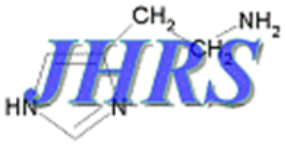

第 20 回日本ヒスタミン学会

The 20th Annual Meeting of Japanese Histamine Research Society

November 24-25, 2016
Kurashiki, Japan

ABSTRACT BOOK

Greetings *written by the chairperson of the annual meeting*

JHRS 20th Anniversary Lecture

written by the president of JHRS

Abstracts *submitted by presenters*

Young Investigator Session

Oral Presentations

NEWSLETTER

Mini Reviews *submitted by Wada Prize Winners*

Next Meeting *announced by the next chairperson*

Greetings

20th Annual Meeting of the Japanese Histamine Research Society

Tamotsu Harada, Chief Organizer
Department of Otolaryngology, Kawasaki Medical School

I am pleased to announce that the 20th Annual Meeting of the Japanese Histamine Research Society will be held at the Kurashiki City Art Museum on November 24 and 25, 2016.

Kurashiki City, located 15 minutes from Okayama Station, is a showcase of culture and magnificent historical buildings. The city's most famous area is the Bikan Historical Quarter, which is home to the Ohara Museum (est. 1930), Japan's oldest museum of Western art with permanent exhibitions. Since coming under the direct control of the central government in the mid-Edo period, the Quarter has been well preserved for about 300 years. The Quarter is only a 5-minute walk from the meeting venue, so I am sure all attendees will enjoy many opportunities for both learning and sightseeing.

I first became interested in histamine when I was working at Osaka University, where I learned about the compound's significance from graduate students supervised by Professor Hiroshi Wada and Dr. Hiroyuki Fukui at the Department of Otorhinolaryngology. Since being appointed to my current position, I have rarely had the chance to conduct basic research on histamine, but I have become more aware of its significance through my clinical research on pollinosis in particular. The study of histamine spans basic to clinical research and involves a wide range of sites in the body, such as the respiratory, digestive, immune, and central nervous systems. The recent development and use of genetically modified mice has provided new insights into the receptors, production, and metabolism of histamine, and consequently, histamine research has entered a new era. Yet, clinical research has not caught up with the rapid advances of basic research.

Against this background, the theme of this year's meeting is "Rapid progress in histamine research, basic and clinical". Our hope is to promote collaboration between basic and clinical researchers and to spur further progress.

To consider the prospects of histamine research from a multidisciplinary viewpoint, the Society has invited Dr. Shigeo Koyasu (Laboratory for Immune Cell Systems, RIKEN) as a special speaker. Dr. Koyasu discovered Th2 cytokine-producing natural helper cells and proposed their strong involvement in the pathology of allergies. We also plan to hold a special lecture on what clinical researchers are focusing on regarding the role of histamine in allergic rhinitis. In addition, Professor Hiroyuki Fukui, the president of the Society, will give a special 20th anniversary lecture. In keeping with the Society's tradition, abundant time will be allocated to presentations by general members and presentations by candidates for the Wada awards. The Society hopes that our 20th meeting to be a special occasion that provides rich opportunities for communication and socializing.

All organizing members are working hard, but what we can do by ourselves is limited. We welcome your suggestions and support to make the 20th meeting a success.

I sincerely hope that we can see all of you at the meeting.

March 8, 2016

JHRS 20th Anniversary Lecture

ヒスタミン研究と歴史と今後の展望

President of JHRS　福井裕行　Hiroyuki Fukui

徳島大学大学院医歯薬学研究部分子難病学分野
Molecular Studies for Incurable Diseases, Institute of Biomedical Sciences,
Tokushima University Graduate School

ヒスタミンの主要薬理作用は、アレルギー作用、末梢血管透過性亢進作用、気管支や腸管平滑筋の収縮作用、末梢血管拡張作用、胃液分泌促進作用などである。これらの作用のうち、胃液分泌促進作用は、ヒスタミン H_2 受容体を介するが、それ以外はヒスタミン H_1 受容体を介する。ヒスタミン H_1 受容体を介する作用がアレルギー疾患に関与し、ヒスタミン H_2 受容体を介する作用が消化性潰瘍に関与することから、多くのヒスタミン研究は病気との関連で進められた。そして、抗ヒスタミン薬、及び、ヒスタミン H_2 受容体拮抗薬の開発に至った。

薬理学の主要生理活性物質のアセチルコリン、カテコラミン、及び、セロトニンの研究においても、多くの治療薬が受容体標的薬から開発されており、ヒスタミン研究の歴史と似かよった歴史が見える。これは、治療薬開発の歴史と本筋と強く関連すると考えられる。

そこで、古い時代の治療薬開発を考えたい。麻黄の主成分であるエフェドリンは気管支喘息野治療薬である。薬理学の教科書には中間型交感神経刺激薬であると説明されている。エフェドリンが同定されたのは 1885 年である。この時代のエフェドリンに関連する学問分野は有機化学であり、薬理学的研究はもっと後の時代である。結論的に、エフェドリンの気管支喘息治療薬開発には、効くか効かないかのレベルで行われたと考えられる。但し、ヒスタミンに関しては古い時代の治療薬開発には関連しなかった。

