

**Center Director's Vision**

**Osaka University Immunology  
Frontier Research Center**

**Shizuo Akira, M.D., Ph.D**

**Director**

## **1. Objectives to be achieved**

The aim of this program is to unveil the whole picture of the dynamic immune system by employing a variety of imaging techniques to visualize the immune cells within live animals. We will attempt to improve the imaging technologies, which will allow us to track the dynamic behavior of immune cells and their communications more directly and to understand how immune cells respond to non-self components, such as pathogens and cancers, in vivo. Based on these basic studies, we will seek to develop new strategies for the diagnosis and treatment of various diseases, including infectious diseases, autoimmune diseases, allergies and cancers. To this end, we will invite 10-20 world-class principal investigators to the Osaka University Immunology Frontier Research Center as core scientists in the project and expand the program by forming links with domestic and overseas institutions that will function as satellites.

## **2. Background**

Immunology is a medical science that investigates the mechanisms of the host defenses that protect the body against microbial infection (1). Although the immune system is essential for eliminating infectious pathogens from the host, malfunction of this system gives rise to various immunological disorders, such as autoimmune diseases, allograft rejection and allergic responses. Effective immune responses to pathogens and protective immunity against reinfection with the same pathogen are generated through a complex immune network exerted by various types of immune cells, including T lymphocytes, B lymphocytes, dendritic cells, macrophages and natural killer (NK) cells. These immune cells distinguish between self and non-self, and elicit integrated host defense responses as a system to efficiently eliminate invading pathogens. The fundamental characteristics of the mammalian immune system are the motility and migratory behavior of immune cells, which are originally derived from bone marrow cells. Immune cells leave the bone marrow and circulate in the blood. Subsequently, some of these cells enter lymphoid organs, such as the thymus and lymph nodes, and some re-enter the blood and move to tissues or organs throughout the body. The cell-cell interactions among different immune cells are also important for immune responses. In particular, cells of the innate and adaptive immune systems communicate extensively in the lymphoid organs, and the communication between the cells determines the overall immune responses in the body. The activities of these immune cells are also influenced by non-immune cells, including fibroblasts, epithelial cells and endothelial cells.

To date, research in the immunology field has either been carried out by isolating immune cells from the body and examining the cells in vitro or by using in

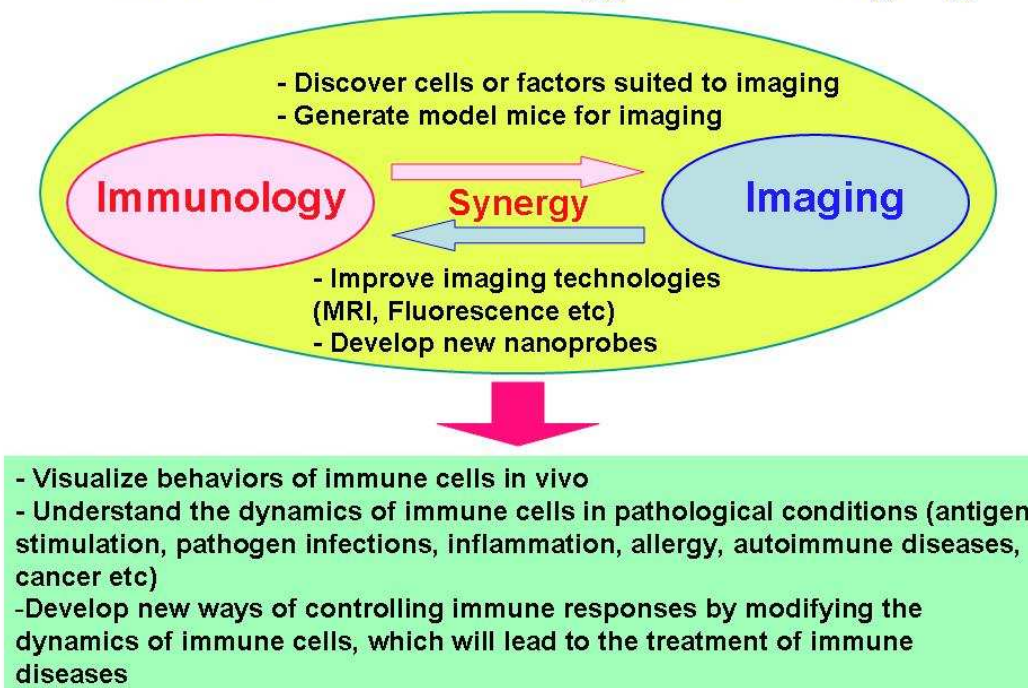
vitro cultured cell lines. Although such studies have provided many new insights into different immunology fields, we are still unable to draw the whole picture of immunological responses or predict the outcome of immune responses when a certain pathogen invades the body. Recently, in order to circumvent such drawbacks, we became aware of the necessity to study such immune responses in a spatiotemporal manner. Given the importance of the spatiotemporal organization of lymphoid organs in the establishment of immune reactions, as well as the importance of analyzing immune cell activation at the single cell level, immune cell and molecular imaging techniques are vital for future developments in immunology research.

