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SPECIAL LECTURES

SL3 | Roles of prostaglandins in regulation of plasticity of lymphatics and lymph nodes

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Angiogenesis is upregulated by prostaglandin (PG)E_2 and prostaglandin E (EP) receptor signaling pathway during the inflammatory processes and the promotion of tumor growth, however, little is known about their involvement in lymphangiogenesis. When we examined the roles of an inducible cyclooxygenase (COX-2) and endogenous PGE_2 on lymphangiogenesis in chronic proliferative inflammation, lymphangiogenesis detected by the double immunostaining of VEGFR-3 and LYVE-1 was upregulated during the development of granulation tissues, which were formed around the Matrigel plugs with inductions of COX-2 and mPGES-1. Administration of a COX-2 inhibitor, celecoxib significantly reduced lymphatic vessel formation in granulation tissues, whereas topical PGE_2 administration enhanced lymphangiogenesis. Lymphangiogenesis was suppressed in the granulation tissues of mice lacking either EP3 or EP4 in comparison with wild type counter parts (WT), suggesting that these molecules are relevant receptors for PGE_2. An EP3-selective agonist increased the expression of VEGF-C and VEGF-D in cultured macrophages, while an EP4-selective agonist increased VEGF-C expression in cultured macrophages and increased VEGF-D expression in cultured fibroblasts. These findings suggest that EP3 and EP4 signaling contributes to plasticity of lymphatics in a chronic inflammation.

The lymphatic system is an important route for cancer dissemination, and lymph node metastasis (LNM) serves as a critical prognostic determinant in cancer patients. A murine model of Lewis lung carcinoma (LLC) cell metastasis revealed that COX-2 is expressed in dendritic cells (DCs) from the early stage in the lymph node subcapsular regions, and COX-2 inhibition markedly suppressed mediastinal LNM. Stromal cell-derived factor-1 (SDF-1) was elevated in DCs before LLC cell infiltration to the lymph nodes, and a COX-2 inhibitor, an SDF-1 antagonist, and a CXCR4 neutralizing antibody all reduced LNM. Moreover, LNM was reduced in mice lacking the PGE2 receptor EP3, and stimulation of cultured DCs with an EP3 agonist increased SDF-1 production. Compared with WT $CD11c^+$ DCs, injection of EP3-deficient $CD11c^+$ DCs dramatically reduced accumulation of SDF-1^+ $CD11c^+$ DCs in regional LNs and LNM in LLC-injected mice. Accumulation of regulatory T cells and lymph node lymphangiogenesis, which may influence the fate of metastasized tumor cells, was also COX-2/EP3-dependent. These results indicate that COX-2-derived PGE_2 modulate lymph node plasticity to form a premetastatic niche.

SYMPOSIUM

SY1-1 | Cerebral microvascular restructuring and microglial adaptation to chronic hypoxia in the animal models

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Neurons, glias, and vascular cells are tightly coupled to maintain structural and functional integrity in the brains. This feature is called neurovascular unit. In response to cerebral hypoxia, parenchymal capillaries dilate significantly and develop a new vascular network. Astrocytes extend their thin processes to enwrap the vessels, following restructuring of the vessels. These observations suggest that vessel sprouts independently to the astrocytic activation during cerebral hypoxia. Here, the present study determined whether microglia, another type of glial cell, is involved in the hypoxia-induced microvascular restructuring. Using CX3CR1-GFP mice in which the microglia expresses green fluorescent protein (GFP), repeated longitudinal two-photon microscopic imaging was conducted for GFP-expressing microglia and microvessels through a closed cranial window over somato-motor

cortex during 3 weeks of hypoxia exposure. Surgical procedures of the cranial window according to a Tomita-Seylaz method had apparently no detectable effects on morphology of the GFP-positive cells and vasculatures up to 28 days of the surgery. Chronic hypoxia induced changes in the GFP-positive cells' morphology and dilation of the parenchymal capillaries. Localization of the GFP-positive cells around the sprouting capillaries was also observed with an increase in number of the GFP-positive cells. These findings indicate that the CX3CR1-GFP expressing cells are involved in the microvascular responses to cerebral hypoxia. Further works are needed to identify a type of the GFP-positive cells which initiate microvascular sprouting and their functional roles in the cerebrovascular restructuring induced by such as hypoxia and ischemia.

SY1-2 | Roles of microglial-astroglial interaction in the pathophysiology of cerebral ischemia

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Background: Under ischemia, both harmful and beneficial roles of astroglia and microglia have been postulated. These two types of glial cells may communicate each other and control the fate of neuronal cells. Both astroglia and microglia express toll-like receptor 4 (TLR4), which plays a pivotal role in the pathophysiology of stroke-induced inflammation. Reactive oxygen species (ROS), nitric oxide (NO), and inflammatory cytokines produced by TLR4 activation play harmful roles in neuronal damage after stroke. Although astroglia exhibit proinflammatory responses upon TLR4 stimulation by lipopolysaccharide (LPS), they may also play cytoprotective roles via the activation of the pentose-phosphate pathway (PPP), reducing oxidative stress by glutathione peroxidase. We investigated the mechanisms by which astroglia reduce oxidative stress via the activation of PPP, using TLR4 stimulation in concert with microglia.