治療薬開発において、古い時代から新しい時代に移る時期は、受容体標的薬などの明確な標的分子を持つ治療薬が開発される時期である。そして、β アドレナリン受容体拮抗薬、ヒスタミン H_2 受容体拮抗薬の開発に対してノーベル賞が授与された。本当は、抗ヒスタミン薬が明確な標的分子を持つ最初の治療薬であると言えると思うが、受容体標的薬が治療薬としての概念が足りないために、真の新しい時代の治療薬と言い切れないと考えられる。

1950 年ころから現在まで、新しい時代の治療薬、即ち、受容体、イオンチャンネル、及び、酵素などを標的とする治療薬開発が行われ、全ての治療薬の約３分の１を占めるようになった。その中で、薬理学の主要研究対象であるアセチルコリンと生体アミンの受容体標的薬は治療薬開発の中で重要な位置を占めている。ヒスタミン受容体については、新しい受容体サブタイプとして、ヒスタミン H_3 受容体、及び、ヒスタミン H_4 受容体が同定され、これらの受容体標的薬から新規治療薬開発に向けた研究が進められている。

ヒスタミン神経の同定とその神経分布が、渡邉教授と故和田教授の研究、及び、パヌラ教授の研究により明らかにされた。そして、ヒスタミン研究の新しい分野として、中枢ヒスタミン神経系機能の研究が進められている。中枢神経系において、ヒスタミン H_1 受容体、H_2 受容体、H_3 受容体サブタイプが発現している。ヒスタミン H_1 受容体、H_2 受容体のヒスタミンに対する親和性は、H_3 受容体の親和性と比較して約 1,000 倍低い。ヒスタミンの濃度の違いに対応した異なる作用機構の存在が示唆される。現在、ヒスタミン H_3 受容体拮抗薬のピトリサントがナルコレプシー治療薬として開発されている。最近、不随意の運動性、音声性チックを主症状とする Tourette 症候群におけるヒスチジン脱炭酸酵素の遺伝子変異が発見され、ヒスタミン機能の関与が注目されている。中枢ヒスタミン神経系の関与する精神神経疾患の病理機構の解明が進展し、病理機構に作用点を持つヒスタミン受容体標的薬による新規治療薬の開発を期待したい。

末梢におけるヒスタミンの主要機能は、アレルギー反応、及び、胃酸分泌のメディエーターである。上述のように、これらの機能は疾患の症状に関与するために、主に疾患との関連で研究された。逆に、アレルギー反応に関与する免疫機能の研究が不十分であったのではないかと考えられる。ヒスタミン H_4 受容体の同定以後、免疫機構におけるヒスタミンの機能解明が期待される。

ここで論を創薬に戻したい。全ての治療薬の約３分の１を占めるに至った受容体標的薬ではあるが、新規治療薬の開発が難しくなってきた。現在は、受容体標的薬から治療薬を開発する方法以外の治療薬開発の方法が求められている時代であると考えられる。私は、創薬のための新しい戦略が求められる時代ではないかと考えている。個々の研究者の独創性が求められるところである。

最後に私なりのヒスタミン研究に対する思いを述べさせていただきたい。近年のライフサイエンスの進歩は、疾患の病理機構解明の研究法が進歩し、その結果、治療薬開発のための作用点を明確にできるようになったと考えられる。ヒスタミンの基礎研究を軽視しているわけではない。基礎研究から創薬に至る研究法を意識すべきと考える。アセチルコリン、及び、生体アミンの研究と同様に、これまでのヒスタミン研究は疾患の治療戦略に役立ってきた。今後も新しい治療戦略に役立つ独創的な研究をすべきだと考える。

ABSTRACTS

Young Investigator Session

Y-1 The role of histamine H3 receptor in neuropathic pain

Takuro Matsuzawa[1], Maria Mogilevskaya[12], Takeo Yoshikawa[1], Tadaho Nakamura[13], Kazuhiko Yanai[1]

[1]Department of Pharmacology, Tohoku University Graduate School of Medicine

[2]Maastrict University School for Mental Health and Neuroscience

[3]Department of Pharmacology, Tohoku Medical and Pharmaceutical University Faculty of Medicine

【Abstract】

Neuropathic pain is a chronic condition following nerve damage and degeneration induced by infection, diabetes, autoimmune diseases and cancers. One of the typical symptoms is allodynia. Allodynia is pain resulting from a stimulus which would not normally provoke pain. Recent research points towards prominent role of histamine H3 receptor in the pathology of neuropathic pain. However, it was not revealed where does hiatamine function to alleviate pain in the central nervous system (CNS). This study aims to investigate the mechanisms and site of action of H3R antagonists on allodynia alleviation in the animal model (SSL) of neuropathic pain.

Histamine concentration was elevated after the local injection of JNJ into posterolateral nucleus of thalamus (VPL), periaqueductal gray (PAG). The local administration of JNJ into VPL, PAG and spinal cord attenuated neuropathic pain. These results indicate CNS histamine regulates pain sensation via histamine receptors in VPL, PAG and spinal cord.

