Abstract

Session 1　Division of Metabolic Disorders

The effects of radiation exposure on multidrug resistance in a non-small lung cancer cell line.

Keita Utsunomiya(Radiology)

Tc-99m MIBI (MIBI) is a substrate that exhibits the same uptake kinetics as cisplatin and doxorubicin. Multidrug-resistance (MDR) is a mechanism that impedes chemotherapy of non-small cell lung cancer (NSCLC).
We investigated the synergistic effects of chemotherapeutics, MDR modulator GG918 (final concentration: 0.1μM, 0.001μM, and 0μM), and irradiation {0 Gy: C (control)-group; 3, 6, 9, 12 Gy: I (irradiation)-group} in the non-small lung cancer cell line, H1299 exhibiting MDR, on MIBI and doxorubicin ABC transporter kinetics, in vitro and in vivo, respectively.
Inhibition of H1299 cell proliferation by irradiation was found to be irradiation dose dependent. The degree and duration of MDR inhibition in vitro in H1299 was also dose dependent. In the cells of both the C-group and 3 Gy I-group, no significant difference of MIBI accumulation was observed. In the 6 Gy I-group, a higher MIBI accumulation was observed at only 7 days after irradiation relative to the C-group. A higher MIBI accumulation in the 9, 12 Gy I-groups with a significant difference from the C-group was observed at 4 to 14 days after irradiation. A significant negative correlation between intracellular MIBI accumulation and cell replication was found. In combination with low-dose MDR modulator, GG918, a synergistic reduction in MDR transport function was observed 48 hours after irradiation. High accumulation and retention of doxorubicin was observed in irradiated tumors in the H1299 xenograft mice group at 2 to 14 days after 9 Gy irradiation compared to the control mice group.
These results provide evidence for a synergistic effect of concurrent chemotherapy and radiotherapy with MDR modulator. Chemotherapy should potentially be reconsidered to include a complementary radiotherapy regime to improve the chemotherapy efficacy through MDR reduction.

Statins, HMG-CoA reductase inhibitors, have a potential to regulate dendritic cell-mediated allergic responses

Phan Thi Xuan Vien(Hematology, Respiratory Medicine &Reumatology)

Thymic stromal lymphopoietin (TSLP), produced mainly by inflammatory epithelial cells, has a pivotal role for initiating and maintaining allergic inflammation. TSLP stimulates myeloid dendritic cells (TSLP-mDCs) to induce the expression of OX40L that induce and maintain Th2 cells and secretion of Th2 cell-attracting chemokine CCL17. In our previous studies, we have shown that HMG-CoA reductase inhibitors, statins, can inhibit the production of type I interferons (IFNs) from plasmacytoid DCs, thus suggesting that statins can be utilized as therapeutic agents to break off the IFN-mediated pathogenic spiral observed in some autoimmune diseases such as systemic lupus erythematosus. Here we have expanded our study to mDCs, particularly to the TSLP-mDCs-dependent Th2 pathway to examine the immunoregulatory role of statins in the allergic responses.
TSLP induced human mDCs to express OX40L on their surface and CCL17 in the culture supernatant, suggesting the promotion of Th2 cell-mediated responses. We found that addition of statins, pitavastatin and simvastatin, into the DC culture, repressed the upregulation of OX40L expression and CCL17 secretion. Furthermore, these inhibitoty responses by statins were counteracted by the addition of mevalonic acid (MVA), indicating that the inhibitory effects of statins are mediated by the action as HMG-CoA reductase inhibitor through MVA pathway in

TSLP-mDCs. Rho Kinase (ROCK) inhibitor, as well as statins, also repressed the TSLP-induced OX40L and CCL17 expression, suggesting that statins may exert the cholesterol-independent inhibitory effect on TSLP-mDCs through the Rho-ROCK pathway. This study suggests a specific role of statins in controlling the TSLP-mDCs-dependent Th2 pathway and the therapeutic potentials for allergic diseases such as SLE.

Circulating cardiac stem cells for cardiac repair

Masayoshi Iwasaki (Medicine II)

Despite significant advances in diagnosis and therapy, the mortality of patients with heart failure still remains high. Stem cell therapy is therefore one of the promising therapeutic options for chronic heart failure.