Methods: *In vitro* experiments were performed using cells prepared from Sprague–Dawley rats. Coexisting microglia (usually less than 10%) in the astroglial culture were chemically eliminated using L-leucine methyl ester (LME). Cells were exposed to LPS (0.01 μg/mL) for 12–15 hours. PPP activity was measured using [1-^{14}C]glucose and [6-^{14}C]glucose. ROS and NO production were measured using fluorescent indicators. The involvement of nuclear factor-erythroid-2-related factor 2 (Nrf2), a cardinal transcriptional factor under stress conditions that regulates G6PDH, the rate-limiting enzyme of PPP, was evaluated using immunohistochemistry and quantitative RT-PCR.

Results: LPS induced ROS and NO production in the astroglial culture containing microglia but not in the microglia-depleted astroglial culture, indicating that major source of ROS/NO was microglia. However, LPS did enhance astroglial ROS production after glutathione depletion, suggesting astroglial potent anti-oxidative roles. Cultured astroglia exposed to LPS elicited 20% increases in PPP flux, and these actions of astroglia appeared to involve Nrf2. Moreover, the chemical depletion of coexisting microglia again eliminated both increases in PPP and astroglial nuclear translocation of Nrf2. U0126, an upstream inhibitor of mitogen-activated protein kinase, eliminated LPS-induced NO production by microglia and LPS-induced PPP activation in astroglial–microglial culture, indicating that microglia-derived NO mediated astroglial PPP activation. S-nitroso-*N*-acetyl-DL-penicillamine, an NO donor, which was confirmed to enhance Nrf2-regulated gene (HO-1) expression by RT-PCR, induced astroglial PPP activation. Elimination of ROS and NO production by mixed astroglial-microglial culture with sulforaphane, a natural Nrf2 activator, confirmed the astroglial protective mechanism.

Conclusions: Astroglia in concert with microglia may play a cytoprotective role for countering oxidative stress in stroke.

SY1-3 | Multiple sclerosis and leukoencephalopathy as primary microgliopathy

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Microglia, one of the main immune cells in central nervous system, is well known for play roles in many kinds of CNS disorders. In the inflammatory site of experimental autoimmune encephalopathy (EAE), which is recognized as multiple sclerosis (MS) model mice, many macrophages differentiated from peripheral monocytes and resident microglia are activated. We found clear differences between resident microglia and infiltrated monocytes in EAE lesion; resident microglia activated before the infiltration of monocytes that indicated contribution of the recruitment of peripheral immune cells. Meanwhile, at the onset phase of EAE, the main source of proinflammatory cytokines was the infiltrated monocytes, Microglia released anti-inflammatory cytokines and contributed to clear myelin debris to facilitate remyelination. These findings indicated regulatory roles of microglia.

Hereditary diffuse leukoencephalopathy with axonal spheroids (HDLS), which is also called as adult onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP), is a neurological condition characterized by adult onset ataxia and dementia. Leukoencephalopathy is one of the main MRI findings in this condition. HDLS is caused by the mutations in the CSF1R gene located on long arm of chromosome 5 (5q32). Colony stimulating factor 1 receptor (CSF1R), also known as macrophage colony-stimulating factor receptor (M-CSFR) and CD115, is a cell-surface protein and acts as the receptor for CSF1 and contributing the differentiation of macrophages. In CNS, microglia express CSF1R and are thought as causable cells. Microglia play rolls in maintenance of CNS milieu, and mutation of CSF1R induce "microgliopathy" which leads to the disease condition.

SY1-4 | The role of microglia in the pathogenesis of Parkinson's disease

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Clinical and experimental researches on Parkinson's disease (PD) pathogenesis has revealed that neuroinflammation associated with glial activation influences the disease progression. Although whether or not PD-onset is triggered by inflammatory mechanisms remains unclear, various experimental evidences mainly using cellular and mice models have suggested that cytokines like IL-1β, TNF-α and IL-6, and secreted α-synuclein from the neurons activate the microglias, resulting in the disease exacerbation. Also, levels of serum/plasma cytokines such as IL-1β, TNF-α, IL-2 and ApoA1 have been reported to be significantly elevated in patients with PD compared to the controls. Also, in our double cohort study with comprehensive Luminex analysis of inflammatory proteins in serum and plasma, we identified several proteins specifically changed in PD compared to the controls. In this presentation, I will report our preliminary results of serum/plasma PD-inflammatory biomarkers summarize the present status of PD pathogenesis associated with neuroinflammation based on the latest reports associated with PD molecular mechanism focusing on the neuroinflammation.

SY1-5 | Critical role of microglia in pathogenesis of Alzheimer's disease – imaging-based study

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Neuroinflammation characterized by activation of microglia is an important pathological event in the progression of Alzheimer's disease (AD) as well as two core pathologies: amyloid-β (Aβ) and tau deposits. Non-invasive imaging with positron emission tomography (PET) radioligands for these pathological changes enables real-time monitoring for them and therefore consistently provides evidence for interactions between pathologies in animal model and diseased human brains. Since the expression of 18-kDa translocator protein (TSPO) is greatly induced in active glial cells, quantitative assessment for TSPO ligands has high value in visualizing neuroinflammation. Aβ and tau deposit-associated activated microglia is well-observed in postmortem AD and mouse model brains. neuroinflammation in AD model mouse with Aβ and tau lesion is captured by TSPO PET imaging spatio-temporally associated with accumulation of Aβ and tau. The intracranial implantation of a microglial cell line with high-level inflammation and TSPO expression accelerate Aβ deposition in AD model mouse brain as compared with that implanted with a low inflammatory level microglial cell line, and monocyte chemotactic protein-1 (MCP-1) might be a critical molecule handling inflammation-induced Aβ deposition based on longitudinal dual imaging for Aβ and TSPO, since intracranial administration with anti-Aβ antibody in combination to anti-MCP-1 antibody showed more efficient decrease in Aβ deposits and only triggered low-level inflammation than sole use of anti-Aβ antibody. Moreover, functional deficiency by TSPO gene knockout (KO) decreased LPS-stimulated MCP-1 release in primary microglial culture. These findings suggest feasibility of control of inflammation by modifying microglial function via targeting TSPO with agonistic or antagonistic ligands.

