恒常性機能解析部門　Division of Analysis of Homeostasis

客員教授　永井　博弐　Visiting Professor　Hiroichi Nagai (Ph.D)

◇研究目的

近年の免疫学の進歩は目覚しく、生体には自然免疫と獲得免疫が存在し、それぞれ相関しながら生体防御に役立っている。しかし、この免疫機構の中で、種々の原因により生理的バランスが崩れると免疫疾患と呼ばれる一連の疾患が発症する。

当研究室では、免疫疾患のうち、特にアレルギーと自己免疫疾患に焦点を当て、治療薬の基礎的検索と発症の分子的機構の解明を試みる。

◇研究概要

Ⅰ）アレルギーおよび自己免疫疾患発症機序の細胞および分子レベルでの研究

研究室で開発した臨床症状を反映した実験モデルを用い、遺伝子工学的手法、分子生物学的手法および薬理学的手法を駆使して、発症に必要な分子および細胞を検索する。

Ⅱ）アレルギーおよび自己免疫疾患治療薬検索の基礎的検討

前述の実験モデルを用い、天然物あるいは合成化合物の中から有効物質を検索し、医薬品としての可能性を検討する。

◇原著論文

1) **Kawakami Y., Inagaki N., Salek-Ardakani S., Kitaura J., Tanaka H., Nagao K., Kawakami Y., Xiao W., Nagai H., Croft M., and Kawakami T. :Regulation of denderitic cell maturation and function by Bruton's tyrosine kinase via IL-10 and Stat3. Proc Natl Acad Sci USA., 103(1): 153-158, 2006.**

Abstract: Btk plays crucial roles in the differentiation and activation of B and myeloid cells. Despite drastic reductions of other Ig isotypes, paradoxically high IgE responses have been known in btk mutant mice. Here we show that btk(-/-) dendritic cells exhibit a more mature phenotype and a stronger in vitro and in vivo T cell-stimulatory ability than wild-type cells. Increased IgE responses were induced by adoptive transfer of btk(-/-) dendritic cells into mice. Consistent with the stronger T cell-stimulatory ability of btk(-/-) dendritic cells, btk(-/-) mice exhibited enhanced inflammation in Th2-driven asthma and Th1-driven contact sensitivity experiments. These negative regulatory functions of Btk in dendritic cells appear to be mediated mainly through autocrine secretion of IL-10 and subsequent activation of Stat3.

2) **Oki T., Kitaura J., Eto K., Lu Y., Maeda-Yamamoto M., Inagaki N., Nagai H., Yamanishi Y., Nakajina H., Kumagai H., and Kitamura T. :Integrin $\alpha_{IIb}\beta_3$ induces the adhesion and activation of mast cells through interaction with fibrinogen. :The Journal of Immunology., 176: 52-60, 2006.**

Abstract: Integrin alphaIIb, a well-known marker of megakaryocyte-platelet lineage, has been recently recognized on hemopoietic progenitors. We now demonstrate that integrin alphaIIbbeta3 is highly expressed on mouse and human mast cells including mouse bone marrow-derived mast cells, peritoneal mast cells, and human cord blood-derived mast cells, and that its binding to extracellular matrix proteins leads to enhancement of biological functions of mast cells in concert with various stimuli. With exposure to various stimuli, including cross-linking of FcepsilonRI and stem cell factor, mast cells adhered to extracellular matrix proteins such as fibrinogen and von Willebrand factor in an integrin alphaIIbbeta3-dependent manner. In addition, the binding of mast cells to fibrinogen enhanced proliferation, cytokine production, and migration and induced uptake of soluble fibrinogen in response to stem cell factor stimulation, implicating integrin alphaIIbbeta3 in a variety of mast cell functions. In conclusion, mouse and human mast cells express functional integrin alphaIIbbeta3.

3) **Kimata M., Ishizaki M., Tanaka H., Nagai H., and Inagaki N. :Production of matrix metalloproteinases in human culutured mast cells: involvement of protein kinase c-mitogen activated protein kinase kinase-extracellular signalregulated kinase pathway. Allergology International., 55(1): 67-76, 2006.**

Abstract: BACKGROUND: Matrix metalloproteinases (MMPs) have been reported to play crucial roles in the migration of inflammatory cells through basement membrane components. To confirm the role of mast cells as a source of MMPs, we investigated the production of MMP and its pathway in human cultured mast cells (HCMC). We also investigated the production of tissue inhibitors of metalloproteinase (TIMPs). METHODS: HCMC was stimulated with phorbor 12-miristate 13-acetate (PMA) and/or calcium ionophore A23187 (A23187), and the resulting MMP production was evaluated by gelatin zymography and western blotting. Expression of MMP and TIMP mRNA was also examined. Granulocyte macrophage-colony stimulating factor (GM-CSF) was measured by ELISA and activation of extracellular signal-regulated kinase (ERK) was evaluated by western blotting. RESULTS: We detected the de novo synthesis of MMP-9 in HCMC after stimulation with PMA and found that the synthesis was mediated through protein kinase C-mitogen activated protein kinase kinase (MEK)-ERK pathway. The MMP-9 production induced by PMA was suppressed by simultaneous treatment with A23187, whereas GM-CSF production was potentiated. We also detected the expression of mRNA for membrane-type 1

(MT1)-MMP, TIMP-1 and TIMP-2 after stimulation with PMA. Glucocorticoids and flavonoids inhibited MMP-9 production, and TIMPs and MMP inhibitors inhibited the gelatinolytic activity of mast cell-derived MMP-9. Furthermore, phenylmethylsulfonyl fluoride, a protease inhibitor, inhibited the conversion from proMMP-9 to active MMP-9. CONCLUSIONS: These results suggest that the human mast cell is a leading member of MMP production, and the production, activation and activity are controllable by pharmacological agents.