The imaging techniques for visualizing the immune system are still in the early stages of development. To extend our understanding of the functions of the immune system in vivo, new technologies are needed to examine cells and molecules that are more deeply located in tissues, fainter and smaller, as well as to clarify the biochemical events that occur on a very rapid timescale. The ability to simultaneously monitor an increasing number of cell types and tissue structures is the key to developing a robust understanding of how tissue organization, extracellular factors and cell movements combine to support the development of useful immune responses. In order to overcome such imaging challenges, interdisciplinary efforts in physics, computer science and immunology are required. Despite such hurdles, the imaging techniques have rapidly been applied to the immunology field worldwide, particularly in the United States. In fact, a conference named “Imaging Immune Responses” was held during this year’s Keystone Symposia. Currently, immunology research in Japan is very strong. We have made great contributions to the progression of immunology research, which are highly appreciated worldwide. However, we have to admit that our imaging of the immune system is behind that of the United States. The next 10 years of development in the immunology field definitely relies on whether or not we can gain priority in imaging of the immune system in vivo. To sustain the international domination of immunology in Japan, I propose to establish a new Immunology Research Center in which immunologists can study the immune system by utilizing in vivo imaging techniques, and produce further innovations in bio-imaging for immunology research through interactions with researchers who are engaged in the development of imaging systems. By integrating the immunology and imaging fields, we will be able to understand the dynamic interactions of immune cells and their activation. This will allow us to manipulate the immune system, leading to the development of vaccines for infectious diseases based on novel strategies, concepts for immune therapies for various infectious diseases and cancers, and methods for treating autoimmune diseases.

### 3. Members and groups participating in the research center

Japanese immunologists have accomplished tremendous contributions to the development of modern immunology. In particular, Osaka University has won worldwide acclaim for its outstanding achievements. The late Yuichi Yamamura, the former President of Osaka University, initiated cancer immunotherapy using bacterial extracts in Japan, and became a founder of the Japanese Society for Immunology. Among the outstanding immunologists who have studied under Dr. Yamamura, Tadamitsu Kishimoto has become an internationally acclaimed specialist in cytokine research through his discovery of interleukin (IL)-6. Researchers disciplined under the supervision of Dr. Kishimoto have also made fine contributions to the field of immunology. For example, Toshio Hirano studied IL-6 biology and its signaling, Shizuo Akira revealed the mechanism of pathogen recognition by innate immunity (2) and Hitoshi Kikutani clarified the role of the semaphorin family in immune responses. Eminent immunologists of Osaka University will participate in the new center. The researchers will include Shizuo Akira (as Director), Tadamitsu Kishimoto, Toshio Hirano, Masayuki Miyasaka, Hitoshi Kikutani, Taroh Kinoshita, Atsushi Kumanogoh, Kiyoshi Takeda and Hisashi Arase. In addition, Shimon Sakaguchi (Kyoto University), Takashi Saito (RCAI) and Tomohiro Kurosaki (RCAI) will be invited to join the center.