SY2-1 | Microbiome of multiple sclerosis

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Multiple sclerosis (MS) is an inflammatory disease of the central nervous system. Genetic data as well as the success of various immunological treatments suggest a key role of autoimmune-mediated pathology. The incidence of MS has significantly increased in the last few decades in developed countries, including Japan. It is difficult to explain such a rapid increase by genetic factors or by established environmental risk factors such as vitamin D, smoking or Epstein-Barr virus. Previously unknown environmental factors may contribute to the increased prevalence of MS. Recent developments in the field of gut immunity and commensal microbiota start to provide important clues. Commensal bacteria profoundly shape mammalian immune system and contribute to the mutualistic relationships between bacteria and hosts. Many studies have proved the important role of gut microbiota in experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Our group and others have reported that changing the composition of microbiota by antibiotic treatment affected the disease course of EAE. A single strain, named segmented filamentous bacterium, was shown to be critical to induce pathogenic T cells in mice. In a spontaneous CNS inflammatory mouse model, the disease did not develop under germ-free conditions, but showed signs of disease after commensal microbiota were induced. Our group demonstrated a moderate dysbiosis (imbalance in the intestinal bacteria) in the gut of relapsing-remitting MS patients (PLOS one, 2015). We compared the gut microbiota of 20 Japanese MS patients and of 40 healthy Japanese. Bacterial 16S ribosomal RNA gene analysis of DNA isolated from fecal samples revealed presence of a moderate dysbiosis in the gut microbiota of patients with MS. Moreover, we detected a significant change in the abundance of several taxa, including depletion of species belonging to Clostridia XIVa and IV clusters. Interestingly, other species classified in the same clusters were reported to induce regulatory T cells, suggesting a possible disease-modifying effect of these bacteria. Further studies are needed to evaluate whether these observations play a role in MS pathogenesis.

SY2-2 | Microbiome and kidney disease-kidney-gut axis

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Intestinal microbiota contributes to the pathogenesis of various chronic diseases, including chronic kidney disease (CKD). We

recently examined the role of gut microbiota in the progression of CKD. Renal failure was induced in six-week-old spontaneously hypertensive rats by 5/6 nephrectomy (Nx). We analyzed the gut microbiota population to identify the relevant species potentially involved in inducing renal damage. Nx rats showed an increase in *Bacteroides* (*Bact*) and a decrease in *Lactobacillus* (*Lact*) species compared with sham-operated rats. *Lact*, but not *Bact*, populations were significantly associated with urinary protein excretion. Treatment of Nx rats with 1×10^{10} CFU/kg/day *Lact* ameliorated increased urinary protein excretion and higher serum levels of the uremic toxins, indoxyl sulfate and p-cresyl sulfate, and serum urea nitrogen levels. *Lact* also attenuated systemic inflammation in Nx rats, as evaluated by serum lipopolysaccharide, interleukin-6, and C-reactive protein levels. Histologically, renal sclerosis in Nx rats was restored by *Lact* treatment. The reductions in tight junction proteins and toll-like receptor 2 (TLR2), a putative *Lact* receptor, in the colons of Nx rats were mitigated by *Lact*. Treatment of human colon Caco-2 cells with indole downregulated tight junction protein expression, which was abolished by exposure to *Lact*. The effects of *Lact* were reversed by treatment with OxPAPC, a TLR inhibitor. Similarly, the increase in the permeability of the Caco-2 cell monolayer was reversed by the administration of *Lact*. *Lact* upregulated TLR2 expression in Caco-2 cells. *Lact* also attenuated the increases in serum IS and urea levels and urinary protein excretion in Nx rats even in the pseudogerm-free environment. We concluded that *Lact* supplementation mitigated the systemic inflammation and proteinuria associated with renal failure, suggesting that the gut microbiota *Lact* plays a protective role against the progression of CKD. More recently, the renal protective effects of small chain fatty acid produced by intestinal microbiota were revealed, which further revealed the mechanisms of novel interaction among the organs, kidney-gut axis. The elucidation of this organ interaction will provide the novel therapeutic strategies against the initiation and progression of CKD.

SY2-3 | Microbiome-oriented supplements for the treatment of dry eye

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Recently, the microbiome is believed to be related to general health. Dry eye is the condition where unstable tear film and decreased tears affect the patient's discomfort and visual function. We found that lactobacillus combined with lactoferrin had a positive effect on the recovery of dry eye in the mouse model. Next, we developed a combination of supplements based on the microbiome modification, and produced a microbiome-oriented supplement. We further performed the clinical study which showed its efficacy in the treatment of dry eye. Microbiome modification is one of the effective ways to treat eye diseases, and one example, the treatment of dry eye, will be presented.

