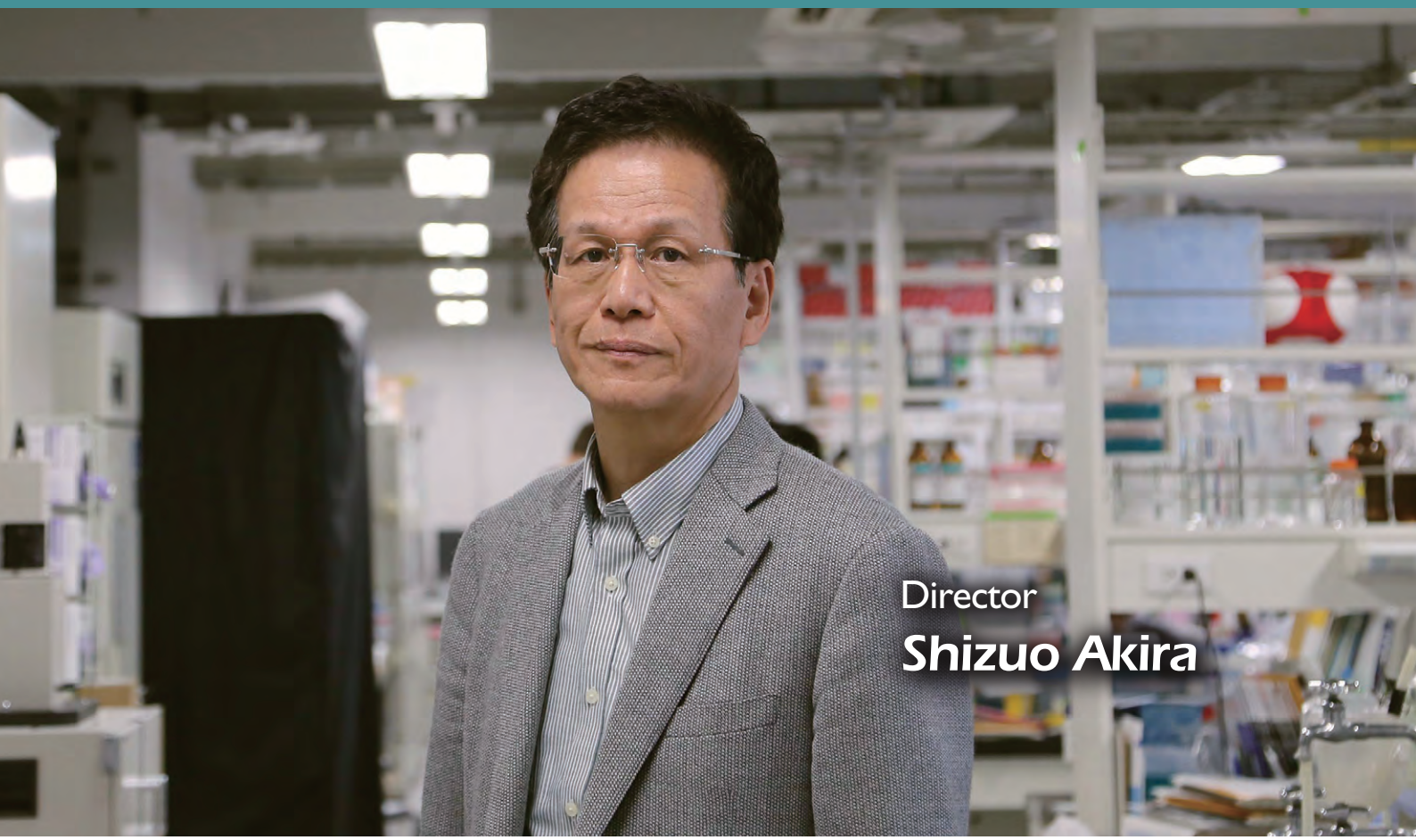
Immunology Frontier Research Center



The pursue of fundamental immunology research and elucidation of immune diseases

Immunology is a field of research that studies the mechanism of the host defense system protecting our bodies from microbial infections. The immune system is essential for eliminating pathogens, and its disorder causes various illnesses such as autoimmune diseases and allergy. Osaka University has a long and distinguished history in immunology researches. IFReC has selected excellent researchers from Japan and overseas, and has actively incorporated imaging science and bioinformatics into immunology. IFReC aims to overcome immune-related diseases by clarifying the whole picture of the immune system.