## Fusion of immunology and imaging



All of these researchers have made great contributions to a variety of fields in immunology. In particular, Shimon Sakaguchi discovered regulatory T cells, which are now a hot topic in immunology, and his joining will enhance our status as a world-premier immunology center. Furthermore, Fritz Melchers will join the center as a principal investigator. He was a director of the Basel Institute for Immunology from 1980-2001, and President of the International Union of Immunological Societies from 1998-2001. He is now a senior research group leader at the Max Plank-Institute for Infection Biology (Berlin, Germany). I believe his joining will definitely strengthen the international prestige of the center.

Toshio Yanagida is renowned for single-molecule imaging in individual cells. Furthermore, he recently set up the High Performance Bioimaging Research Facility in Osaka University, which houses an 11.7 Tesla magnet Magnetic Resonance Imaging (MRI) system. Junji Seki and Yoshichika Yoshioka are specialists in imaging techniques, while Takeshi Jin and Yutaka Hata (University of Hyogo) are experts in developing molecular probes for imaging and computational image processing, respectively.

In an attempt to promote bioimaging techniques in our center, we plan to set up six satellites in foreign laboratories (all in the United States), which have made great advances in bioimaging. These researchers will include: Mark Davis (Stanford University), a specialist in single-cell imaging; Ronald Germain (NIH), Michael Dustin (New York University), Jason Cyster (UCSF) and Ulrich H. von Andrian (Harvard University), experts in intravital two-photon imaging; and Scott Fraser (California Institute of Technology), a specialist in MRI.

#### **4. Planned activities of the center**

##### **4.1. Generation of immuno-imaging core facilities**

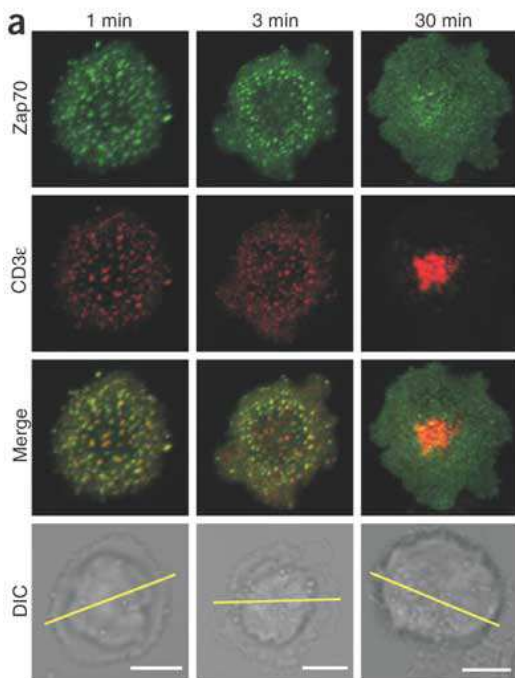
Immune responses against pathogens within the body proceed as follows: 1) pathogen recognition by innate and adaptive immune cells, and subsequent activation of these cells; 2) communication between immune cells and their maturation in lymphoid organs, such as the lymph nodes and spleen; and 3) systemic and local immune responses. For imaging of the immune reactions in each step, it is necessary to apply appropriate and different techniques. The imaging techniques now being utilized in the immunology field is composed of three parts.

First, single-molecule imaging is used for visualizing the activation of intracellular signaling cascades in a single cell. Second, confocal and multiphoton microscopy techniques combined with surgical methods have recently enabled dynamic imaging of immune cells in situ. Third, various techniques, including positron emission

tomography (PET), MRI, computer tomography (CT) and luminescence imaging, can possibly be applied to the visualization of immune reactions in vivo in a non-invasive manner, although these technologies still require improvement in terms of their spatial and temporal resolutions. I will establish immuno-imaging core facilities in the new research building and utilize the High Performance Bioimaging Research Facility in Osaka University housing the 11.7 Tesla magnet MRI system. In the immuno-imaging core facilities, we will attempt to develop novel techniques for the imaging of immune responses in a single cell, in situ intravitaly and in vivo in a non-invasive manner. Furthermore, these techniques will be applied to research in the immunology field by tight collaboration between the imaging groups and immunology groups.