SY2-4 | Gut microbiota and cardiovascular diseases

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Atherosclerosis is a chronic inflammatory disease and an intervention to the inflammatory process could be new therapeutic strategies for preventing coronary artery diseases (CAD). We had already shown that oral administration of anti-CD3 antibody or an active form of vitamin D_3 decreased atherosclerosis in mice by inducing Tregs and tolerogenic dendritic cells in the gut-associated lymphoid tissues. These research findings implied that we could prevent atherosclerosis via modulating the intestinal immunity. Under these background, we have been further interested in the gut microbiota, which are reported to be highly associated with the intestinal immunity and systemic metabolism. Recent basic and clinical studies implicated the involvement of gut microbiota in development of metabolic disorders including obesity and type 2 diabetes. We investigated the relationship between the susceptibility to CAD and the gut flora type by analyzing the stool with terminal-restriction fragment length polymorphism (T-RFLP) method. Human gut flora type was shown to divide three types and called "entero types" (*Bacteroides* dominant type I, *Prevotella* dominant type II, and *Ruminococcus* dominant type III) in Nature in 2011. We investigated the entero type and demonstrated CAD patients were predominant in entero type III. We proved that the order *Lactobacillales* was increased and the phylum *Bacteroidetes* (Bacteroides+ Prevotella) was decreased in CAD patients. There was also an association between the percentage of *Lactobacillales* and the severity of CAD. These data imply the possibility that the gut microbiota composition and modulation of them could be used for the diagnostic tool and therapeutic strategies of CAD.

We further examined the effect of gut microbiota on plasma cholesterol metabolism and atherosclerosis using germ free apolipoprotein E-deficient mice. Germ free condition was shown to increase plasma cholesterol levels via mainly modulating a bile acid metabolism, but to decrease atherosclerotic lesion formation compared to specific pathogen free mice.

We would like to review the state of art in this research area and demonstrate the prospects for clinical application.

YOUNG INVESTIGATORS

Y-01 | RICE (Rapid Image Contrast Enhancement): a method enabling quantitative intergroup comparison of local metabolite distributions acquired by MALDI imaging mass spectrometry

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Background: Examination of differences in abundances of specific metabolites among different experimental groups without losing spatial information of organs is important in life sciences. Although matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) excels in visualizing tissue distributions of biomolecules within a single specimen, however, this method cannot be applied for comparing differences between two specimens. Such limitation motivated us to develop a visualization platform, RICE (Rapid Image Contrast Enhancement) enabling "spatio-quantitative" comparisons between multiple samples "at-a-glance".

Results: RICE utilizes ANALYZE7.5 dataset, a widely used format, and maximally exploits the ability of ImageJ. RICE mainly consists of following three steps. At first, RICE discriminate real null-values acquired as non-detected mass peaks against null-values created during the data conversion process to ANALYZ7.5. Next, RICE compile content matrices from all experimental groups into one file. Finally, RICE construct intuitive color-coded content maps by sorting out compiled values into color-coded bins by adapting histogram equalization method. To evaluate RICE, we compared a color-coded content map of ATP constructed by RICE against that by SIMtools, using a pair of datasets from control and ischemic brains of mice. Firstly, RICE clearly delineated tissue boundaries, whereas SIMtools failed to do so. Secondly, RICE unraveled low contents of ATP in the stroke brain, whereas SIMtools failed to demarcate them. Thirdly, RICE, but not SIMtools enabled spatio-quantitative comparison such as thalamic ATP contents between control and ischemic brains.

Conclusions: RICE enhanced the contrast of rendered images. It made it possible to spot spatio-quantitative differences in metabolic profiles between two groups at-a-glance. Since RICE materializes a common format, ANALYZE7.5, as a starting source, it can be applied to IMS dataset acquired by many different mass-spectrometers. RICE has the ability to help researchers to characterize complex metabolic interactions between distinct regions of tissues such as damaged vessels and normal vessels. The strategy will provide means for elucidating molecular mechanisms for disease progress.

Y-04 | Rivaroxaban reduced the sizes of fibrin emboli in the intracranial arteries in mice even after embolic stroke occurred

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Objective: Direct oral anticoagulants (DOACs) have been shown to be effective in the prevention of ischemic stroke in patients with non-valvular atrial fibrillation by inhibiting intra-cardiac thrombus formation. Some reports also suggest the thrombolytic effects of DOACs on intra-cardiac thrombi. Therefore, it is possible that DOACs help in recanalization of the occluded arteries, leading to good outcomes. We evaluated the effects of rivaroxaban on intracranial fibrin thrombus formation in a mouse embolic stroke model.

Methods: We created a fibrin thrombus *ex vivo* and labeled it with rhodamine dextran. Then, we injected the labeled thrombus through the common carotid artery into male C57BL/6 mice and observed the embolization process in the intracranial arteries on the brain surface through a cranial window by fluorescence microscopy. We compared mice treated with rivaroxaban (n = 7) with those not treated with rivaroxaban (n = 9) as controls. Feeding of the mice of laboratory chow mixed or not mixed with rivaroxaban was started a week before the thrombus injection, and the mice had free access to food until just before the thrombus injection. The cranial window was made the day before the injection. We measured the sizes of the emboli immediately and 3 hours after the thrombus injection in the same animal, and calculated the rate of reduction of the embolus sizes in each group. Furthermore, we measured the serum levels of the coagulation and thrombolysis markers in each group using other mice.

Results: Two mice that died within 3 hours of the thrombus injection were excluded from the study. The Rivaroxaban group (n = 6) exhibited a higher rate of reduction of the embolus sizes than the control group (n = 8) (p = 0.0196, Mann Whitney's U test). There were no differences in the serum levels of the coagulation markers such as TAT, D-dimer, protein C/S or plasmin between the two groups.