Previously we isolated and characterized circulating mesoangioblasts (cMABs) from patients undergoing cardiac surgery. These are a subset of mesenchymal stem cells, express CD73, KDR and Nkx2.5, and are capable of differentiating into endothelial cells, smooth muscle cells and cardiomyocytes in vitro. When transplanted in vivo, these cells enhanced functional recovery following myocardial infarction or hindlimb ischemia in mice (1,2). We also found that hepatocyte growth factor (HGF) induces cMAB mobilization in rats (2).

To establish a feasible method for the isolation of human cMABs, we tested the hypothesis that heparin induces cMAB mobilization by increasing serum HGF concentration in patients undergoing cardiac catheterization. Indeed, heparin increased serum HGF levels and the number of outgrowing cMAB colonies in a dose-dependent manner. Simultaneous blood sampling from aortic sinus, coronary sinus and right atrium revealed that cMABs are derived from the heart.

Our results demonstrate that heparin mobilizes human heart-derived cMABs during cardiac catheterization. We are planning to characterize the localization of cMABs in the heart using CD73-BAC-EGFP transgenic mice.

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Analysis of neutrophil infiltration in type 1 and type2 autoimmune pancreatitis.

Kazushige Uchida (Gastroenterology & Hepatology)

One of the histopathological characteristics of type 2 autoimmune pancreatitis (IDCP) is granulocyte epithelial lesions (GEL). However, infiltration of neutrophils is well-known I type 1 AIP (LPSP). To clarify pathogenesis of type 2 AIP, we investigated mechanism of neutrophil infiltration in type 1 and type 2 AIP.

The number of neutrophils around the interlobular pancreatic ducts was significantly higher in IDCP than in alcoholic chronic pancreatitis (ACP), and LPSP. There was no significant difference in the number of neutrophils around the intralobular pancreatic ducts among ACP, LPSP and IDCP. The expression of neutrophils chemoattractants granulocyte chemotactic protein-2 (GCP-2) in the interlobular pancreatic duct epithelia was significantly higher in IDCP than in ACP and LPSP. There was no significant difference in the expression of IL-8 in the interlobular pancreatic duct epithelia among ACP, LPSP and IDCP.

Our data showed that GCP-2 has an important roll in the neutrophil infiltration around interlobular pancreatic duct in type 2 AIP.

1. Mitsuyama T, **Uchida K**, Sumimoto K, Fukui Y, Ikeura T, Fukui T, Nishio A, Shikata N, Uemura Y, Satoi S, Mizuno N, Notohara K, Shimosegawa T, Zamboni G, Frulloni L, **Okazaki K**. Comparison of neutrophil infiltration between type 1 and type 2 autoimmune pancreatitis. Pancreatology. 2015;15:271-80.

2. **Okazaki K**, **Uchida K**. Autoimmune Pancreatitis: The Past, Present, and Future. Pancreas. 2015;44:1006-16.

Activation of hypoxia-inducible factor 1 confers the metabolic reprograming in the myeloid THP-1 cells

Kiichi Hirota (Anesthesiology)

Hypoxia induces expression of a select set of genes encoding glycolytic enzymes and glucose transporters that generate ATP under specific conditions such as anaerobic circumstances. At the cellular level, the adaptation includes a switch of energy metabolism from oxidative phosphorylation to anaerobic glycolysis, increased glucose uptake and the expression of stress proteins related to cell survival and death. At the molecular level, the adaptation involves changes in gene expression. The transcription factor hypoxia-inducible factor 1 (HIF-1) plays an essential role in the maintenance of O_2 homeostasis. HIF-1 is a heterodimer consisting of HIF-1α and HIF-1β subunits that bind to specific regulatory sequences known as hypoxia response elements. Expression of a line of enzymes involving glucose metabolism and oxidative phosphorylation (OXPHOS) are under regulation of HIF-1. Activation of myeloid cells such as macrophages by pro-inflammatory stimuli causes them to undergo a metabolic switch towards glycolysis and away from OXPHOS because activated myeloid cells are recruited to diverse sites of inflammation, such as wounds, arthritic joints, and necrotic tumors, which all share in common a reduction in O_2 tension below levels present in normal tissues. However, it is only recently that the mechanisms responsible for this metabolic reprogramming have been elucidated in more detail. HIF-1 plays an important role under conditions of both hypoxia and normoxia and interference with this process actually abolishes the function of macrophages.
In this talk, we would like to demonstrate the involvement of HIF-1 in the metabolic reprograming process during the differentiation process of the myeloid THP-1 cells.