#### 4.1.1. Single-molecule imaging

Single-molecule imaging is a technique for visualizing fluorescently-labeled single molecules by fluorescence microscopy, mostly in living cells. High-sensitivity video microscopy has allowed us to monitor the behavior of multiple fluorescent molecules in individual cells. Furthermore, this technique provides information about the structures of the molecules themselves, as well as the microstructures surrounding



Visualization of microclusters formed by TCR complexes in T cells.  
Yokosuka et al. Nat. Immunol. 6: 1253-1262, 2006

the molecules. Intra- and inter-molecular fluorescent resonance energy transfer (FRET) between single fluorescent molecules provides information on the molecular structure. Thus, this technique is useful for visualizing signal transduction in immune cells by monitoring the behavior of signal transduction molecules. Considering the importance of signaling cascade activation in the responses to microbial infection and cytokines, broad application of this technology is vital for the future development of immunology research.

Dr. Yanagida is a pioneer in the

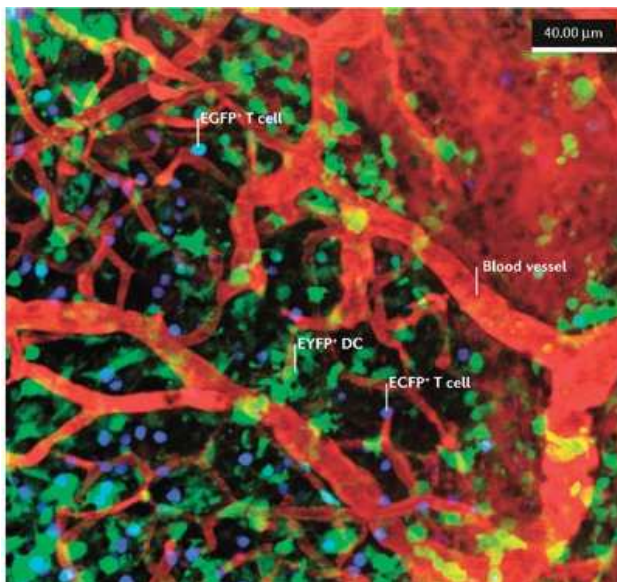
development of single-molecule imaging, and has established various techniques. His group has succeeded in the direct detection of kinesin molecular motion, and also

visualized epidermal growth factor (EGF)-EGF receptor complexes in living cells (3).

Dr. Saito has succeeded in the imaging of T cell receptor (TCR) microclusters and their behavior (4). In the proposed center, we will attempt to introduce these techniques in the immunology field. Dr. Saito will continue his research in the imaging of TCR signaling at RCAI, and maintain close contact with Dr. Yanagida's group in order to further improve the single-molecule imaging technology. For this purpose, we will have a satellite in the group of Mark Davis, an expert in this field, to introduce state-of-the-art technology from his laboratory and exchange information regarding single-molecule imaging.

#### 4.1.2 Imaging of cellular behavior and interactions in situ

Immune cells, including lymphocytes and dendritic cells, communicate with each other in response to infection with pathogens, and proper interactions of immune cells are critical for the establishment of immune responses against pathogens. Immune cells show a specific migratory behavior. After they leave the bone marrow, some cells enter secondary lymphoid tissues, such as the spleen and lymph nodes, or the thymus. The migration and trafficking of these cells are dynamically regulated. Furthermore, communication with T lymphocytes and stromal cells is critical for the proper differentiation and maturation of T cells in the thymus. Although previous studies have been performed using static methods, the importance of dynamic analyses of the immune system has been recognized.