Conclusion: Our study suggest that treatment with rivaroxaban even after embolic stroke has occurred will be effective for reducing the sizes of fibrin emboli in the intracranial arteries in patients with embolic stroke.

FREE COMMUNICATIONS

F-01 | Nicotine ameliorates colonic inflammation via inhibition of leukocyte interaction to colonic microvessels and down-regulation of MAdCAM-1

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Introduction and Aim: Adhesion molecules play important roles in inflammatory bowel disease (IBD), and modulation of gut-specific adhesion molecules like α4β7-integrin is now commercially available. Epidemically, nicotine absorbed by smoking is reported to work protectively in certain cases of ulcerative colitis. However, its mechanism of action was poorly understood. We investigated the effect of nicotine on dextran sulfate sodium (DSS)-induced murine colitis in terms of microvascular leukocyte trafficking and adhesion molecules. We examined particularly focusing on the following parameters; (1) leukocyte adhesion to colonic microvascular endothelium *in vivo*, (2)

expression of α4β7-integrin on lymphocytes, and (3) expression of adhesion molecules on vascular endothelium.

Method: C57BL/6J mice were treated with 3% DSS in drinking water for 7 days. Some mice were treated with 0.1 mg/mL nicotine in drinking water simultaneously. Disease activities of colitis were assessed by DAI score, which consists of body weight loss, stool consistency and bleeding. Mice were injected FITC-labeled splenocytes obtained from control mice via cervical vein. The number of splenocytes adhering to colonic submucosal vasculature was counted with an intravital fluorescence microscope. The expression of α4β7-integrin on splenocytes derived from DSS treated mice with or without nicotine treatment was analyzed with a flow cytometer. The expression of adhesion molecules in the colonic mucosa was assessed by immunohistochemistry by using the labeled streptavidin biotin method.

Result: The DAI score was increased by DSS treatment, and the increase was significantly attenuated by additional nicotine treatment. In intravital microscopic study, the number of splenocytes adhering to vascular endothelium was increased by DSS treatment, and the increase was significantly attenuated by additional nicotine treatment. On the other hand, expression of α4β7-integrin on splenocytes was not affected by nicotine.

Conclusion: Colonic damage induced by DSS and enhanced leukocyte interaction to colonic microvessels in the inflamed colonic mucosa was significantly ameliorated by nicotine treatment.

F-02 | Orally administered redox nanoparticle (RNP^{O}) attenuates intestinal ischemia-reperfusion injury in mice

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Objective: Intestinal ischemia-reperfusion (I-R) injury is an abdominal emergency leading to multiple organ failure with high mortality. However, the treatment for intestinal I-R injury remains to be established. Recently, the therapeutic effect of orally administered RNP^{O} was reported for intestinal injury. RNP^{O} is composed of hydrophilic PEG (polyethylene glycol) segment for shell and central hydrophobic poly (styrene) segment possessing reactive oxygen species (ROS) scavenging potential of nitroxide radicals. To this end, we investigated whether RNP^{O} could attenuate intestinal I-R injury.

Methods: Before 1 hour of intestinal ischemia, C57BL/6J mice were orally administered saline, RNP^{O} and nRNP (nanoparticle without ROS scavenger), respectively. Mice small intestines were challenged with ischemia by occluding superior mesenteric artery for 45 minutes. Inflammatory responses in the small intestine were assessed 4 hours following reperfusion by measuring luminal hemoglobin, tissue levels of TNF-alpha, IFN-gamma and KC mRNA expression (RT-PCR), polymorphonuclear neutrophil tissue accumulation (MPO assay), histology (hematoxylin & eosin staining), lipid peroxidation (MDA assay). Additionally, fecal microbiota was assessed (T-RFLP profiling) after I-R with consecutive 7 days administration of RNP^{O}.

Results: Induction of I-R caused significant increase in luminal inflammatory markers such as luminal hemoglobin, tissue levels of TNF-alpha, IFN-gamma and KC mRNA expression, and subsequent PMN accumulation in the small intestine. The above changes were significantly attenuated by the treatment with RNP^{O} but not by nRNP. Treatment with RNP^{O} resulted in down-regulation of I-R induced increase in lipid peroxidation as well as modulation of fecal microbiota.

Conclusion: These findings indicate that orally administrated RNP^{O} attenuates interesting I-R injury in mice via decrease in oxidative stress-related inflammation and leukocyte recruitments, suggesting that RNP^{O} may be a possible therapeutics for intestinal I-R injury.

F-05 | Deterioration of slit diaphragm in an early diabetic rat

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Because of Westernization of life style of Japanese people, the prevalence of metabolic syndrome has been increasing. One of the contributing factors of metabolic syndrome is diabetes. Diabetes causes three major complications, retinopathy, neuropathy and nephropathy. Diabetic nephropathy is a leading cause of renal failure, which includes a breakdown of glomerular filtration barrier. The barrier consists of mainly fenestrated endothelial cells, glomerular basement membrane and podocytic foot processes. The narrow gaps between the neighboring foot processes are bridged by the glomerular slit diaphragm, another pore size barrier. Previously, we reported that there are hyperfiltration and a leakage of large molecules of the size of albumin even from the early stage of diabetes. We also found that there is a progressive deterioration of slit membranes along the duration of being diabetic, demonstrating gradual decrease of slit membrane component proteins, podocin, nephrin and CD2AP. Thus, the leakage of large molecules is closely connected to the structural changes of the glomerular filtration barrier. The purpose of this study was to examine whether or not the deterioration of slit membrane structure is attributed to gene alteration. We used Wistar rats. Type I Diabetes (2 months model) was induced by administration of streptozotocin (5omg/kg) from the tail vein. Samples of renal cortex where the glomeruli were harvested from control (n = 3) and diabetic rats (n = 3). After homogenization, we performed gene analysis by RT-PCR on podocin, nephrin and CD2AP. There was not any difference in gene expression of these genes between the control rats and diabetic rats. We then examined protein production of podocin by Western blotting. There was a decrease of podocin production in diabetic rats. In conclusion, slit membrane

deterioration in diabetic rats is due to decreased production of slit membrane component protein but not due to gene alteration. It is considered that decrease of slit membrane component protein production caused the leakage of proteins at the early stage of diabetes.