Session 2 Division of Neuroscience

Alterations of Mirror Neurons in Patients with Schizophrenia

Yukiko Saito(Neuropsychiatry)

Background and aim
The neural basis of impaired mirror neurons in schizophrenia is not completely understood. The aim of this study is to evaluate the white matter microstructure of the tracts of mirror neurons in patients diagnosed with schizophrenia. A secondary aim is to test for an association between measures of white matter microstructure and Social function Scale. Mirror neurons are known to contribute to creating a sense of intimacy, and there is evidence that dysfunction of mirror neurons may be one of the core deficits of socially isolating disorders such as autism and schizophrenia. To the best of our knowledge, this is the first study to investigate the structural connectivity of the mirror neuron system in first episode schizophrenia.
Methods
Diffusion tensor imaging (DTI) scans were acquired on 16 patients and 10 matched controls. Fractional anisotropy (FA) and Trace were measured. In this study, we evaluate the fiber tracts generated using two-tensor tractography algorithm. This method makes it possible to follow tracts that pass through branching and crossing regions of the brain.
Results
Independent t test revealed a significant effect of group on Trace, with higher values in the patients compared with healthy ($p = .05$). Patients with schizophrenia showed a significant negaitive correlation between Trace in the fiber tract on the left hemisphere and the scores of

Social Fanction Scales (*rho* =-.648, *p* = .007).

Conclusions

These results may have implications for novel therapeutic approaches, and prognosis of impaired social communication in patients with schizophrenia.

Analysis of synaptic plasticity in the basal ganglia network by regional quantitation of mRNA in 6-OHDA-lesioned rats

Mitsuaki Oki(Neurology)

Background: Parkinson's disease (PD) is a neurodegenerative disease presenting progressive motor dysfunction. Its major neuropathological feature is dopaminergic neuronal loss in the substantia nigra pars compacta. Dopaminergic treatment is effective initially, but it becomes less effective and motor complications such as dyskinesia and wearing off appear in the advanced stage of the disease. Not only presynaptic neuronal loss at nigrostriatal dopaminergic synapses, but also postsynaptic dysfunction is considered to cause the decreased effectiveness. Non-dopaminergic neurotransmitters, such as glutamate, endocannabinoids and adenosine have been attracting attention recently to participate in the pathophysiological process of the postsynaptic dysfunction of Parkinson's disease. To evaluate postsynaptic changes of nigrostriatal dopaminergic synapses, we analysed mRNA expressions of non-dopaminergic neurotransmitter receptors in 6-OHDA-lesioned rats and levodopa-induced dyskinesia (LID) rats.

Methods: 6-OHDA was stereotaxically injected into the unilateral medial forebrain bundle of SD rats to make unilateral PD model rats. These rats were subdivided into three groups and treated as follows; 1) no medication, 2) continuous levodopa infusion, and 3) intermittent levodopa injection. Two weeks after the treatment, involuntary movements such as dyskinesia were scored. Frozen brains were coronally sectioned at 1 mm thickness. Approximately 0.7 mg of tissue from putamen was die-cut by 18 guage non-bevel needle. Random hexamer-generated cDNA was applied to real time PCR.

Results: Dopamine D1 and D2 receptor mRNAs were not elevated on the operated side of the putamen in untreated PD model rats. Dopamine D2 receptor mRNA was elevated in continuously levodopa infused rats. Both dopamine D1 and D2 mRNAs were elevated in intermittently levodopa injected rats. Endocannabinoid CB1 receptor mRNA was increased on the operated side of putamen in all rats regardless of treatment. LID was observed only in rats treated with levodopa intermittently. Adenosine A2a receptor (A2A) mRNA was increased on the 6-OHDA-treated side of putamen in these rats.

