and, coupled with the discovery of antibiotics, allowed us to completely escape the threat of infectious diseases. We may be able to defeat cancer with PD-1 inhibition through immunotherapy in the twenty-first century. Humanity should put our serendipities to use and contribute to the sustained development of the world (Fig. 42).

To summarize, scientists were able to solve part of the puzzle of acquired immunity due to a great series of serendipities. The AID enzyme that creates antibody memory was discovered and its molecular structure was determined. The PD-1 molecule, which acts as a brake on immunity, was discovered, and treatment of cancer by inhibiting PD-1 was proved to be possible. Finally, my outlook on the future is that these unbelievable serendipities of acquired immunity may enable humanity to defeat cancer.

The many Japanese and international institutions listed here have supported this research for many years. I am very grateful to them.

My research would not have been possible without the assistance of about 600 research collaborators, staff members, students, postdocs, technical staff, and administrative assistants. Collaborative work with many researchers outside my department, from countries such as Sweden, the United States, and France, has also been of great assistance. I would like to take this opportunity to thank these collaborators.

I would also like to thank my wife and children for kindly supporting my self-indulgent academic lifestyle.

This concludes my presentation. Thank you for your attention.
PD-1抗体による抗腫瘍効果の持続

“6か月で投与を停止したが
31名中20名がその後1年半以上再発なし”

Fig. 31

PD-1抗体の長期間有効性

(Ono et al. ASCO 2015)

Fig. 32

無治癒メラノーマ患者に対するオプジーボと
ダカルバジンアルキ化剤投与の無作為化試験

オプジーボ

Fig. 33

オプジーボ投与を受けたホジキンリンパ腫患者で
は23例で全例有効

Fig. 34

がん細胞は遺伝子変異頻度が高く
免疫系が異物と認識する

Fig. 35

PD-1抗体治療の薬事承認（日本）

2014 根治切除不能メラノーマ
2015 難治性非小細胞性肺がん
2016 難治性腎がん

Fig. 36
PD-1抗体治療はがん治療法の第一選択になるだろう

1. 初期に使う方が効果大
   （メラノーマでは生存率70% VS 30%）
2. 化学療法、放射線、手術は免疫力を弱める
3. 副作用が少ない
4. 短期間の投与で長期に渡る効果 - 病変も

がん免疫治療の当面の課題

1. 基幹研究の課題
   1) 有効例と無効例の診断前後の判定
   2) 有効率の向上
2. 臨床現場の課題
   1) がん専門医の教育
   2) 副作用の対応プロトコルの充実

PD-1抗体治療の強化

CLOSED IN ON CANCER
PD-1抗体治療によってがん治療は大きな転機を迎えた
Andy Coghlan (New Scientist, 5 March 2016)
ジェネティック社 がん専門医 Don Chen 氏は、「
「我々は今、がんにおけるペニシリンの発見とも
言うべき時期にいる」と述べた

獲得免疫が与えた想定外の幸運

・獲得免疫の仕組みは脊椎動物が病原体
   から生命を守るためにも進化した。
   その結果脊椎動物の寿命は飛躍的に延伸
・幸運なことに、免疫細胞も悪性の癌細胞で
   異物となり獲得免疫のターゲットとなる

獲得免疫力を持った人類の幸運

20世紀 感染症を克服
   抗生物質の発見の成果
   感染
   抗生物質

21世紀 PD-1阻害によるがん免疫
   治療法でがんの克服の可能性