Research Center's Information (FY 2015)

Center Director: Shizuo Akira
Principal Investigators (PI): 27 (including 5 overseas researchers and 1 female researcher)
Other Researchers: 100 (including 35 overseas researchers and 15 female researchers)
Research Support Staff: 66
Administrative Division:
Administrative Director: Nobuo Sakaguchi
Administrative Staff: 39 (percentage of bilingual staff:54%)
Satellites and Cooperative Organizations: Institute for Frontier Medical Sciences, Kyoto University, Japan; RIKEN Center for Integrative Medical Sciences, Japan; National Institute of Biomedical Innovation, Health and Nutrition, Japan; Seoul St. Mary's Hospital, Catholic Medical Center, Korea; Indian Institutes of Science Education and Research, India; Maurice Wilkins Centre, The University of Auckland, New Zealand; Pohang University of Science and Technology, Korea
URL: http://www.ifrec.osaka-u.ac.jp/en/

WPI Osaka University iFReC

Major Research Achievements

1. **New models for immune regulations**
"Brake" and "Accelerator" for the regulation of inflammation.

2. **Regulatory T cell (Treg); an important immune regulator**
New concepts of immune regulation and therapies for various diseases.

3. **Fighting with parasites**
Infection by parasites is still an enormous problem of the world.

4. **Toward the novel vaccine developments**
Basic principle of vaccination revealed by latest researches.

5. **Guts are important organs for immunity**
The gut is a fertile field for immune reactions.

6. **Observation of Immune cells by MRI**
MRI performance at IFReC reached a stage where each immune cell is visualized.

7. **Dynamic observation of osteoporosis**
Newly developed fluorescent dye and 4D imaging enabled to depict the state of individual osteoclasts.

8. **Neural activity affects immune reactions**
"Illness starts in mind" is truth in some aspects.

Research Paper's Information

Number of Research Papers:	1,090
Top 10% Papers:	27.3%
Top 1% Papers:	5.3%
Internationally Collaborative Research Papers:	46.4%

(Database: WoS between 2007-2015)

Second Fl. IFReC Research Building, Osaka University
3-1 Yamadaoka, Suita, 565-0871 Osaka, Japan
Phone: +81-6-6879-4275(General Affairs),
+81-6-6879-4917(Accounting),
+81-6-6879-4777(Research Planning & Management)
Email: ifrec-office@ifrec.osaka-u.ac.jp

Background Picture: Microscopic images of mouse spleen where lymphocytes mature and "Immune synapse" formed on a T lymphocyte

Introduction

Immunology has made significant advances since the 19th century. Scientists such as Edward Jenner, the pioneer of the small pox vaccine and Louis Pasteur, who discovered treatments for rabies and other infectious diseases, appear frequently in textbooks. After these pioneer researches, immunology has achieved tremendous discoveries by continually introducing advanced scientific techniques. For example, developments in culturing methods led to the isolation of pathogens such as cholera, typhus and dysentery. Optical microscopy permitted the identification of pathogenic microbes and electron microscopy led to confirmation of the existence of viruses. Immunology attained its success by incorporating the techniques of microbiology, genetics, and computer science.

Research into infection and immunology at Osaka University began with Ogata Koan, the founder of Tekijuku and promotor of the smallpox vaccine in Japan. Later, in the 1960's, researchers inspired by Yuichi Yamamura, Professor of the Medical School, became the core of immunology studies at Osaka University. One of the peaks of that research was the discovery of interleukin-6 (IL-6) by Tadamitsu Kishimoto. Kishimoto's group clarified that IL-6 is implicated as an essential factor in inflammatory response of the immune system. In the 1990s, Shizuo Akira and others determined that innate immune cells such as dendritic cells detect pathogens by a receptor protein group called Toll-like receptors and elucidated the mechanism causing inflammation after infection. At this stage, most immunology researches were undertaken with T cells that had been isolated from the body. As such, it was not possible to draw conclusions on how immunity operated within the complex system of a living organism.