Discussion: 1) Real time RT-PCR provided regional quantitation of mRNA expression in the basal ganglia of PD model rats. 2) Increased A2A mRNA was associated with LID, but whether it is a cause or a result of LID is not yet determined.

Three-dimensional distribution of sensory stimulation-evoked neuronal activity of spinal dorsal horn neurons analyzed by *in vivo* calcium imaging

Kazuhiko Nishida(Medical Chemistry)

The spinal dorsal horn comprises heterogeneous populations of interneurons and projection neurons, which form neuronal circuits crucial for processing of primary sensory information. Although electrophysiological analyses have uncovered sensory stimulation-evoked neuronal activity of various spinal dorsal horn (SDH) neurons, monitoring these activities from large ensembles of neurons is needed to obtain a comprehensive view of the SDH circuitry.

To analyze the global distribution pattern of SDH neuronal activity in response to sensory stimulation, we established *in vivo* calcium imaging of SDH neurons by using a two-photon microscope. For *in vivo* calcium imaging, FRET-based calcium indicator protein, Yellow Cameleon, which is insensitive to motion artifacts of living animals was introduced specifically

into SDH neurons by *in utero* electroporation. This method enabled us to monitor the activities of more than 200 SDH neurons at a single cell resolution across a wide region localized 1.4 mm along the rostrocaudal axis and 150 □m in depth. Moreover, we extracted three-dimensional neuronal activity maps of these neurons in response to cutaneous sensory stimulation.

Based on these technological backgrounds, we further examined three-dimensional distribution of SDH neuronal activity in response to different sensory stimulation and cutaneous pinch stimulation toward different points. These results provide a clue to understand neuronal processing of sensory information in the spinal dorsal horn.

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Visualization of oligodendrocyte differentiation processes by using imaging mass spectrometry

Yukie Hirahara **(**Anatomy and Cell Science)

Myelin is rich in two glycosphingolipids, galactosylceramide (GalC) and its sulfated form, galactosylceramide I3-sulfate (sulfatide). GalC is 23 wt. % and sulfatide is 4wt. % of the myelin total lipid in the central nervous system. These glycosphongolipids are necessary to consist the multilayered myelin sheath (1) and to differentiate oligodendrocyte (OL) (2). Sommer and Schachner characterized OL specific monoclonal antibodies O1 and O4 that were generated by fusion of mouse myeloma with spleen cells from mice immunized with bovine corpus callosum (3). The following studies showed the antibody specificity of O1 is GalC and of O4 is sulfatide. The O4-positive oligodendroblast was characterized by proliferative capacity but entering terminal differentiation with non-migratory ability. However, the O4 antigen, sulfatide, has not been detected at this stage by biochemical analysis. Therefore, the antigen reacting with the O4 antibody at this stage has been suspected to differ from sulfatide (4). In the present study, we showed by imaging mass spectrometry that sulfatide existed in restricted regions of the spinal cord at chick embryo stage 32, where oligodendroblasts first appear. At this stage, short-chain sulfatide with 16 and 18 carbon fatty acids was predominant. The C18 sulfatide was also detected in embryonic mouse spinal cord at E15.5, and gradually expands from the restricted region to lateral part in madula and pons at E18.5. In addition to C18 sulfatide, C22 sulfatide appear in ventral zone of spinal cord at E19.5, while, C24 sulfatide became dominant in adult spinal cord. Moreover, the sulfatide variant in mouse adult brain were compared with in cerebroside sulfotransferase null mouse brains (5), which were not synthesized sulfatide variants. The major sulfatide species were absent. These results show that the mass imaging signals shows exact sulfatide signal. These data strongly suggest that the sulfatide with short chain fatty acids exist at oligodendroblast stage and the different fatty acid chain of sulfatide species change during OL maturation. Together, these results demonstrate that the sulfatide with short chain fatty acids species regulates the early OL development. We could use sulfatide variants as the target molecule to trace OL differentiation and this tool would help to understand the OL precursor cell maturation and contribute the therapy of neurological diseases including Multiple Sclerosis.