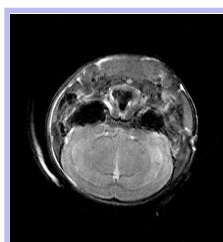
An example of two-photon microscopy imaging.  
Germain et al. Nat. Rev. Immunol. 6: 497-507, 2006

Recent advances in the imaging of organs in situ, especially implemented in the United States, have enabled us to visualize cellular behavior and cell-cell interactions (5). The application of confocal and multiphoton fluorescence microscopy instruments has been combined with new surgical methods and techniques for fluorescent labeling of cells. In these imaging techniques, a cell type of interest is labeled using appropriate dyes or by expression of fluorescent proteins and then visualized using confocal and two-photon or multiphoton

microscopy. In small animals like mice, explant or intravital imaging is now possible in several immune organs, such as the lymph nodes, bone marrow and spleen.

However, these techniques have not been extensively applied in Japan, particularly in the field of immunology. We believe that this is due to the lack of opportunities to fuse the immunology and imaging fields in Japan. Therefore, in our center, I will create a research group that focuses on the imaging of immune cell dynamics. The group will consist of a group leader and two postdoctoral fellows. This group will work under the supervision of Dr. Saito, and intimately collaborate with the principal immunologists at the center. The group will be technically supported by Dr. Yanagida as well as the overseas satellites, including the groups of Ronald N. Germain, Michael Dustin, Jason Cyster and Ulrich von Andrian. A new facility equipped with multiphoton fluorescence microscopes and related apparatus for imaging will be established in the new research building. In this facility, several trained technicians will be hired to help with the imaging experiments carried out by immunologists.

#### 4.1.3. Non-invasive monitoring of immune responses in vivo



The 11.7T MRI system at the High Performance Bioimaging Research Facility, Osaka University (left). Sample imaging of a mouse head using the 11.7T MRI (Bruker) (right).

Our ultimate goal is to develop techniques for non-invasive monitoring of the immune reactions to various stimuli in vivo. In order to understand dynamic immune responses, it is necessary

to develop systems that effectively monitor how pathogens invade and spread in the body and how immune cells migrate and interact with each other in response to invading pathogens. To these ends, we need to improve the imaging techniques involving

luciferase imaging, CT and MRI. Luciferase imaging is currently the best system for tracking the movement of cells in the body, although the image resolution needs to be increased. The new center will apply a luciferase

real-time in vivo imaging system to monitoring the immune responses after pathogen infections in living mice. We will also use CT to monitor the inflammatory responses in mice. MRI is the most attractive technique for imaging of the immune system in the



whole body, although the spatiotemporal resolution needs to be greatly improved. This imaging technique is currently in its early stages, and is expected to make progress in the near future through the development of new nanoprobe that can detect immune cells and the generation of mice expressing a target protein for imaging (6). It is likely that MRI technology will become an indispensable tool for basic research as well as diagnosis. Dr. Yanagida will organize the imaging groups to make efforts toward improving the MRI techniques to allow monitoring of the dynamics of individual immune cells in live animals without invasion, through the use of the 11.7 T MRI available in Osaka University and collaboration with Scott Fraser, one of the world's leading experts in MRI.

#### **4.1.4. Development of novel bioprobes**

For successful immune cell imaging *in vivo*, excellent bioprobes need to be newly developed. Commonly used fluorescent dyes have limitations regarding their brightness and stability. Quantum dots are one of the recently developed fluorospheres that can potentially overcome these problems. Dr. Jin is an expert on quantum dots and will work on the development of new bioprobes for fluorescence. Furthermore, novel bioprobes for MRI detection urgently need to be developed.

#### **4.1.5. Simulation and modeling**

Data obtained by single-molecule imaging, multiphoton microscopy and MRI analysis should be integrated to generate a model based on the characteristic behavior of immune cells. However, given that dynamic 3D imaging data are so large, it is necessary to develop bioinformatic approaches for integrating such data. Dr. Hata is an expert in computing medical imaging data, such as MRI and CT. He will work with immunology groups to develop novel systems for modeling immune responses. This group will be technically supported by the group of Ronald N. Germain, an expert in the generation of models by applying systems biology approaches to the interpretation of imaging data. Once a dynamic model has been generated, it will be verified by the immunology groups with experimental approaches. Appropriate modeling will enable us to simulate the immune responses to specific pathogens *in silico*.