IFReC has advanced research with the aim of controlling immune response to pathogens and autoimmune diseases as well as cancer since its inception as a WPI center in 2007. We aim to determine the dynamism of individual immune cells and further elucidate live immune response of the whole body. This requires visualization (imaging) to track the infinite movements of cells during a living immune response. Technology to analyze the simultaneous and diverse responses is also vital. IFReC is advancing interdisciplinary research to probe these new realms by promoting and encouraging a team of excellent researchers combining immunology, bioimaging and bioinformatics.

Following is a sample of the remarkable results published by IFReC researchers over the past 10 years.

1 New models for immune regulations

Shizuo Akira (PI), Tadamitsu Kishimoto (PI)

The human body functions through the activities of various proteins. Cytokines (cell-to-cell mediators) are a group of proteins that have a vital role in immune response. They are produced inside cells based on genome information. In other words, mRNA is read (transcribed) as a messenger from the DNA in the nucleus, and then the cytokine proteins are produced in the cytoplasm (translated) and subsequently secreted from the cell.

If the mRNA of an inflammatory cytokine such as IL-6 is stabilized, the inflammationwas expected to continue; if it becomes unstable, then the inflammation was expected to recede. The discovery of this mechanism, at the level of mRNA, that finely controls inflammatory response was first in the world and was demonstrated by two independent approaches (Fig.1).

Regnase-1, discovered by Shizuo Akira, is an enzyme that diminishes the protein causing inflammation after transcription. If Regnase-1 activity is strong, it defeats the mRNA inflammation gene and suppresses the inflammatory response. In other words, it is an inhibiting factor for inflammation. Arid5a, discovered by Tadamitsu Kishimoto, binds to the mRNA of the proteins that cause inflammation and eventually regulate the inflammatory response. It is an accelerator of inflammation.

Matsushita et al. Nature 458, 1185 (2009).
Masuda et al. PNAS 110, 9409 (2013).

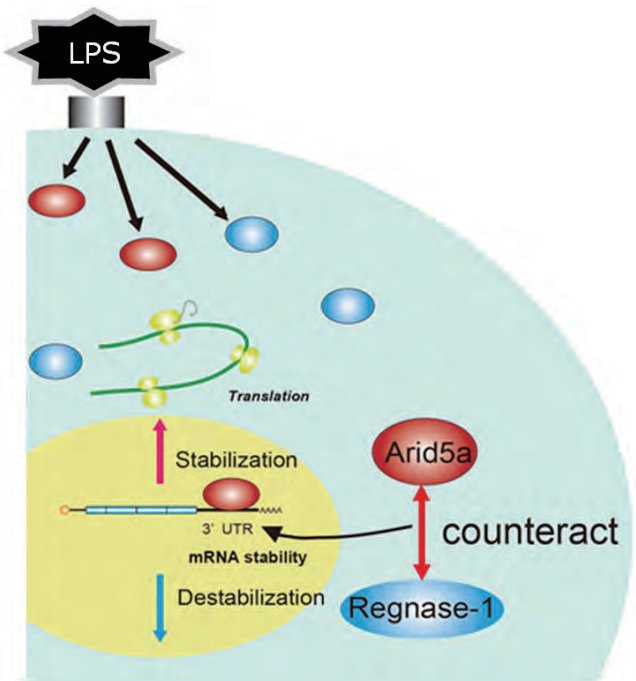


Fig. 1 Regnase-1 and Arid5a are "Blake" and "Accelerator" for inflammation respectively.

2 Regulatory T cell (Treg); an important immune regulator

Shimon Sakaguchi (PI)

Lymphocytes are the main player of acquired immunity that specifically recognize pathogens and remember the pathogenicity of them. T-lymphocytes (T cells) are known as the command center of immunity and are vital for control of immune response. T cells, during cell differentiation, express proteins called T cell receptors that have an infinite repertory of antigen specificity at the clonal level through genomic DNA recombination of the T cell receptor genes. This is the reason that the immune system can specifically identify enormous kinds of pathogens however, a substantial number of T cell clones express the receptor that responds to self. Most of the self-reactive T cells are removed in the thymus while some remain. A long-term puzzle in immunology is the question of "How are the T cells that attack the self inhibited?"