4) **Oida Y., Kitaichi K., Nakayama H., Ito Y., Fujimoto Y., Shimazawa M., Nagai H., and Hara H. :Rifampicin sttenuates the MPTP-induced neurotoxicity in mouse brain. Brain Research, 1082: 196-204, 2006.**

Abstract: Rifampicin, an antibacterial drug, is highly effective in the treatment of tuberculosis and leprosy. Recently, it has been reported to have neuroprotective effects in in vitro and in vivo models. This study was designed to elucidate its neuroprotective effects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity (known as an in vivo mouse model of Parkinson's disease). Mice were injected intraperitoneally (i.p.) with MPTP (10 mg/kg) four times at 1-h intervals, and brains were analyzed 3 or 7 days later. Rifampicin at 20 mg/kg (i.p., twice) had protective effects against MPTP-induced neuronal damage (immunohistochemical changes in tyrosine hydroxylase) in both the substantia nigra and striatum. Rifampicin also protected against the MPTP-induced depletions of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) in the striatum. The maximal concentrations of rifampicin between 30 and 240 min after a single rifampicin injection (20 mg/kg, i.p.) were 2.6 μM (at 30 min) in plasma and 0.77μM (at 60 min) in striatum. Next, the effects of rifampicin on oxidative stress [lipid peroxidation in mouse brain homogenates and free radical-scavenging activity against diphenyl-*p*-picrylhydrazyl (DPPH)] were evaluated to clarify the underlying mechanism. At 1μM or more, rifampicin significantly inhibited both lipid peroxidation in the stratum and free radical production. These findings suggest that in mice, rifampicin can reach brain tissues at concentrations sufficient to attenuate MPTP-induced neurodegeneration in the nigrostriatal dopaminergic neuronal pathway, and that at inhibitory effect against oxidative stress may be partly responsible for its observed neuroprotective effects.

5) **Takayama G., Arima K., Kanaji T., Toda S., Tanaka H., Shoji S., Andrew N. J. McKenzie., Nagai H., Hotokebuchi T., and Izuhara K. :Periostin: A novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals. J. Allergy Clin. Immunol., 118 (1): 98-104, 2006.**

Abstract: BACKGROUND: Subepithelial fibrosis is a cardinal feature of bronchial asthma. Collagen I, III, and V; fibronectin; and tenascin-C are deposited in the lamina reticularis. Extensive evidence supports the pivotal role of IL-4 and IL-13 in subepithelial fibrosis; however, the precise mechanism remains unclear. We have previously identified the POSTN gene encoding periostin as an IL-4/IL-13-inducible gene in bronchial epithelial cells. Periostin is thought to be an adhesion molecule because it possesses 4 fasciclin I domains. OBJECTIVE: We explore the possibility that periostin is involved in subepithelial fibrosis in bronchial asthma. METHODS: We analyzed induction of periostin in lung fibroblasts by IL-4 or IL-13. We next analyzed expression of periostin in patients with asthma and in ovalbumin-sensitized and ovalbumin-inhaled mice. Furthermore, we examined the binding ability of periostin to other extracellular matrix proteins. RESULTS: Both IL-4 and IL-13 induced secretion of periostin in lung fibroblasts independently of TGF-beta. Periostin colocalized with other extracellular matrix proteins involved in subepithelial fibrosis in both asthma patients and ovalbumin-sensitized and ovalbumin-inhaled wild-type mice, but not in either IL-4 or IL-13 knockout mice. Periostin had an ability to bind to fibronectin, tenascin-C, collagen V, and periostin itself. CONCLUSION: Periostin secreted by lung fibroblasts in response to IL-4 and/or IL-13 is a novel component of subepithelial fibrosis in

bronchial asthma. Periostin may contribute to this process by binding to other extracellular matrix proteins. CLINICAL IMPLICATIONS: Periostin induced by IL-4/IL-13 shows promise in inhibiting subepithelial fibrosis in bronchial asthma.