Y-2 Effect of the purinergic signaling on FcεRI-induced degranulation of human basophils and skin mast cells

Matsuo Yoshimi, Yuhki Yanase, Tomoko Kawaguchi, Kaori Ishii, Michihiro Hide
Department of Dermatology, Graduate School of Biomedical and Health Sciences, Hiroshima University

【Abstract】

Purinergic signaling is a form of extracellular signaling mediated by purine nucleotides and nucleosides such as adenosine and extracellular adenosine 5'-triphosphate (ATP). ATP is released from activated or damaged cells. Extracellular ATP is rapidly converted to adenosine. Mast cells and basophils express IgE receptor (FcεRI). When FcεRIs are crosslinked by antigens, mast cells and basophils release several chemical mediators such as histamine. However, the effect of extracellular ATP and adenosine on degranulation of basophils and skin mast cells remained unclear. In this study, we investigated the potential role of ATP and adenosine in FcεRI-mediated degranulation of basophils and skin mast cells. We found that adenosine suppressed the degranulation in human skin mast cell, while ATP activated antigen-induced human basophils degranulation. These results suggest that the purinergic signals play important roles for regulation of mast cells and basophils activation.

Y-3 Role of histamine as a progression factor of septic major end-organ failure: study using H1 - / H2 - receptor double knockout mice

Mizuki Hattori[1,2], Wakana Ohashi[1], Toshio Fujimori[2], Mituaki Yamazaki[2], Yuichi Hattori[1]
[1]Dept. Mol. Med. Pharmacol., [2]Dept. Anesthesiol., Grad. Sch. Med. Pharm. Sci., Toyama Univ.

【Abstract】

Histamine is recognized as an important mediator in diseases related to inflammatory immune responses. However, it's contribution to the pathology and symptoms of sepsis has not been fully understood. Previously, we reported histidine carboxylase knockout mice attenuated septic multi-organ failure. In this study, H1 - / H2 receptor double knockout (H1/H2R-DKO) mice were prepared and investigated the susceptibility to sepsis. Sepsis was rendered by cecal ligation and puncture (CLP) in H1/H2R-DKO mice and its littermate (background: C57BL / 6J). Significant elevation of blood cytokines (IL-1β, IL-6, MCP-1) by CLP was alleviated in H1/H2R-DKO mice. Cytokine mRNA levels in lung, liver and kidney also decreased. Histological damage of major organs by CLP was also attenuated. ALT, BUN and creatinine levels in the blood were also decreased. In sepsis, it was suggested that histamine contributes as an exacerbating factor to the progression of major organ failure due to this syndrome via H1 and H2 receptors.

Y-4 Development of a technique for real-time monitoring of vascular permeability *in vitro*

Yuhki Yanase, Reiko Irifuku, Tomoko Kawaguchi, Kazue Uchida, Michihiro Hide
Hiroshima University

【Abstract】

Impedance sensor detects sensor surface impedance, which reflects area of adhesion and morphology of the cells on the electrodes as cell Index (CI). This technique can sensitively detect changes associated with disruption of tight and adherent junctions without any labeling in full agreement with the permeability assay with Boyden chamber. In this study, we performed impedance-based analysis of HUVEC for real-time evaluation of vascular permeability *in vitro*. For impedance analysis, HUVEC was seeded on E-Plates. The E-plate was then set onto iCELLigence and CI was measured. CI decreased in response to histamine and the decrease of CI was blocked by olopatadine, H_1 receptor antagonist, but not famotidine, H_2 receptor antagonist. Moreover, CI decreased in response to FXa and FIIa, but not to FVIIa. The decreases of CI were blocked by SCH79797, a PAR-1 antagonist. These results suggest that the impedance-based analysis of HUVEC would be a useful tool for real time evaluation of vascular permeability *in vitro*.

Y-5 Functional histamine H1 receptor synthesized by a wheat germ cell-free protein synthesis

Yasuyuki Suzuki, Kazutaka Maeyama
Department of Pharmacology, Ehime University Graduate School of Medicine

【Abstract】

Histamine H1 receptor (HRH1) belongs to the G-protein-coupled-receptor superfamily. Wheat germ cell-free protein synthesis (WGCFS) could produce a massive amount of proteins in vitro. Previously, we synthesized the HRH1 (1.59 mg in one synthesis reaction) by WGCFS and HRH1 was inserted into liposome directly during protein translation. However, 0.02 % of synthesized HRH1 had a ligand binding ability by this method. Therefore, we hypothesized that detergent-mediated reconstitution could improve synthetic efficiency of functional HRH1. We solubilized HRH1 by 20 mM Triton X-100 and reconstituted HRH1 proteoliposomes by removing detergents with Bio Beads SM-2. After reconstitution, a sucrose density gradient centrifugation showed a shift of the HRH1 proteoliposome band toward higher density. We demonstrated that reconstituted HRH1 proteoliposome had a ligand binding ability by [^{3}H] mepyramine binding assay. However, the reconstitution efficiency was low and we need further investigation about reconstituting method.