## **4.2. Application of imaging techniques to studies of immunology**

The new techniques developed in the imaging core laboratory and new equipment introduced in the core facility will be applied by groups of immunology researchers. Details of the projects to be worked on by these immunology research

groups are described below.

#### **4.2.1. Imaging of innate immune responses**

The innate immune system directly recognizes infected pathogens, and plays an important role in the activation of adaptive immunity mediated by lymphocytes (2). Toll-like receptors (TLRs) and cytoplasmic receptors, such as NOD-like receptors and RIG-I-like receptors, are critical for the direct recognition of microbial components. These receptors trigger specific signal transduction cascades in response to their ligands (microbial components), leading to the production of proinflammatory cytokines and type I interferons. Dr. Akira's group will utilize single-molecule imaging techniques to visualize the behavior of the TLR molecules themselves, as well as TLR signaling molecules. This project will allow us to understand the dynamic molecular interactions of TLR signaling molecules. In addition, Dr. Akira will analyze the behavior of cytokine- and interferon-producing cells in response to pathogen infections in lymphoid organs and local infectious tissues using multiphoton microscopy. In addition, his group will maintain close contact with Dr. Yanagida's group to establish an MRI technique for imaging the time course of innate immune responses in vivo.

Dr. Takeda is a specialist in mucosal immunology, which maintains tolerance to nonpathogenic antigens. His group will pursue the imaging of immune cells at mucosal surfaces by generating reporter mice in order to monitor the regulatory functions of mucosal immune cells and analyze them intravitaly using multiphoton microscopy.

Dr. Arase is an expert in the biology of NK cells and their specific receptors. His group will try to image the behavior of NK cells in response to viral infection using multiphoton microscopy.

Dr. Kinoshita clarified an essential role of GPI-anchor in host cell protection from autologous complement. To understand why GPI-anchored form of complement regulatory proteins is important for the self-protection, his group will investigate dynamic behaviors of complement proteins and GPI-anchored complement regulatory proteins on the cell surface using imaging approach.

Dr. Hirano has identified that zinc functions as a second messenger in the signaling pathway in dendritic cells. His group will attempt to visualize zinc behavior in response to immune activation using MRI techniques.

#### **4.2.2. Imaging of acquired immune responses**

The cells involved in acquired immunity, namely T and B lymphocytes, possess rearranged antigen receptors for recognizing invading pathogens. T and B cells with

proper antigen receptors are activated and undergo maturation for rapid responses that eliminate reinfection with the same pathogen.

Dr. Saito has been working on the imaging of T cell receptor (TCR) and its signaling molecules, and has revealed that the formation of TCR microclusters is a critical step for T cell activation. His group will further pursue the imaging of TCR signaling molecules using mouse genetics, and monitor T cell activation in situ by multiphoton microscopy. He will also attempt to generate MRI techniques for in T cell science.

Dr. Kurosaki is an expert in B cell signaling and immunology. His group will work on imaging of B cell receptor (BCR) signaling by single-molecule imaging. In addition, his group will analyze the B cell maturation process, known as the germinal center reaction, by multiphoton microscopy.

Appropriate activation of lymphocytes is mediated not only by antigen receptors, but also by signaling of co-stimulatory molecules. Drs. Kikutani and Kumanogoh have worked on CD40 and semaphorin family members in the activation of immune responses. Dr. Kumanogoh will investigate the roles of semaphorin family members in immune cell interactions using multiphoton microscopy, while Dr. Kikutani will analyze the effects of costimulatory molecules on lymphocyte trafficking in response to viral infection.