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Seeing is believing
-Novel approach to in-situ analysis using mass spectrometry -

Shuichi Shimma(Advanced Science and Biotechnology,
Graduate School of Engineering, Osaka University)

Molecular imaging technique using mass spectrometry (imaging mass spectrometry: IMS) can provide a number of visualizing targets of biomolecules because of the non-labeled feature. This means ionization on sample tissue surfaces is essential for IMS technique. Recently, several instruments which can perform IMS using different ionization technique are available. Mass Microscope (iMScope, Shimadzu) is the novel IMS instrument which is a combination of microscope and tandem mass spectrometer, which is three-dimensional ion trap and time-of-flight mass spectrometer. The author was one of the members in iMScope R&D group from 2004 to 2007.

Many cutting-edge instruments are commercialized, however, only such expensive instruments are insufficient for successful IMS. There are a lot of experimental steps including sample preparation in IMS. The sample preparation and data interpretation are much more difficult in IMS experiment than instrument operation. Especially, to improve ionization efficiency on the tissue surface is one of the most important points due to non-labeled feature.

Generally matrix-assisted laser desorption ionization (MALDI) is equipped in IMS instrument. Conventionally, matrix solution is applied directly onto the tissue surface by spraying. Using this method, insufficient (inhomogeneous) matrix crystal layer is formed on the tissue surface. This inhomogeneous layer might become an artifact for imaging results. Of course, such insufficient matrix crystals prevent high ionization efficiency as well as high spatial resolution.

To overcome these problems in conventional method, the author developed two-step matrix application method (two-step method)[1)]. Two-step method is a combination of vaccuum sublimation of matrix crystal prior to matrix solution spraying. Provided tiny and homogeneous crystals work as seeds for crystal development in the second step. Two-step method can improve quality of matrix crystal layer on the tissue surface, so that high ionization efficiency and non-artifact imaging are available. Additionally, I performed on-tissue derivatization to improve ionization efficiency for low polarity molecules such as steroid hormons. On-tissue derivatization will become a promising technique to expand application areas in IMS.

In this presentation, the author will present the new sample preparation methodologies and their applications in pharmaceuticals and steroids visualization.

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Session 3　Division of Cancer

Clinical applications of fluorescent imagings in liver surgery

Masaki Kaibori(Surgery)

Background: Indocyanine green (ICG) and the porphyrin precursor 5-aminolevulinic acid (5-ALA) have been approved as fluorescence imaging agents in the clinical setting. The aim of this study was to evaluate the usefulness of fluorescence imaging with both ICG and 5-ALA in the intraoperative identification of latent small liver tumors.

Methods: The subjects were 67 patients who underwent hepatic resection for liver tumors. 5-ALA hydrochloride (1 g) was orally administered to patients 3 hours before surgery. ICG (0.5 mg/kg), which had been intravenously injected within 1 month prior to surgery. Intraoperatively, after visual inspection, manual palpation, and ultrasonography, fluorescence images of the liver surface were obtained with ICG and 5-ALA prior to resection.

Results: With ICG, the sensitivity and specificity for detecting the preoperatively identified main tumors were 94% and 60%, respectively. Twelve latent small tumors were newly detected on the liver surface using ICG, five of which proved to be carcinomas. With 5-ALA, the sensitivity and specificity for detecting the main tumors were 48% and 100%, respectively. Five latent small tumors were newly detected using 5-ALA; all were carcinomas. Overall, five new tumors were detected by both ICG and 5-ALA fluorescence imaging; two were hepatocellular carcinomas and three were metastases of colorectal cancers.

Conclusions: Although the sensitivity and specificity of ICG fluorescence imaging for main tumor detection were relatively high and low, respectively, the opposite was true of 5-ALA imaging. Fluorescence imaging using 5-ALA may provide greater specificity in the detection of surface-invisible malignant liver tumors than using ICG fluorescence imaging alone.

Fluorescence nanoparticles for histological diagnosis

Koji Tsuta(Pathology and Clinical Laboratory)

Various antibody therapies, such as breast cancer targeting the HER2 receptor, can also be used for the treatment of other malignancies. The immunostaining can be influenced by factors such as time duration for each reaction and concentration of antibody dilution. We recently developed fluorescence nanoparticles of phosphor-integrated dot (PID), which show high luminance and high dynamic range compared to conventional fluorescent dyes.