Y-6 Development of Impedance based living cell analysis for clinical diagnosis of type I allergy

Reiko Irifuku, Yuhki Yanase, Tomoko Kawaguchi, Kaori Ishii, Takaaki Hiragun, Michihiro Hide

Department of Dermatology, Graduate School of Biomedical and Health Science, Hiroshima University

【Abstract】

In conventional diagnostic tests for type I allergy, there are problems, such as low reliability or risk of anaphylactic shock. To overcome these problems, we developed a new allergy diagnosis using the impedance sensor (iCELLigence). It detects impedance change derived from cell reactions on the electrodes as the change of Cell Index (CI). When IgE-sensitized RBL-2H3 cells on the electrodes were stimulated with various concentrations of antigen, dose dependent CI increases were detected by iCELLigence. We next tested the effect of various kinds of activators and inhibitors. We confirmed the impedance sensor showed morphological changes rather than degranulation. Moreover, we investigated the reaction of RBL-48 cells sensitized with sera of sweat allergy patients. We obtained a large CI change when the cells were stimulated with the sweat antigen. This system makes up for conventional tests' defects, and can be a useful tool for high reliability and high throughput diagnostic test of type I allergy.

Y-7 Molecular mechanism of suppression of NFAT signaling by pyrogallol

Tomohiro Nakano[1], Hiroyuki Mizuguchi[1], Tomohira Ito[1], Noriko Kitamura[2], Osamu Kaminuma[2, 3], Masayuki Uchida[4], Hiromichi Fujino[1], Hiroyuki Fukui[5]

Department of [1]Molecular Pharmacology, and [5]Molecular Studies for Incurable Diseases, Institute of Biomedical Sciences, Tokushima University Graduate School; [2] Allergy and Immunology Project, Tokyo Metropolitan Institute of Medical Science; [3]Center for Life Science Research University of Yamanashi; [4]Food Science Research Laboratories, Division of Research and Development, Meiji Co., Ltd.

【Abstract】

Histamine H_1 receptor (H1R) gene is an allergic diseases sensitive gene and suppression of H1R signaling alleviates the nasal symptoms. NFAT signaling is also responsible for the pathogenesis of the nasal symptoms and suppression of both signaling markedly alleviates nasal symptoms. We found that extract from Awa-tea suppressed NFAT signaling and pyrogallol was identified as an anti-allergic compound in Awa-tea. Treatment with pyrogallol in combination with epinastine also showed significant alleviation of nasal symptoms. However, the mechanism underlying its anti-allergic activity remains unknown. Here, we investigated the molecular mechanism of the suppression of NFAT signaling by pyrogallol. Pyrogallol suppressed NFAT signaling-dependent IL-9 gene expression in RBL-2H3 cells, although it did not inhibit calcineurin protein phosphatase activity. Pyrogallol also suppressed ionomycin-induced dephosphorylation and nuclear translocation of NFAT through the strengthening of calcineurin-NFAT interaction.

Y-8 Suppression of allergic diseases sensitive gene expressions by Sho-seiryu-to

Takako Esu[1], Hiroyuki Mizuguchi[1], Shiho Naniwa[1], Yuki Konishi[1], Yoshiaki Kitamura[2], Noriaki Takeda[2], Hiromichi Fujino[1], Hiroyuki Fukui[3]
Department of [1]Molecular Pharmacology, [2]Otolaryngology, and [3]Molecular Studies for Incurable Diseases, Institute of Biomedical Sciences, Tokushima University Graduate School

【Abstract】
We have demonstrated that histamine H_1 receptor (H1R) gene and IL-33 gene are involved in the pathogenesis of acute and chronic inflammations, respectively. As the expression of both genes are PKCδ-dependent, it is suggested that compounds targeted for PKCδ could alleviate symptoms of not only acute inflammations but also chronic inflammations. Here, we studied the effect of sho-seiryu-to (SST) on H1R and IL-33 gene up-regulation. Extracts from 7 out of 8 constituent plants of SST were dose-dependently suppressed up-regulation of both H1R and IL-33 gene expressions. The IC_{50} values of each plants for H1R gene suppression is highly correlated with that for IL-33 gene expression. These results suggest that active compounds target for common signal proteins. SST suppressed the up-regulation of both H1R and IL-33 gene expression and could be a good therapeutics for both acute and chronic inflammations. Suppression of SST on IL-33 gene expression also suggests that SST could be useful for eosinophilic inflammations.

Y-9 Role of eosinophils in murine experimental allergic rhinitis

Taro Saika[1], Yukiyoshi Hyo[1], Masakazu Hamamoto[1], Ayano Yahagi[2], Katsuhiko Ishihara[2], Tamotsu Harada[1]
Department of [1]Otorhinolaryngology and [2]immunology and Molecular Genetics, Kawasaki Medical School

【Abstract】Eosinophil is a key player in allergic disease. Recently, eosinophils have become recognized as a regulator of acquired immunity. Although eosinophils involve in allergic rhinitis (AR), the roles for eosinophils are not clear. To determine the pathophysiological functions of eosinophils in experimental AR with ovalbumin (OVA), we used eosinophil-deficient mice (Δdbl GATA). Serum OVA specific IgE production was higher in Δdbl GATA than wild type. The frequency of sneezing was significantly lower in Δdbl GATA than in wild type. There were no differences in the number of goblet cells between wild type and Δdbl GATA. Lack of eosinophils enhanced the antigen specific IgE production. Suitably, eosinophils have an important role for the late phase. But induction of goblet cell hyperplasia doesn’t associate with eosinophils in allergic rhinitis.