Sakaguchi discovered that some of the helper T cells, which are a type of lymphocyte, have that function: He called them regulatory T cells. The discovery caused a global sensation and raised interest in this field.

Sakaguchi's group found that the role of regulatory T cells in the human body is to suppress autoimmune response (autoimmune diseases). In a healthy person, damage to the self is prevented by making self-reactive killer T cells immunologically unresponsive (allergy). Namely, they elucidated the importance of regulatory T cells in immunological tolerance (Fig. 2).

Sakaguchi's group discovered that regulatory T cells derive from the same cells as normal helper T cells and various gene expression patterns are controlled by acquired factors (epigenetic information). Regulatory T cells occur not only in innate immune response but also under acquired immune response. If this condition is utilized to enhance the function of regulatory T cells, it would be possible to safely suppress autoimmune disease through immunoregulation. In contrast, weakening regulatory T cells could raise specific immune function toward cancer cells. Immune regulation, as explained, has become a realistic option for treatment of various diseases.

Maeda et al. Science 346, 1536 (2014).
Ohkura et al. Immunity 37, 785 (2012).

3 Fighting with parasites

Masahiro Yamamoto (PI), Cevayir Coban (PI)

In a hygienic, advanced nation such as Japan, infection by parasites is rare but if we look at the rest of the world it is an enormous problem. For example, there are over 200 million malaria patients a year and 2 million deaths (WHO 2013). Most parasites are multicellular organisms compared to viruses and bacteria with a minimum unit or a single cell. Parasites are large and cannot be eliminated by a simple reaction from a living organism. Once parasites enter the body they survive by using the energy and nutrition of the host. They also extend their existence as parasites by keeping the host alive. In Japan, infection is usually due to domestic pets we keep, overseas travel, swimming in a dirty river or eating uncooked food.

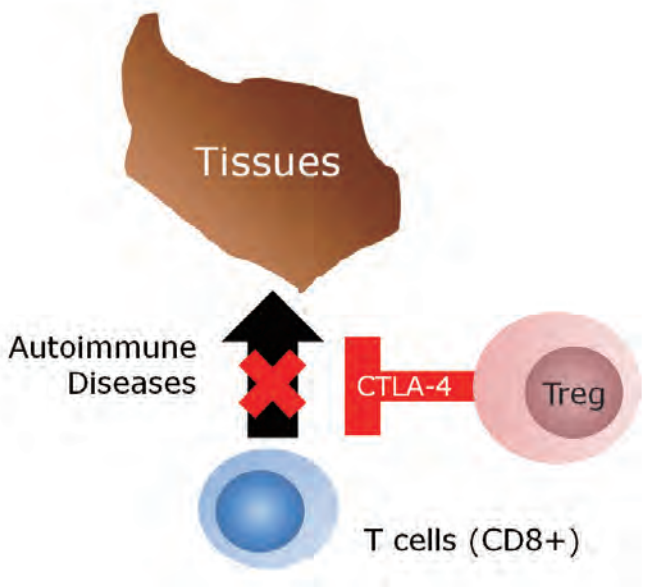


Fig. 2 Usual immune suppression by Tregs.

Amongst these, Toxoplasma can cause lethal encephalopathy and myocarditis in weakened patients. Infection from eating insufficiently heated meat and touching cat feces has been confirmed. The parasite is of concern for pregnant women as infection can cause miscarriage and hydrocephalus in newborns.

Yamamoto's group found that Toxoplasma directly activates the cells of the host (infected mouse) that assembles neutrophils (white blood cells) in inflammatory response at the site of Toxoplasma infection. Toxoplasma opportunistically uses the assembled neutrophils for its survival and to spread throughout the body. It uses the immune response of the organism like the capture of the Trojan horse and takes advantage to spread infection from the original site to the rest of the body (Fig. 3).

Ma et al. J Exp Med 211, 2013 (2014).
Zhao et al. Cell Host & Microbe 15, 551 (2014).