Programmed death-1 (PD-1) is significantly upregulated on activated cancer-specific T cells. The PD-1/PD-L1 signaling pathway plays an important role in escape antitumor immunity. Recent anti-PD-1/PD-L1 drugs showed that blockage of PD-1 and PD-L1 interactions can reverse T-cell exhaustion and restore antigen-specific T-cell responses.

This current study evaluated that the utility of the PID method for PD-L1 protein expression by comparison with the conventional DAB method.

Materials and methods

We chose 5 types of cultured cell lines at various PD-L1 protein expression levels and used the western blot data as the gold standard. We developed each cell block from cultured cell lines and performed PID staining and DAB staining for the PD-L1 antibody. PID signals were counted with an automatic analysis device Pidanalyzer (Konica Minolta). The intensity of the DAB staining was measured with an Aperio system.

Results

Among the three assessment procedures, the PID data correlated well with the western blot data. One of the significant reasons was that the PID signal was countable in the density range of DAB that was difficult to visualize using the naked eye.

Future perspectives

We conducted expression analysis of PD-L1 for 300 cholangiocarcinoma samples using this method and plan to conduct a comparison with the clinical information.

Identification and Characterization of Lineage$^-$CD45$^-$Sca-1$^+$ VSEL Phenotypic Cells Residing in Adult Mouse Bone Tissue

Ryusuke Nakatsuka(Hygiene)

Murine bone marrow (BM)-derived very small embryonic-like stem cells (BM VSELs), defined by a lineage-negative (Lin-), CD45-negative (CD45-), Sca-1-positive (Sca-1+) immunophenotype, were previously reported as postnatal pluripotent stem cells (SCs). We developed a highly efficient method for isolating Lin-CD45-Sca-1+ small cells using enzymatic treatment of murine bone. We designated these cells as bone-derived VSELs (BD VSELs). The incidences of BM VSELs in the BM-derived nucleated cells and that of BD VSELs in bone-derived nucleated cells were 0.002% and 0.15%, respectively. These BD VSELs expressed a variety of hematopoietic stem cell (HSC), mesenchymal stem cell (MSC) and endothelial cell markers. The gene expression profile of the BD VSELs was clearly distinct from those of HSCs, MSCs and ES cells. In the steady state, the BD VSELs proliferated slowly, however, the number of BD VSELs significantly increased in the bone after acute liver injury. Moreover, GFP-mouse derived BD VSELs transplanted via tail vein injection after acute liver injury were detected in the liver parenchyma of recipient mice. Immunohistological analyses suggested that these BD VSELs might transdifferentiate into hepatocytes. The present study demonstrated that the majority of the Lin-CD45-Sca-1+ VSEL phenotypic cells reside in the bone rather than the BM. However, the immunophenotype and the gene expression profile of BD VSELs were clearly different from those of other types of SCs, including BM VSELs, MSCs, HSCs and ES cells. Further studies will therefore be required to elucidate their cellular and/or SC characteristics and the potential relationship between BD VSELs and BM VSELs.

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ATL development in HTLV-1 infected humanized mouse model

Jyunichi Fujisawa (Microbiology)

Development of ATL-like symptoms was successfully recapitulated in HTLV-1 infected humanized mouse model system that was established by intra-bone marrow transplantation of NOG mice with CD133+ hematopoietic stem cells purified from human cord blood[1)]. In addition to the clonal expansion of HTLV-1 infected CD25(+) CD4 T-cell, the induction of anti-HTLV-1 immune responses such as anti-Gag/Env antibodies and the CTL against a viral transforming protein Tax was observed in the HTLV-1 infected humanized mice. By using this system, we examined various candidates for ATL treatment and the vaccine against HTLV-1 infection and leukemogenesis.