6) **Inagaki N., Shiraishi N., Igeta K., Itoh T., Chikumoto T., Nagao M., John Fan Kim., and Nagai H. :Inhibition of scratching behavior associated with allergic dermatitis in mice by tacrolimus, but not by dexamethasone. European Journal of Pharmacology, 546: 189-196, 2006.**

Abstract: Itching is the most important problem in many allergic and inflammatory skin diseases especially in atopic dermatitis. However, animal models for allergic dermatitis useful for the study of itching have rarely been established. We established a mouse allergic dermatitis model involving frequent scratching behavior by repeated painting with 2,4-dinitrofluorobenzene (DNFB) acetone solution onto the mouse skin, and comparatively examined the effects of tacrolimus and dexamethasone on the dermatitis and associated scratching behavior. Repeated DNFB painting caused typical dermatitis accompanied by elevated serum immunoglobulin E (IgE) and frequent scratching behavior. An apparent thickening of the epidermis and dermis, and the significant accumulation of inflammatory cells were observed. Increased interferon (IFN)-gamma mRNA expression and the induction of interleukin (IL)-4 and IL-5 mRNA expression were also observed in the skin lesion. The scratching behavior was inhibited by dibucaine and naloxone. Although tacrolimus reduced the increased expression of IFN-gamma and IL-4 mRNA, dexamethasone potently depressed that of IFN-gamma, IL-4 and IL-5 mRNA. Dexamethasone inhibited the accumulation of lymphocytes and eosinophils, although tacrolimus did not. Both drugs failed to inhibit the elevation of serum IgE levels. Tacrolimus significantly inhibited the scratching behavior that was associated with the inhibition of nerve fiber extension into the epidermis, whereas dexamethasone failed to have any effect. The mouse dermatitis model seems to be beneficial for the study of itching associated with allergic dermatitis, such as atopic dermatitis, and tacrolimus seems to exhibit an anti-itch effect through the inhibition of nerve fiber extension at least in part.

7) **Akabane H., Murata M., Kubota M., Takashima E., Tanaka H., Inagaki N., Horiba M., and Nagai H. :Effects of salmeterol xinafoate and fluticasone propionate on immunological activation of human cultured mast cells. Allergology International, 55(4): 387-393, 2006.**

Abstract: BACKGROUND: The clinical efficacy of combination therapy comprising a long acting beta(2)-agonist (LABA) and corticosteroid is widely recognized for the treatment of adult asthma. Here we examine the effect of salmeterol xinafoate (SX) and fluticasone propionate (FP) alone and in combination on the immunological activation of human cultured mast cells (HCMC)in vitro. METHODS: HCMC were passively sensitized with IgE antibody and then activated by challenging with anti-IgE antibody. The effect of drugs on the activation of mast cells was examined by measuring the amount of released chemical mediators (histamine, leukotrienes (LT) and prostaglandin D(2) (PGD(2))) and granulocyte macrophage colony stimulating factor (GM-CSF). RESULTS: The release of each chemical mediator was inhibited by 10-9-10-8M SX but not by 10-10-10-7M FP. The production of GM-CSF was inhibited by a concentration of 10-8M in both drugs and the inhibition was augmented by combined treatment with 10-11M of each drug. CONCLUSIONS: The immunological release of chemical mediators (histamine, LT, PGD(2)) from HCMC was inhibited by SX but not by FP. SX and FP inhibited the production of GM-CSF by HCMC and both drug showed synergistic inhibition in the production of GM-CSF.

8) **Gao Xinkun., Tanaka H., Inagaki N., Teramachi H., Tsuchiya T., and Nagai H. :Immunopharmacological studies on the effects of Juzentaihoto and Hochuekkito on experimental autoimmune encephalomyelitis in rats. J. Trad. Med., 23: 196-202, 2006.**

Abstract: Experimental autoimmune encephalomyelitis (EAE) is a prototype experimental model for

human multiple sclerosis (MS). This study was conducted to research the effects of Juzentaihoto and Hochuekkito on the onset and development of EAE. Both Juzentaihoto and Hochuekkito suppressed the onset and development of EAE. Suppressive mechanisms of both Kampo medicines are mainly based on the immunosuppressive and anti-inflammatory activity. In addition, Hochuekkito showed immunomodulating activity in the central nervous system. These findings will contribute to consider a new therapy of human MS.

◇総　説

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3) 永井博弌：アレルギー性炎症とリモデリング．耳鼻免疫アレルギー，24: 5-7, 2006.

◇学会報告　(*: 特別講演、シンポジウム、ワークショップ等)

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2) 永井博弌：大森健守：アレルギー治療薬の将来展望－開発の現場から．第 18 回日本アレルギー学会春季臨床大会，2006, 5.30-6.1, 東京.
3) 永井博弌：アレルギー性炎症の免疫薬理学的解析．第 36 回日本皮膚アレルギー学会・第 31 回日本接触皮膚炎学会合同学術大会，2006, 7.15-16, 兵庫.
4) 永井博弌：和漢薬からの抗アレルギー薬開発研究の試み．第 23 回和漢医薬学会大会，2006, 8.26-27, 岐阜.
5) 永井博弌：アレルギー疾患治療標的としての脂質メディエーター．第 56 回日本アレルギー学会秋季学術大会，2006, 11.2-4, 東京.

◇その他

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3) Nagai H :The possible role of prostaglandins in allergic inflammation. AR Forum 2006, 2006, 8, 東京.
4) 永井博弌：基礎の面から見た抗アレルギー薬の併用療法．アレルギー性鼻炎治療研究会 entry 第 6 回セミナー，2006, 10, 東京.
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