4 Toward the novel vaccine developments

Tomohiro Kurosaki (PI), Ken Ishii (PI)

The rule of infection that states; "once one has had an infection it is harder to get it again" is recorded as far back as Ancient Greece. Later research showed that many illnesses are caused by microbe infection and after a first infection the organism becomes resistance to further infection. Furthermore, it was found that the injection of an attenuated microbe can create the same resistance as a prior infection. This knowledge led to our current vaccines.

The nature of vaccines is to leave a memory of illness in the immune system to enable quick resistance upon a second infection. How this immune memory is created and how vaccines work best is still largely a mystery. Kurosaki's group used a mouse model to elucidate that the decreased expression of a protein called Bach2 in B cells is the primary factor for generating memory B cells, which are the main immune cells that govern immunological memory, in order to respond quickly upon re-infection and release antibodies. Bach2 temporarily suppresses the differentiation of B cells into antibody producing cells after contact with an antigen (bacteria, virus). Once Bach2 has decreased, memory B cells rapidly differentiate into antibody producing cells in large numbers after a second contact with the antigen. The over-suppression of Bach2 causes hyper-activation of memory B cells into antibody secreting cells, potentially resulting in allergy and autoimmune disease. Understanding the function of memory B cells is vital to developing effective vaccines against diseases such as influenza.

Ishii Ken's group is investigating additives or adjuvants that raise the effectiveness of vaccines. The most often used adjuvant to date is alum, an aluminum compound. Alum adjuvants, once taken up into the immune cell induce the cell's death. It has been found that the unwound double-helix structure DNA, then released from the host cell, revitalizes natural immunity and is indispensable for the vaccine effect. The released DNA activates a protein called interferon regulatory factor (IRF3), and subsequently causes the allergic reaction with IgE antibody as a side effect of adjuvants. However, the antibody reaction with IgG that is a main effect of the vaccine is unaffected. This research is expected to lead to the development of new adjuvants that suppress the excessive release of DNA and vaccines with fewer side-effects.

Kometani et al. Immunity 39,136 (2013).
Marichal et al. Nat Med 17, 996 (2011).

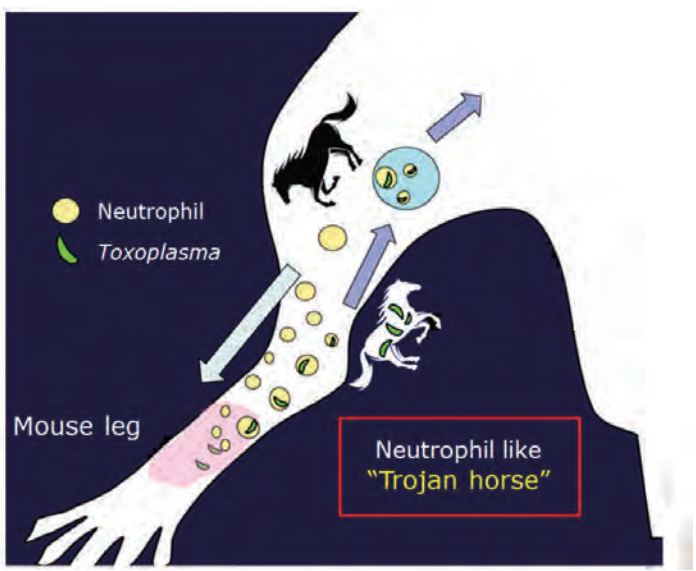


Fig. 3 "Trojan horse strategy" by Toxoplasma gondii.

5 Guts are important organs for immunity
Kiyoshi Takeda (PI)

A large quantity of microorganisms lives in our intestines in the form of intestinal bacteria. The number exceeds the total number of human cells constituting our whole body. This assembly of intestinal bacteria is also known as microflora. Meanwhile, the gut is also gaining attention as a fertile field for immune reactions as a variety of good and microorganisms pass through from the outside.

Takeda's group found that in a mouse lacking a protein called Lypd8, which is abundant in the epithelial cells of the intestine, many intestinal bacterial penetrate to the inner mucous layer resulting in more severe enteritis than a wild-type mouse. They revealed that Lypd 8 binds specifically to intestinal bacteria with flagella and prevents bacterial invasion by suppressing motility (Fig. 4).

