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Editorial

Development of *in vivo* imaging

In *Allergology International* (AI) Vol. 65 Issue 3, we feature a set of review articles entitled "Development of *in vivo* imaging" as well as an invited review article, original articles, and letters to the editor. This issue will update you on novel aspects of immune responses of peripheral immune tissues, such as the skin and central nervous systems, via *in vivo* live imaging using two-photon (TP) microscopy.

The immune system constitutes a dynamic network; immune cells actively move throughout the peripheral organs to scan for external pathogens. In the past, intravital imaging of peripheral immune responses was performed using confocal or fluorescence microscopy; however, these techniques have relatively low depth of light/laser penetration. Therefore, intravital imaging studies of cutaneous immune responses have been confined to the superficial areas of peripheral organs.

TP microscopy, using scanning probe microscopes, was first demonstrated by Denk[1] and has been in common use for about 10 years. Two-photon excitation is a fluorescence process in which a fluorophore is excited by the simultaneous absorption of two photons. Compared with conventional single-photon excitation microscopes, two-photon excitation allows deeper tissue penetration with less photodamage. TP microscopy therefore allows us to achieve high spatio-temporal resolution of target tissues.[2]

TP microscopy has several advantages for observing living tissue. First, some biological components, such as hair shafts and elastic fibers, are detectable without exogenous probes because of their own auto-fluorophores.[1] In addition, biological tissues contain many sub-cellular components composed of (a) vitamins or vitamin derivatives — such as retinol, cholecalciferol, riboflavin or pyridoxine — that emit in the visible light range or (b) aromatic amino acids such as tyrosine, phenylalanine, and tryptophan that emit in the UV range.[3] Second, collagen fibers, microtubules (tubulin), and muscle myosin are highly polarizable and are visualized via second harmonic generation (SHG). Third, some fluorescent-labeled reagents are available to label tissue components. Boron dipyrromethene (BODIPY), a lipophilic fluorescent dye, is useful for intravital tissue imaging. Intravenous injection of BODIPY labels sebaceous gland and adipocytes.[4] In addition, fluorescence-labeled dextran has an interesting application. It has been reported that the injected fluorescence-labeled dextran was engulfed by macrophages,[5] which allows us to monitor tissue under attack by phagocytic cells.

Transgenic animals that express fluorescent reporter proteins in specific tissues or cells are useful for intravital imaging. Many transgenic reporter mice have been developed and are used for *in vivo* imaging. Ubiquitous reporter strains such as CAG-enhanced green fluorescent protein (EGFP) are available to label immune cells.[6]

TP microscopy is at its best observing cell-mediated immune responses because immune cells represent high motility and rapid changes in their morphology.[7,8] Various kinds of subset-specific reporter strains have been developed that express fluorescent tags under the control of subset-specific promoters.[9] With these strains, intravital imaging analyses have elucidated cell behaviors in immune responses.

In this issue, Ishii *et al.* show us intravital TP imaging of various tissues and organs, such as live bone, bone marrow, thymus, blood vessels, and secondary lymphoid organs under the steady or inflammatory conditions.[10] More specifically, Honda *et al.* describe how skin immune cells cooperate to maintain skin homeostasis or to exert immune responses against foreign antigens that lead to allergic skin inflammation, such as contact hypersensitivity response.[11] Intriguingly, they found that leukocyte cluster formation during contact hypersensitivity is essential to activate memory T cells in the skin. In addition, Kawakami summarizes recent imaging studies of autoreactive T cell infiltration into the central nervous system.[12] Initially, autoreactive T cells interact with endothelial cells in the airways of the lung or with splenocytes in order to acquire a migratory phenotype to infiltrate the central nervous system (CNS). Then they interact with endothelial cells before passing through the blood–brain barrier. Lastly, CNS-infiltrating T cells are activated by recognizing endogenous autoantigens produced by local antigen-presenting cells.

Recently, analysis of human biological samples is gaining prominence. In western countries, TP microscopy is being currently applied to human skin *in vivo*.[13,14] TP microscopy is now used as a non-invasive diagnostic tool for human tissue diseases and changes, including tumors, aging, and connective tissue diseases.[15] The attempt to visualize tissue immune responses is, however, just getting started. Because immune cells emit no autofluorescent signals, *ex vivo* imaging of biopsy samples in conjunction with whole-mount immunofluorescence staining techniques will play a pivotal role. At present, clinical applications using TP microscopy are limited to the skin,[4,16] but TP microscopes can be used with other human organs as novel systems are developed, such as lensless TP imaging through a multicore fiber with coherence-gated digital phase conjugation.[17]

Progress in basic science has improved our understanding of the molecular mechanisms behind various diseases, including allergic diseases. This has led to a tremendous shift in viewing diseases, a shift from "phenotypes" to "endotypes". Focusing on "endotype" is defined as what uncovers molecular mechanisms underlying observable characteristics known as "phenotype". Development of appropriate "biomarkers" is required for this shift toward the typing of diseases based on endotypes. "Precision medicine" is defined as medicine taking individual variability into account and is based

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on the recent development of large-scale biological databases, powerful methods for characterizing patients, and computational tools.[18] Developing medicines based on endotypes would make precision medicine a reality. Professors Ioana and Cezmi discuss the concepts of endotypes, phenotypes, biomarkers, and precision medicine with relation to allergic diseases,[19] suggesting directions to take in using precision medicine in treating these diseases.

The exhaled nitric oxide fraction (FeNO) is widely used as a biomarker for airway inflammation and as a guide for anti-inflammatory therapy in asthma. However, it has remained unclear whether a persistently high FeNO in patients with controlled asthma is associated with the progression of lung dysfunction. In this issue, Matsunaga *et al.* report the results of a three-year prospective study of the correlation between the changes of FEV1 and FeNO in 140 patients with controlled asthma.[20] They found that the cut-off value of 40.3 ppb for FeNO identified the rapid-decline patients in FEV1 with 43% sensitivity and 86% specificity. This suggests using FeNO with well-controlled asthma patients to identify patients at risk of a progressive loss of lung function.

The 2012 edition of the Japanese Pediatric Guideline for the Treatment and Management of Asthma (JPGL) recommends salmeterol/fluticasone combination therapy (SFC) as a step 3 to 4 treatment to control moderate to severe asthma in children aged 5–15 years.[21] However, the optimal step-down approach following stabilization with SFC remains unclear. Akashi *et al.* report the results of a randomized controlled study of step-down approaches for asthmatic children controlled by SFC.[22] They surveyed 131 subjects who were assigned to the half-dosing group (salmeterol 25 μg/fluticasone 50 μg b.i.d., SFC group) or the fluticasone alone group (fluticasone 100 μg b.i.d., FC group) and followed for 12 weeks. They found that although the FC group showed decreased lung function compared to the SFC group, there was no significant difference in FeNO levels or C-ACT scores between them. These results suggest that, irrespective of the effects on lung functions, both halving the dose of SFC and switching to FP alone are optimal step-down approaches.

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Conflict of interest

The authors have no conflict of interest to declare.

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