Oral Presentations

O-2 Compound 48/80, a Mast Cell Stimulator, Enhances Synthesis of IgE and IgG Induced by Intranasal Application of Ovalbumin in Mice

Nobuaki Matsui [1], Daisuke Ito [1], Yukari Takabatake [1], Shingo Tada [1], Masato Kanagawa [1], Nobuyuki Fukuishi [2], Masaaki Akagi [1]

[1] Department of Pharmacology, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, 180 Nishihama bouji, Yamashiro-cho, Tokushima 770-8514, Japan; [2] College of Pharmacy, Kinjo Gakuin University, 2-1723 Ohmori, Moriyama-ku, Nagoya 463-8521, Japan.

【Abstract】

Recent studies have suggested that activated mast cells enhance local immunoglobulin E (IgE) synthesis in the nasal mucosa of allergic rhinitis patients. Here, we examined the effect of compound 48/80 (c 48/80), a mast cell activator, on IgE and immunoglobulin G (IgG) synthesis. Female Balb/c mice were intranasally administered a mixture of ovalbumin (OVA) (1-10 μg/nose) and c 48/80 (1-100 μg/nose) on days 0, 7, 14 and 21 and on consecutive days from day 28 to day 42. Intranasal administration of c 48/80 with OVA increased serum OVA-specific IgE and IgG. Double staining with OVA and IgE- or IgG-specific antibody demonstrated the presence of OVA-specific IgE- or IgG-producing cells in the nasal mucosa of sensitized mice. Moreover, intranasal administration of c 48/80 with OVA increased the nasal mucosal interleukin (IL)-4 level and enhanced the OVA-induced symptom of sneezing. These results suggested that simultaneous activation of mast cells with antigen exposure enhances local IgE and IgG synthesis.

O-3 Effects of dexamethasone on IgE-independent activation of mast cells

Satoshi Tanaka[1], Keiko Yamada[1], Hitomi Sato[1], Mayumi Kamada[2], Yasushi Okuno[2], Nobuyuki Fukuishi[3], Kazuyuki Furuta[1]

[1]Department of Immunobiology, Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University, [2]Department of Clinical System Onco-Informatics, Graduate School of Medicine, Kyoto University, [3]Department of Pharmacology, College of Pharmacy, Kinjo Gakuin University

【Abstract】

It remains largely unknown how steroidal anti-inflammatory drugs affect the characteristics of cutaneous mast cells, in particular IgE-independent degranulation. We investigated the effects of dexamethasone on the process of maturation using murine bone marrow-derived cultured mast cells, which were co-cultured with fibroblasts in the presence of stem cell factor. Treatments with dexamethasone significantly suppressed the proliferation of the co-cultured mast cells and IgE-independent degranulation induced by compound 48/80 or substance P, whereas it drastically augmented the histamine storage. It had no effects on degranulation upon IgE-dependent antigen stimulation. Dexamethasone inhibited the induction of $G_{\alpha i1}$ expression during the co-culture period. Daily cutaneous application of dexamethasone for 6 days in mice augmented the amount of cutaneous histamine but suppressed degranulation induced by compound 48/80 and IgE-dependent antigen stimulation.

NEWSLETTER

Mini Reviews

Next Meeting

The role of histamine in promoting organ dysfunction in sepsis

Mizuki Hattori
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Sepsis is a highly fetal medical condition in patients who have various infections in intensive care units (ICU). In February 2016, a new definition of sepsis (Sepsis-3) was established by the special committee of the Society of Critical Care Medicine [1]. Thus, the definition of sepsis has been changed from the conventional "Systemic inflammatory response syndrome (SIRS) by infection" to "life-threatening organ dysfunction caused by a dysregulated host response to infection" (Figure.1). The in-hospital mortality rate when meeting its new criteria was assessed as exceeding 10% at present [2]. Since multiple organ failure poses a definite threat to the survival of patients, it's urgent to find out the pathophysiological mechanism for the development of organ dysfunction in sepsis.

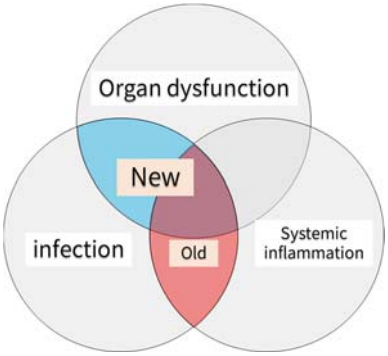

Figure.1 Schema of old and new definition of sepsis

A new definition of sepsis is "life-threatening organ dysfunction caused by a dysregulated host response to infection". Compared to the old definition of "systemic inflammatory response syndrome (SIRS) by infection ", organ dysfunction is emphasized.