In addition, the group found that lymphoid tissue present in the appendix, which was thought to be redundant in humans, is important for production of the antibody protein IgA known to play an important role in mucosal immunity as it is involved in the control of intestinal bacteria. Namely, it is now considered that the appendix is an important organ for the suppression of inflammatory bowel disease caused by an imbalance of intestinal bacteria. Thus, the claim that the cecum is redundant is refuted from an immunological standpoint.

Okumura et al. Nature 532, 117–121 (2016).
Masahata et al. Nat Commun 5, 3704 (2014).

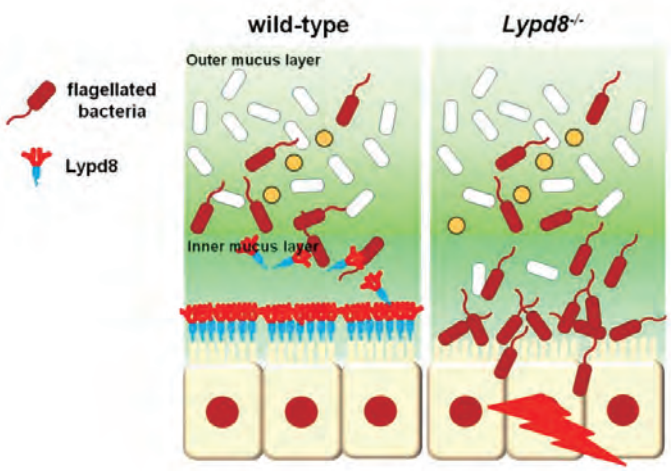


Fig. 4 Important function of Lypd8 in gut homeostasis.

6 Observation of immune cells by MRI
Yoshichika Yoshioka (PI)

Nuclear Magnetic Resonance Imaging (MRI) is an analytical method capable of photographing and stereoscopically displaying the inside of a human body. It is based on a completely different principle from X-ray CT. Improvements to electromagnetic coils and peripheral equipment has dramatically improved MRI performance. It has now reached a stage where each immune cell can be visualized.

Yoshioka's group has successfully continuously observed macrophages (phagocytes) in the mouse brain using the world's highest level MRI installed at IFReC (Fig. 5).

In mice injected with lipopolysaccharide (LPS), which causes inflammation, macrophages in the brain increase over time peaking after several days. However, after one week it returned to normal as if nothing had happened. It was surprising that so many immune cells were present in the brain without direct invasion of toxin into the brain. Thereafter, MRI imaging has been further enhanced with three-dimensionalization and time resolution capability, the world of immunity that could be observed previously is being revealed in living animals.

Mori et al. Sci Rep 4, 6997 (2014).

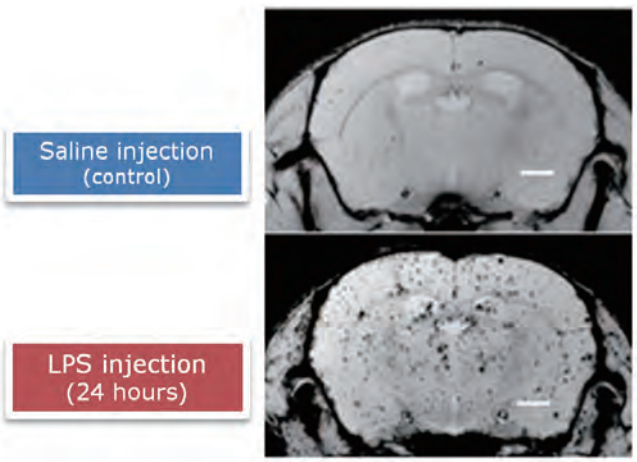


Fig. 5 Macrophages appearing in the brain in response to the toxin.

7 Dynamic observation of osteoporosis

Masaru Ishii (PI), Kazuya Kikuchi (PI)

The constituents of our body are replaced daily by metabolism on a daily basis. Bones are no exception, bones are formed by osteoblasts while osteoclasts destroy and consume bones. Osteoporosis occurs when osteoclasts, a type of immune cell, excessively destroy bones and this balance breaks down.