Dr. Sakaguchi is renowned for the discovery of regulatory T cells and the mechanism of immune suppression. Regulatory T cells play important roles in preventing aberrant activation of immune responses. His group will work on imaging the behavior of regulatory T cells as well as their interactions with other immune cells using multiphoton microscopy and MRI techniques.

#### **4.2.3. Imaging of immune cell trafficking**

Multiphoton microscopy techniques have traditionally been used to monitor the migration and trafficking of immune cells. However, the current techniques are not sufficient to clarify the reason why specific cell populations enter the secondary lymphoid organs through the endothelium. Dr. Miyasaka will develop a technique for monitoring the endothelial changes that occur in specific venules or lymphatics during leukocyte trafficking in real time. He will generate reporter mice to monitor the high endothelial venules and use these mice to visualize the endothelial transmigration of immune cells.

#### **4.2.4. Development of new technologies for the assessment of autoimmune diseases**

## **by imaging**

Dr. Kishimoto discovered IL-6, an essential cytokine for inflammation, and has clarified its importance in immune responses against infection and the development of autoimmune diseases. He previously generated a monoclonal antibody that blocks the IL-6 receptor, and this is now used to treat human autoimmune diseases, such as rheumatoid arthritis and Newcastle disease. However, the molecular mechanism of the action of this antibody is still under investigation. Dr. Kishimoto will apply luciferase imaging and MRI techniques to elucidate the severity of experimental autoimmune diseases, and will also establish new methods for evaluating such diseases.

### **4.3. Establishment of domestic and overseas satellites**

In an attempt to promote bioimaging techniques in our new center, we will have one domestic satellite and five satellites in foreign laboratories (all in the United States) that have made great advances in bioimaging.

First, we will have a satellite in RIKEN, RCAI (Yokohama). From this satellite, Takashi Saito and Tomohiro Kurosaki will participate in the establishment of the center as principal investigators. Dr. Kurosaki will move to Osaka University, while Dr. Saito will maintain groups in both the RCAI and Osaka University. I believe that this satellite is the key to the success of our research center, since many excellent researchers in the imaging field belong to RIKEN. For instance, Makio Tokunaga and Yasushi Seko are experts in single-molecule imaging, and Atsushi Miyawaki is famous for developing fluorescence molecules. Given that Dr. Saito is a core member for our research center for developing novel imaging techniques for immunology, I believe that he can collaborate with these researchers in RIKEN toward fusing these two fields.

The overseas satellite members will include Mark Davis (Stanford University), Ronald Germain (NIH), Michael Dustin (New York University), Jason Cyster (UCSF), Ulrich H. von Andrian (Harvard University) and Scott Fraser (California Institute of Technology). The researchers allotted to these satellites will work under the supervision of a chief investigator in each individual laboratory, and also exchange information on *in vivo* imaging with the members of our imaging groups to help improve our bioimaging techniques. The chief investigators and satellite researchers will regularly visit our center for discussions and seminars.

I will organize an international symposium on bioimaging of the immune system every year, at which the satellite leaders will present their updated findings. This international symposium will enhance the mutual interactions among our center, RCAI and foreign satellites, and definitely make our center's mission visible worldwide.

## **5. Recruitment of foreign researchers and organization of the international symposium**

I will organize an international symposium on bioimaging of the immune system every year, which will definitely make our center's mission visible worldwide.

I will set up two groups consisting of a foreign professor or associate professor and several researchers working under their direction. These two principal researchers will be selected by international public advertisements, and their research expenses will be provided for 5 years from this grant.

In the new building, I will secure space for visiting scholars who will perform short-term collaborative studies.

## **6. Summary**

Finally, the main aims in this center are to promote the participation of principal immunologists in imaging research, and encourage them to regularly utilize such techniques in their own immunology research. I hope that new in vivo imaging techniques developed in the center will be utilized worldwide, and that our center will be recognized as one of the world-renowned immunology centers, particularly in bio-imaging. Studies performed in this center will lead to the generation of novel concepts for understanding the dynamics of immunology.

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