F-06 | Cyclooxygenase-2/microsomal prostaglandin E synthase-1 axis induces blood flow recovery by accumulating regulatory T cell

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It was reported that cyclooxygenase 2 (COX-2) derived Prostaglandin E2 (PGE_2) induce angiogenesis, especially in tumor angiogenesis. But the precise of mechanism of COX-2 derived PGE2 on ischemic recovery is not well understood. We evaluated this phenomenon by using model of acute hind limb ischemia of microsomal prostaglandin E synthase-1 deficient mice (mPGES-1KO) and wild type mice (WT). In order to confirm whether COX-2 involved in ischemic recovery, aspirin and COX-2 inhibitor were administrated after surgical treatment. Compared to vehicle treated mice, mice treated with aspirin and treated with selective COX-2 inhibitor mice were significantly suppressed blood flow recovery ($p < 0.05$). The mRNA expression of COX-2, mPGES-1 and FOXP3, specific transcription factor for Treg, in the ischemic muscle was enhanced in vehicle treated mice but not in aspirin and Cox-2 inhibitor treated mice. Compared to WT, blood flow recovery was significantly suppressed in mPGES-1KO. Furthermore, real time PCR analysis and immunohistochemical analysis showed that the expression of FOXP3+ cells in the ischemic muscle was suppressed in mPGES-1KO compared to WT. These results suggested that COX2/mPGES-1 axis induced blood flow recovery from ischemia by accumulating Treg.

F-07 | Development of antibody-drug conjugate against refractory cancer by utilizing molecular imaging

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In oncology, development of the next generation therapeutic antibodies such as antibody-drug conjugate (ADC), radioimmunotherapy (RIT) or bispecific antibody have made rapid progress during the past decade. Among them, ADC has been already approved for the treatment of relapsed lymphoma or metastatic breast cancer. However, it has not been proved in treating refractory cancer such as brain cancer glioblastoma multiforme (GBM) or pancreatic cancer at this time. These types of cancer possess abundant stroma that hinders the distribution of the antibodies. In the brain, blood brain barrier (BBB) also prevents most molecules including antibody from entering it through blood vessels. Thus, controlled drug delivery has become increasingly important to overcome such drawbacks. Molecular imaging using fluorescent or radiolabeled antibodies provides the information of pharmacokinetics, biodistribution and tumor uptake of them. Accordingly, we can select the appropriate one to deliver the drug into the tumor. In addition, efficacy of ADC is also depending on the action of released drug in the targeted site. Therefore, it is necessary to evaluate it in distinguishing between free and conjugated drug. Our new drug imaging approach by using microscopic mass spectrometry allows the observation of the free drug and its distribution distinctly. We will present our recent progresses of ADC research against the refractory cancer by utilizing the molecular imaging technologies.

F-08 | The laser speckle flowgraphy applied to the facial skin blood flow for evaluating its physiological functions

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Objective: In order to especially maintenance and promote Japanese female's skin care of face with aging, we have tried to develop further non-contact and real-time Laser Speckle Flowgraphy (LSFG) by Fujii et al.(2002), as being able to measure accurately skin blood flow and to compare it visually between individuals.

Methods: The stability of skin blood flow measurements was examined between two distances (25 and 50 cm) and angle (from −45 to +45°). Facial skin blood flow was compared among those subjects using the thermal diffusion method (TDM) and strain-gauge plethysmography (SPG), which is an established blood flow measurement technique.

Results: The MBR from the LSFG showed a significant strong negative correlation between those two distances ($r^2 = 0.998$, $p < 0.001$) and between those angular distances ($r^2 = 0.928$, $p < 0.001$). The slope of temperature from the TDM showed a significant negative correlation with the MBR ($r^2 = 0.791$, $p < 0.001$). Furthermore the SPG showed a strong positive correlation with the MBR ($r^2 = 0.980$, $p < 0.001$). Age-related changes in the MBR demonstrated a significant negative correlation ($r^2 = 0.254$, $p < 0.001$) to the blowout time (BOT), which represents the MBR as the ratio between the half width and the time of one heartbeat.

Conclusions: It was important to evaluate the effect of distance and angle on MBR, because the LSFG system was able to use continuous and non-contact in real time. It was demonstrated that independence effects of two dimensional experiment on the MBR were measured

accurately and that the results of MBR with aging is confirmed by previous studied. It is suggested that the LSFG applied to face is a useful device for evaluating physiological properties of face skin on the basis of the MBR. We are considered to be applicable not only to the clinical but also to human health care fields.