Masaru Ishii's group and Kikuchi's group are collaborating to investigate this function of osteoclasts by using their proprietary live imaging technology. Initially, multiphoton microscopic imaging was only able to show osteoclasts as "shiny particles". Eventually, however, individual shapes and movements became apparent. Furthermore, using the newly developed functional fluorescent molecule and 4D imaging (three-dimensional + time), they are able to depict the activity of individual osteoclasts, that is, "how actively they consume bone, do they take rests?" (Fig. 6).

These results are only achievable at IFReC with the combination of immunology, microscope system and multiple imaging technologies such as the development of fluorescent dye. Based on such data, control of the activity balance of osteoclasts could lead to new treatment strategies for bone-destroying diseases such as osteoporosis and cancer metastasis. If we can control the activity balance of osteoclasts based on such data, it will be a new strategy to treat bone destruction due to osteoporosis and cancer metastasis.

Maeda et al. Nat Chem Biol 12, 579 (2016).

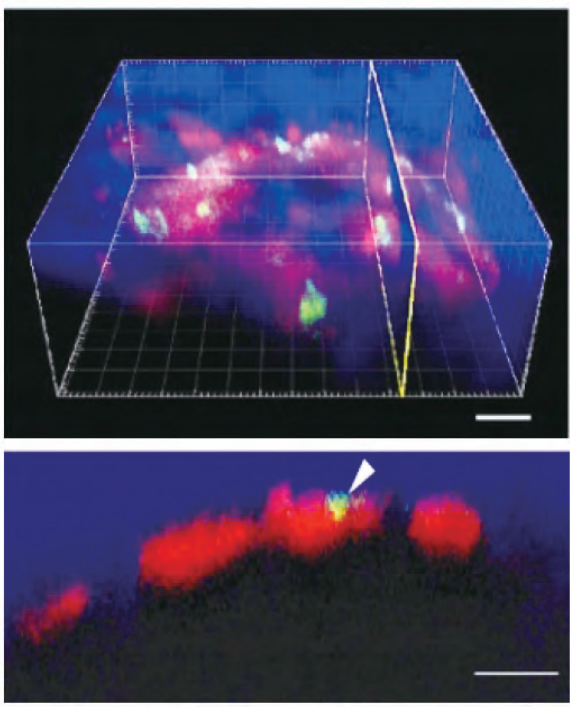


Fig. 6 Direct 4D imaging of bone-eating osteoclasts.

8 Neural activity affects immune reactions

Kazuhiro Suzuki (PI)

Have you ever come down with a cold as soon as a period of stress is over? The connection between the nervous system and immunity is fascinating but it seems difficult to prove academically.

Kazuhiro Suzuki's group found that noradrenaline secreted by stimulation of the sympathetic nerve, which is an autonomic nerve, suppresses the release of lymphocytes from the lymph node (they are retained in the lymph node). The autonomic nerve plays an important role to maintain the balance of the body when it is having cold sweats and panting in times of nervousness. In this study, the group succeeded in demonstrating that information from the nervous system affected the function of the immune system (Fig. 7).

Furthermore, it was found that in a mouse model of multiple sclerosis, an autoimmune disease of the nervous system, nervous system signals hindered the arrival of lymphocytes at the site of inflammation. That is, inflammation (immune reaction) was suppressed to some extent when nerves were in tension.

Nakai et al. J Exp Med 211, 2583 (2014)

The future of IFReC

In the 10 years since its establishment, IFReC has achieved steady results to high international acclaim. Osaka University was ranked No. 1 in the world (number of citations per paper) in "Research Institution Ranking in the 10 Years (Immunology Field)" published by Thomson Reuters in 2014. IFReC researchers make a significant contribution to this ranking. In addition, comparing IFReC article citation data with several top laboratories in Europe and the United States, it was indeed the world's leading research institute of immunology.

Some of the major awards received since establishment include; The Keio Medical Science Prize (Akira and Sakaguchi), Person of Cultural Merit (Akira), The Asahi Prize (Sakaguchi), The Canada Gairdner International Award (Akira and Sakaguchi), The Crafoord Prize (Kishimoto and Hirano), and The Japan Prize (Kishimoto and Hirano). As of 2016, four IFReC members; Akira, Sakaguchi, Nagata and Kishimoto are foreign members of the American Academy of Sciences this is very rare for research institutions around the world.