Treatment with the combination of anti-viral agents, zidovudine (AZT) and interferon-alpha (IFN), has been reported to be effective to certain type of ATL but the mechanism of action is totally unknown. We, therefore, examined the efficacy and the *in vivo* mechanism of AZT/IFN treatment in the humanized mouse system[2)]. HTLV-1 infected humanized mice were inoculated daily with AZT and IFN from two to four weeks post infection and the number of infected cells and proviral loads (PVL) were analyzed. Treatment with AZT and IFN suppressed the growth of infected T-cells in PBL and the PVL remained low throughout lifetime even after withdrawal of drugs, while mock-treated mice were died of leukemia after 8 weeks post infection. These results suggested that the drug treatment resulted in the cell death of vigorously growing infected-T-cells but not of infected-T-cells with dormant growth, probably depending on the viral expression.

The humanized mouse model was further applied for the examination of the Tax-peptide vaccine to

restrict the HTLV-1 infection and leukemogenesis[3]. Humanized mice were subcutaneously or intranasally inoculated with the Tax-peptide vaccine and then infected with HTLV-1 by the intraperitoneal injection of γ-irradiated HTLV-1 producing Jurkat cells. While the control mice died of leukemia within two months, the leukemic growth of infected lymphocytes in the vaccinated mice were retarded and two out of five mice stayed alive long with low rate of HTLV-1 infection. It was thus concluded that Tax peptide vaccination can elicit protective immunity against HTLV-1 infection and/or ATL developments.
Thus, the HTLV-1 infected humanized mouse model should provide an invaluable tool to understand the mechanism of ATL leukemogenesis and to develop curable drug systems and the preventing vaccine against the onset of leukemia.

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Single molecule imaging of LFA-1/ICAM-1 interaction at immune cell contact site

Naoyuki Kondo(Molecular Genetics, IBS)

Lymphocyte integrin LFA-1 play a critical role on the migration and antigen-dependent stopping of T cells in response to the T cell receptor- and chemokine receptor-mediated signaling. Regulation of LFA-1 by small GTPase Rap1 and its downstream molecules, such as Mst1, Talin and Kindlin-3 play a critical role on the T cell homing and the suppression of autoimmunity.
LFA-1 forms mainly three types of conformations, low-, intermediate- and high-affinity conformer, which correlate with the affinity for its ligand, ICAM-1; however how these conformers are regulated by Rap1-related factors and the precise role of each conformer on T cell function is still elusive. In this study, we setup the single molecule measurement system of LFA-1/ICAM-1 binding event on supported planner lipid bilayer and assessed the distribution and the function of conformer in T cell-antigen presenting cell (APC) contact site. We demonstrate that low/intermediate conformers are dominated in conformers, whereas high-affinity conformers were present as a rare population. Interestingly, decrease of the rare population by gene knock-out of Rap1 or Mst1/2 resulted in drastic reduction of cell adhesion frequency and formation of mature immunological synapse (IS), a layered supramolecular activation clusters (SMAC) formed at T-APC interface.
Simultaneous imaging of active form of Rap1 and single molecule LFA-1/ICAM-1 binding events showed activated Rap1 accumulated at inner areas of peripheral SMAC (pSMAC), in which Mst1 was colocalized. To investigate the relationship between Rap1 signaling and key integrin regulatory molecules, Talin and Kindlin-3 in IS formation, we performed 3D immunofluorescent staining of T cells forming IS. Rap1b or Mst1 KO T cells showed defective recruitment of Kindlin-3, which binds to the β2 subunit to stabilize high-affinity LFA-1 conformation, to the contact plane, while Kindlin-3 in wild-type T cells localized at contact planes, in particular at inner pSMAC areas. These results indicate that Rap1 signaling to Kindlin-3 controls the formation of rare high-affinity LFA-1 conformers at the inner pSMAC, which is required for efficient adhesion to APC and mature IS formation.

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Closing Remarks

Hiroo Ueno

Generation of chimeras, which is now a standard technic for producing gene modified mutant mice, was originally developed as a tool for studying developmental biology. However, application of conventional single marker chimeric mice for studying developmental study had been limited. This situation has been dramatically changed by development of multicolor chimeric mice using various kinds of fluorescent proteins. Now by our technology, up to ten different clones could be distinguished in situ by their colors, which enable us to perform more accurate statistical analyses and lineage tracing experiments than by conventional methods. The method could be applied to visualize not only turnover of normal stem cells but also development of malignant tumors in vivo. As closing remarks, we will briefly introduce our progress of stem cell research in the last three years using our technology.