F-09 | Role of endogenous H_2O_2 during reactive hyperemia in dogs *in vivo* by an infrared fluorescence microscope study

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We have newly developed an infrared white fluorescence microscope with a CMOS high vision camera by attaching an object lens to laparoscope. We have demonstrated that endothelium-derived hydrogen peroxide (H_2O_2) is an EDHF in dogs. In 7 open-chest anesthetized dogs, subepicardial arterioles (SA) were visualized with the needle probe of microscope. We evaluated the relation between SA diameters obtained by infrared fluorescent images with indocyanin green (ICG) and non-fluorescent images. We examined an important regulatory mechanism of endogenous H_2O_2 as an endothelium-derived hyperpolarizing factor (EDHF) during reactive hyperemia (RH) *in vivo*. SA (<100 μm) were continuously observed by an infrared fluorescence microscope with ICG during RH (reperfusion after 20 seconds of left anterior descending artery, LAD occlusion) in the presence of ibuprofen (cyclooxygenase blockade) under the following two conditions (n = 5 each): control and apamin+charybdotoxin (K_{Ca} channel blocker). An excellent correlation (r = 0.9, p < 0.01) was found between the two methods (fluorescence and non-fluorescence) for measuring diameters. Fluorescent diameters slightly larger than non-fluorescent diameters (p < 0.05) in SA. It is clear to evaluate the margin of the SA in infrared fluorescent images compared with non-fluorescent images. Vasodilatation from baseline during RH under control conditions were significantly increased and were significantly decreased by apamin+charybdotoxin (both p < 0.05). Endogenous H_2O_2 plays an important role in RH-induced canine coronary vasodilation *in vivo*. An infrared fluorescence microscope is useful for *in vivo* observation of EDHF/H_2O_2.

F-10 | Activation of microglia after vascular endothelial injury observed with confocal microscopy

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Objective: Microglia is involved in the maintenance of the parenchymal environment and may also contribute to the maintenance of blood-brain barrier (BBB) when the blood vessel endothelial cells (ECs) are damaged together with macrophage which transmigrates from the blood to the parenchyma. In this study, we aimed to elucidate the role of brain microglia and blood monocyte in the protection and repair of BBB by their long-term observation with confocal microscopy.

Methods: Experiments were performed in adult male B6·129P-Cx3cr1 mice encoded with enhanced green fluorescent protein in the locus of the chemokine CX3CL1 receptor CX3CR1. The chemokine receptor CX3CR1 is specifically expressed in microglia in the brain. ECs in a 350 μm-long segment of cortical branch of the middle cerebral artery (MCA) were damaged by a photochemical reaction of intravenously injected rose bengal and laser beam illuminated through the cranial window. Morphological changes of the microglia were repeatedly observed in the identical region with a laser-scanning confocal microscopy before, 1 and 7 days after the endothelial damage.

Results: Microglia showed ramified structure in resting state. Photochemical damage of BBB caused reduction of the microglial projection and enlargement of the cell body. Further damage induced amoeboid structure. Microglia in the resting state were evenly distributed in the cerebral cortex before photochemical injury. However, 1 day after injury, increased number of activated microglia were seen around the lesioned vessel. In addition, some mouse showed migration of macrophage from blood to the brain parenchyma. Finally, 7 days after ischemia, restoration process of the cell body and relocation of microglia were observed.

Conclusion: Activation of microglia and their restoration was observed. Transmigration of macrophage might contribute to the repair process of microglial system.

F-11 | Dynamic flow velocity mapping based on transit time measurements of fluorescent dye

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The present study aimed to map microcirculatory flow velocity using conventional video-rate fluorescence microscopy. The idea proposed is to calculate a propagation speed of fluorescent dye intravenously injected and captured with a microscope thorough the organ microvascular networks. To test a feasibility of the idea, a parallel plate flow path model was used by applying a pre-calibrated constant flow. Flow velocity was calculated by measuring a mean time of the dye propagation over a certain distance in each pixel basis, and a propagation distance was automatically calculated within the vessel region using a custom written Matlab software. The image was captured using a

EM-CCD camera with a fluorescence microscope, and a small amount of fluorescent Rhodamine 6G was injected to the flow path. We observed a good linear correlation for the measured mean and maximum flow velocities with the actual velocities tested (R = 0.94 and R = 0.99, respectively). Then, animal experiments were conducted using the isoflurane-anesthetized rats and mice. To stain blood plasma, a small amount of fluorescent dye was intravenously injected, while the flowing dye in the cerebral microcirculation was visualized through a closed cranial window with confocal laser scanning fluorescence microscopy. The average flow velocity measured was 4.4 ± 1.2 and 2.4 ± 0.5 mm/s in the pial artery and vein of the rats, respectively, and 4.9 ± 1.4 and 2.0 ± 0.9 mm/s in those of the mice, respectively. These results were in good agreement with the flow velocities measured using a cross-correlation method. In conclusion, flow velocity in the microcirculation can be mapped based on transit time measurements of intravenously-injected fluorescent dye with video-rate fluorescence microscopy.