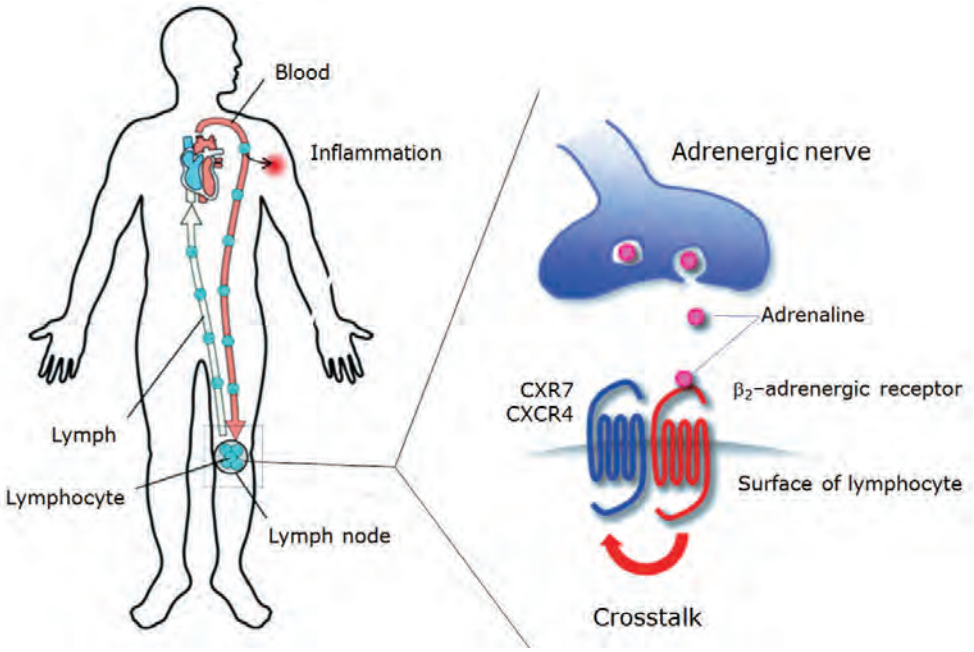


Fig. 7 Neuronal excitation affects immunity.

Promotion of integrated research in IFReC has become the foundation of significant developments in fundamental immunology research and has produced many excellent results. In the field of multiphoton excitation microscope and MRI imaging, the results obtained were as mentioned above. The results exceeded initial expectations due to advances in technological innovation during this period.

In addition to this, many researchers are developing research that incorporates bioinformatics, and it has already become a major trend in IFReC, including multiple papers published in Cell, Nature and their sister journals. In IFReC, fusion research of immunology with imaging and informatics is no longer a goal but a natural part of our daily research method.

There is no doubt that this research system has achieved the "environment in which researchers from different fields gather under one roof" promoted by the WPI program.

The research environment of IFReC is an achievement that could not exist without the WPI program. In 2017, IFReC, with a variety of financial support including from private companies, will increase human resources and extend research concepts and diversity. Even then, the devoted efforts of IFReC researchers who emphasize basic research will continue.

In addition, IFReC will continue as a WPI center to make new approaches to continuing to disseminate excellent research results to the world. The fusion with bioinformatics made it possible to comprehensively analyze research data. It will become possible to make data analysis, which until now has depended on human effort, far more reliable and it will be possible to apply it to ultra-high resolution analysis images etc. The development of AI technology as an artificial brain is also expected to advance quickly. The trend of scientific research in the world will develop with these technological innovations meaning young talented persons will be essential for the future of IFReC. Therefore, IFReC hopes to expand as a center that can provide a wide field for collaborative research. We will advance the Open Innovation Laboratory Concept which aims to gather the intelligence of industry and academia with international researchers in various fields. It will be a place for the circulation of global brains that focuses the power of young people. IFReC will continue its activities as a WPI center in line with the currently proposed Open Campus Initiative of Osaka University.

Jun Sakanoue (IFReC), Catherine Nakamichi (IFReC)