Histamine is widely recognized as a major chemical mediator of various disorders of inflammation and immune responses. However, the role of histamine in the development of multiple organ injury or failure in sepsis is not well understood. Therefore, we have been focused on the histamine and its related molecules as an aggravating factor of sepsis.

Previous study reported that histamine concentration elevated in the plasma of patients in association with severity of sepsis [3]. In our previous studies, we demonstrated the highly plasma and tissue histamine levels associated with the prolonged increase in histidine decarboxylase (HDC) gene expression in the animals rendered septic by lipopolysaccharide (LPS) and cecal-ligation and puncture(CLP) [4–7] (Figure. 2).

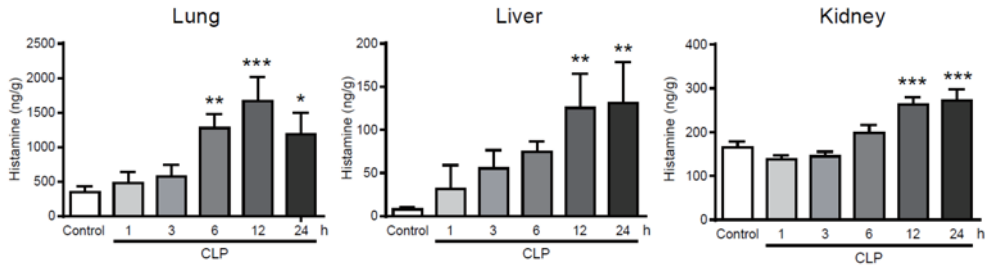

Figure. 2 Changes in histamine synthesis in lung, liver, and kidney tissues from mice after CLP-induced sepsis. All values are provided as means ± SEM. (n = 8/group), *P< 0.05, **P < 0.01, and ***P < 0.001 vs. control. (Hattori, et al. Intensive Care Med Exp 2016)

In our recent study [7], we assessed the response to sepsis caused by CLP in histamine decarboxylase knockout (HDC-/-) mice and histamine H1-/H2- receptor double knockout (H1R-/-/H2R-/-) mice. Knockout mice of histamine-related genes showed lower levels of serum aminotransferase activity, serum creatinine, and serum and tissue pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, and MCP-1) than WT mice when the animals were rendered septic by CLP. Histopathological examinations showed significantly reduced acute lung, liver, and kidney injury after CLP in HDC-/- and H1R-/-/H2R-/- mice. The histamine-mediated development of major end-organ injury was associated with an increase in the nuclear factor-kB signaling pathway (Figure 3).

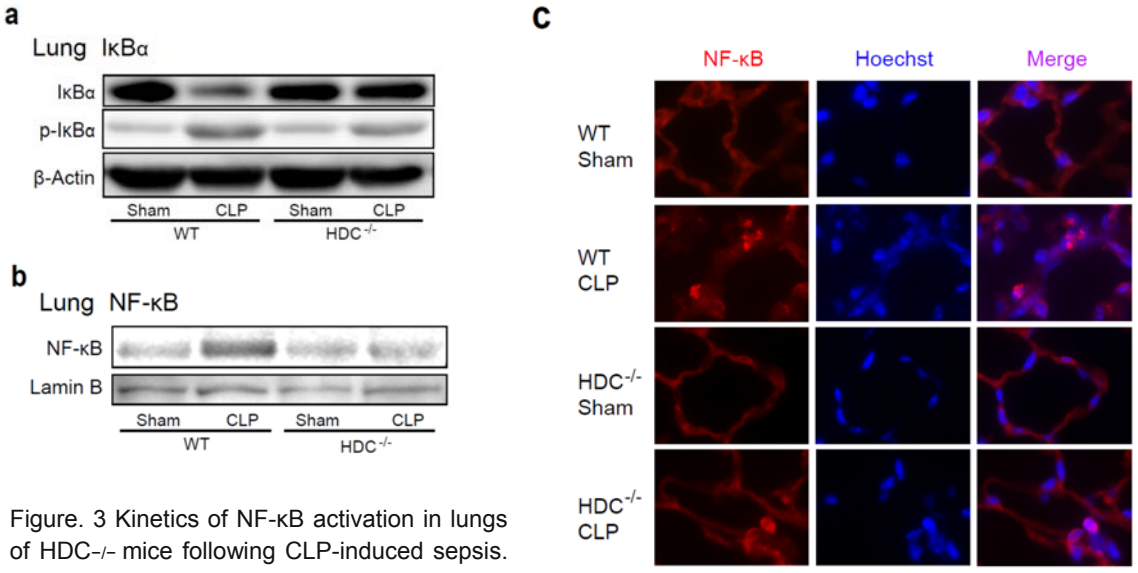

Figure. 3 Kinetics of NF-κB activation in lungs of HDC-/- mice following CLP-induced sepsis. Lung tissues were harvested from sham-operated and CLP-induced septic mice 18 h after surgery. **a** Western blot analysis using anti-IκBα antibody and anti-phospho-IκBα antibody. β-Actin served as loading control. **b** Nuclear proteins were extracted, and then NF-κB p65 was detected by Western blot analysis. Lamin B served as a nuclear marker. **c** Immunofluorescent images for NF-κB p65 (red) in lung sections. Nuclei were counterstained with Hoechst 33342 dye (blue). Original magnification, x400.
(Hattori, et al. Intensive Care Med Exp 2016).