F-12 | *In vivo* imaging and quantification of glucose transfer using 2-NBDG in the mouse cortex with two-photon microscopy

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Fluorescently-labeled glucose derivatives have been used for understanding glucose transfer in *in vivo* brains. However, the current method is limited to qualitative assessment, which is a major drawback compared to the gold standard method with radioactive tracers. The purpose of this study is to establish a quantitative method to characterize regional glucose transfer rate in the cerebral microcirculation using a fluorescent D-glucose derivative 2-NBDG. Tie2-GFP mice in which the vascular endothelium expressed green fluorescent protein (GFP) were used in the experiments, and a closed cranial window (3 mm in diameter) was preliminary made over the left somatomotor cortex. 2-NBDG dissolved in saline (2 mM) was intravenously administered for 5 minutes at a rate of 0.04 mL/min under either awake or 1% isoflurane-anesthetized conditions, while time-lapse imaging was performed every 2–60 seconds in the cortex up to a depth of 0.3 mm with two-photon microscopy. The mean pixel intensity measured in respective vascular (i.e., an input) and tissue regions was converted to a concentration of the 2-NBDG based on a pre-determined calibration curve. Then, a rate constant of blood to tissue transfer and net influx rate were calculated with a graph analysis method. We observed that about 1.5-fold increase in the rate constant of 2-NBDG transfer under awake relative to the anesthetized conditions, and the net influx was 2.2 and 1.8 fold higher in the parenchyma (depths of 50–300 μm) than those of the cortical surface (depths of 0–20 μm) under awake and anesthetized conditions, respectively. These results showed that the method presented successfully characterize the rate of glucose transfer in *in vivo* brain microcirculation.

F-13 | Heterogeneous response of microcirculation to spreading depolarization evoked during middle cerebral artery occlusion in anesthetized mice

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Spreading depolarization (SD), which is a self-propagating wave of mass depolarization and breakdown of transmembrane ion gradients in neurons and astrocytes, sometimes occurs in patients with ischemic or hemorrhagic stroke and trauma. SD accompanying hemodynamic response and metabolic dysfunction may be pathologically harmful during ischemic lesion. In this study, we evaluated cerebral blood flow (CBF) changes during passage of SD at locations with various levels of ischemia following by transient occlusion of middle cerebral artery (MCA) for 45 minutes with a monofilament that had been passed under the artery through a burr hole in the temporal bone in isoflurane anesthetized C57BL/6J mice (male, n = 18). CBF was continuously recorded over the ipsilateral parietal bone with a laser speckle flowgraphy, and the spatial response was evaluated with ImagePro software. SD was determined by measuring DC potential at the caudal area of the temporo-parietal bone. Upon MCA occlusion, CBF decreased by −60.5 ± 5.2% in the core region and recovered by approximately 20% per 1 mm away from the core region. At 1–30 minutes after occlusion, SD was spontaneously or mechanically occurred and concentrically propagated from the core region in 83% of mice. CBF was markedly decreased (−27.1 ± 13.9% from pre-SD level) in the core region. Decrease (−30.3 ± 8.5%) and slight increase (+18.3 ± 14.9%) was seen in the intermediate ischemic zone (1.0 mm away from the core region). Transient decrease (−23.1 ± 10.1%) and increase (+12.0 ± 11.0%) followed by long-lasting oligemia (−29.7 ± 12.4%) were seen in the non-affected zone (2.5 mm from the core). SD spontaneously re-occurred and propagated around the ischemic area in 39% of mice. Marked decrease, transient decrease and increase, and marked increase of CBF were observed in the core, intermediate ischemic and non-affected zones, respectively. CBF recovered after releasing the occlusion in 56% of mice. Thus, we could detect CBF changes associated with SD and propagation of SD wave under ischemia. This model should be useful for investigation of blood flow changes during transient ischemia.

F-14 | Acceleration of red blood cells in intraparenchymal capillaries and dilation of arterial diameter in response to hypercapnia were diminished after passage of cortical spreading depression in anesthetized mice

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Hypercapnia induces vasodilation and increases cerebral blood flow (CBF). Cortical spreading depression (CSD) includes mass depolarization of neuron and astrocytes, accompanying dramatic changes in CBF and metabolism. We have previously reported vasoreactivity of penetrating arteries synchronized with CSD-induced CBF response and abolition of vasodilatory response to hypercapnia after CSD. To further understand the microcirculatory response, we conducted two-dimensional spatial analysis of changes of red blood cell (RBC) velocity in parenchymal capillaries before and after passage of CSD. In urethane-anesthetized, artificially ventilated Tie 2-GFP mice, which have fluorescence in endothelial cells, intraparenchymal images were obtained using a high-speed camera laser-scanning confocal fluorescence microscope at 125 frames/s for 30 seconds through cranial window, simultaneously recording of CBF with laser Doppler flowmeter (24 trials in 19 mice). The velocity of FITC-labeled RBCs in each capillary was automatically evaluated with our original Matlab domain software (KEIO-IS2) before and during inhalation of 5% CO_2. Changes of velocity vs. pre-hypercapnia level in individual capillaries were evaluated (10 ± 4 capillaries per mouse). Arterial diameter was evaluated by averaging the same images. Hypercapnia elicited significant increases of CBF (26 ± 14%), RBC velocity (27 ± 33%) and arterial dilation (13 ± 14%). The change of RBC velocity and vasodilation were significantly correlated with CBF increase (r = 0.450 and 0.536) and were negatively correlated with the respective pre-hypercapnia levels (r = −0.494 and −0.577). KCl-induced CSD elicited an increase of RBC velocity with a transient drop accompanied with arterial vasoreaction, and repeated CSDs also increased RBC velocity and vasodilation. After passage of several CSD waves, the changes of CBF (11 ± 8%), RBC velocity (7 ± 19%) and arterial diameter (3 ± 6%) to CO_2 inhalation were significantly diminished and the above correlations were almost abolished. These results suggest that CSD may impair the circulatory responsibility to hypercapnia. This simultaneous evaluation of RBC flow in multiple capillaries along with arterial diameter and CBF might suggests that microcirculatory responses are closely interlinked.

Ethics are the responsibility of the authors and their administering institutions.