These results suggest that endogenous histamine acting on H1- and H2- receptors is identified as an aggravating mediator to contribute to the development of major end-organ injury in sepsis. Our data may also offer the validity and feasibility of the use of histamine receptor antagonists to septic organ injury.

Acknowledgement
I wish to thank Prof. Yuichi Hattori and Prof. Mitsuaki Yamazaki for giving me the opportunity to conduct this study.

[1] Mervyn S, Clifford S, et al. JAMA (2016); 315(8): 801-810.
[2] Shankar M, Gary S, et al. JAMA (2016); 315(8): 775-787.
[3] Neugebauer E, Lorenz W, et al, Crit Care Med (1996); 24: 1670–1677.
[4] Matsuda N, Hattori Y, et al. Naunyn-Schmiedeberg's Arch Pharmacol (2002); 366: 513–521.
[5] Matsuda N, Hattori Y, et al. J Physiol Lung Cell Mol Physiol (2004); 287: L1248–L1255.
[6] Matsuda N, Hattori Y, et al. J Pharmacol Exp Ther (2010); 332: 730–737.
[7] Hattori M, Hattori Y, et al. Intensive Care Medicine Experimental (2016); 4: 36.

A large scale synthesis of functional G-protein-coupled-receptors using wheat germ cell free protein synthesis system and its reconstitution in liposome.

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Human histamine H1 receptor (HRH1) belongs to G-protein-coupled-receptors (GPCRs) superfamily. GPCRs are membrane proteins distributing on various cell surface and known as major drug targets. However synthesizing GPCRs in vitro were usually a challenging task, because membrane proteins synthesized in a hydrophilic environment aggregate very easily.

Recently, some cell-free protein synthesis system could produce a massive amount of membrane protein. These systems were made from various cell extracts, for example E.coli, rabbit reticulocyte, insect and wheat germ. Prokaryotic cell extract system, such as E.coli, provides the highest protein yields because this translating speed is high. However, synthesized proteins in E.coli system was often misfolded. On the other hands, the eukaryotic system, such as rabbit reticulocyte, insect and wheat germ, produced appropriate transcription speed and prevented from misfoldings. Especially, wheat germ cell free system (WGCFS) can provide the highest yield in eukaryotic cell free system and is a useful method to synthesize human proteins.

Nevertheless WGCFS provide a large scale protein synthesis, membrane proteins such as GPCRs synthesized in hydrophilic reaction mixture easily aggregate. Ando et.al reported that adding liposome which acted as a hydrophobic environment into synthesis reaction mixture prevented membrane protein from aggregations and inserted membrane protein into liposome membrane [1]. Arimitsu et.al showed that synthesized dopamine D1 receptor by WGCFS combined with asolectin liposome had a ligand binding ability [2]. We had synthesized HRH1 by this method, however synthesized HRH1 did not have a ligand binding ability. After sucrose density gradient centrifugation, as well as empty asolectin liposome, synthesized HRH1 proteoliposomes were distributed at lower density fraction. Therefore, we hypothesized that more high efficiency of GPCR insertion into membrane was needed to improve ligand binding ability of synthesized HRH1 proteoliposome.

We tried to insert GPCRs into the lipid membrane by detergent-mediated reconstitution method. Synthesized HRH1 by WGCFS was solubilized by 20 mM Triton X-100 and reconstituted HRH1 proteoliposomes by removing detergents with Bio Beads SM-2. After reconstitution, a sucrose density gradient centrifugation showed a shift of the HRH1 proteoliposome band toward higher density fraction (fig.1). We demonstrated that reconstituted HRH1 proteoliposome had a ligand binding ability by [^{3}H] mepyramine binding assay (fig.2). Further investigation about producing various functional GPCRs by our synthesis and reconstitution methods will be very important for future drug discovery, especially creating a new antibody drug or an aptamer drug to orphan GPCRs.

We acknowledge Tomio Ogasawara, Hiroyuki Takeda, Tatsuya Sawasaki and Yaeta Endo at the division of Cell-free Sciences, Proteo-Science Center, Ehime University for providing valuable technical suggestions on the synthesis of GPCRs by WGCFS.

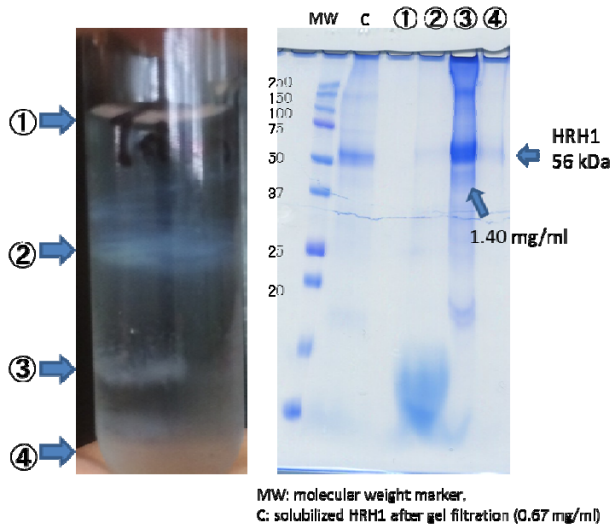

Figure 1
Sucrose density gradient centrifugation and SDS-PAGE after reconstitution HRH1 proteoliposome

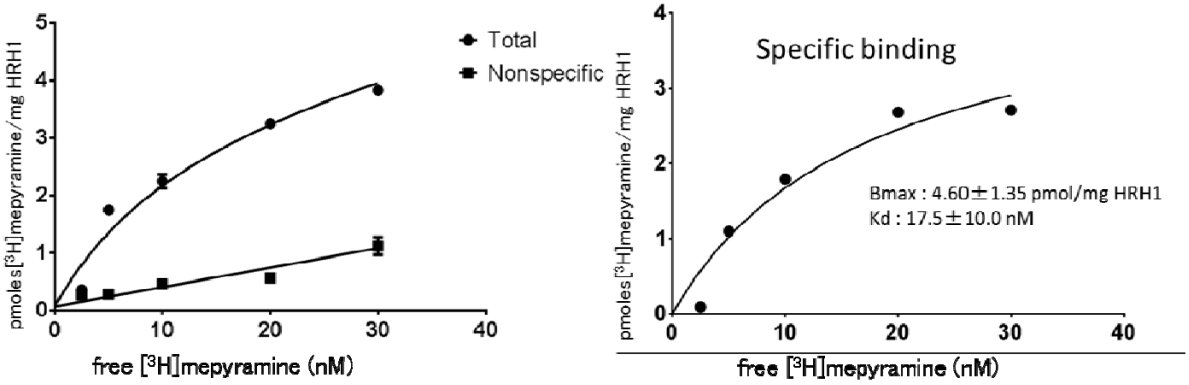

Figure 2
[³H] mepyramine binding assay to reconstituted HRH1 proteoliposome

References

[1] Ando, M., et al. "Liposome chaperon in cell-free membrane protein synthesis: one-step preparation of KcsA-integrated liposomes and electrophysiological analysis by the planar bilayer method." *Biomaterials science* 4.2 (2016): 258-264.

[2] Arimitsu, Eiji, et al. "The ligand binding ability of dopamine D 1 receptors synthesized using a wheat germ cell-free protein synthesis system with liposomes." *European journal of pharmacology* 745 (2014): 117-122.

次大会に向けて

第 21 回日本ヒスタミン学会

代表幹事　櫻井栄一
（徳島文理大学薬学部　教授）

現在、「ヒスタミン」に関する研究は世界的にますます盛んになっており、国際的レベルでの学会・会議が頻繁に開催されております。なかでも、日本における研究は世界をリードしております。その情報発信源である日本ヒスタミン学会も今回で 21 回を迎え、「ヒスタミン研究の新たなる出発のとき」をテーマに阿波徳島で開催いたします。

ヒスタミンの生合成、トランスポーター、代謝、生理機能、薬理作用、創薬、さらに花粉症などアレルギーを中心とした臨床研究など、共通性のある分野の研究者が、基礎と臨床を両輪に、自由で闊達な意見交換を行います。幅広い、多数の演題を募集致します。

特別講演では、深水昭吉先生（筑波大学　生命環境系／生命科学学際領域研究センター）と原田　保先生（川崎医科大学　耳鼻咽喉科学）をお招きし、ご講演を賜ります。演題等は後日掲載させていただきます。

多くの方々のご参加をお待ち申し上げております。

日時：2017 年 12 月 21 日（木）　13 時～17 時 30 分、
　　22 日（金）　9 時～13 時（予定）

会場：徳島文理大学国際会議場（21 号館 2 階）
　〒770-8514　徳島市山城町西浜傍示１８０（徳島キャンパス）
　TEL：088-602-8466

懇親会：12 月 21 日（木）18 時より、祥雲閣（学会場から徒歩 3 分）にて行います。

ホームページ：http://www.jhrs.umin.jp/jhrs21st/index.html

1st Announcement of 21st Annual Meeting of Japanese Histamine Research Society (JHRS 2017)

It is our great pleasure to announce that "21st Annual Meeting of Japanese Histamine Research Society (JHRS 2017)" will be held in Tokushima, Japan, from December 21 to 22 in 2017.

The main theme of this meeting is "Taking a new perspective on histamine research endeavour". We will be a very nice opportunity for all participants to exchange of ideas between basic and clinical research finding and discuss the way to develop new histamine-related drugs in allergy/inflammation and central nerve system.

In this meeting, two distinguished researches: Dr. Akiyoshi Fukamizu (Life Science Center of Tsukuba Advanced Research Alliance) and Dr. Tamotsu Harada (Kawasaki Medical School, Otorhinolaryngology) will be invited for the special lectures.

We hope you will join us in attending this scientific meeting and share the progress histamine research.

Sincerely,

Eiichi Sakurai, Ph.D.
Organizer, JHRS 2017
Prof., Faculty of Pharmaceutical Sciences, Tokushima Bunri University

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