様　式　Ｃ－１９、Ｆ－１９、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

平成 ２７ 年　 ６ 月 １７ 日現在

機関番号：１３２０１
研究種目：基盤研究(C)
研究期間：2012～2014
課題番号：２４５９０８７９

研究課題名（和文）免疫修飾作用を有する漢方薬の樹状細胞機能に対する作用の解明と治療効果の科学的検証

研究課題名（英文）Examination of the effects of constituents of traditional Japanese herbal (Kampo) medicines on the migration of plasmacytoid dendritic cells to develop a new therapeutic target

研究代表者

門脇　真（KADOWAKI, MAKOTO）

富山大学・和漢医薬学総合研究所・教授

研究者番号：２０３０５７０９

交付決定額（研究期間全体）：（直接経費）　　4,000,000円

研究成果の概要（和文）：樹状細胞はその機能を発揮するためには、適切なタイミングで適切な場所へ遊走することが非常に重要であるが、これまで時空間的な樹状細胞機能に対する創薬研究は殆どない。本研究では、自己免疫疾患などに関与する形質細胞様樹状細胞の遊走能に着目し、80種類の漢方方剤含有化合物の作用を検討した。
その結果、アストロガロサイドIV、オキシマトリン、ベルベリン、クルクミン、イソフラキシディンに強力な抑制作用を見出した。さらに、アストロガロサイドIV及びオキシマトリンは古典的樹状細胞の遊走能には影響を与えず、形質細胞様樹状細胞に対する特異性が明らかにされ、自己免疫疾患に対する選択的創薬シードとなる可能性が示唆された。

研究成果の概要（英文）：The involvement of the plasmacytoid dendritic cells (pDCs), but not the conventional dendritic cells (cDCs) in autoimmune diseases have been reported, but there are few reports about the regulation of the migration of pDCs and no drug available with selective inhibitory activity on pDC functions. Therefore, we screened 80 constituents of traditional Japanese (Kampo) formulae which are frequently used in Japan using bone marrow-derived DCs (BMDCs) for inhibitory activities on DC migration. As a result, astragaloside IV, berberine, curcumin, isofraxidin and oxymatrine markedly exerted inhibitory effects on CCL21-stimulated BMpDC migration. Furthermore, oxymatrine and astragaloside IV had no effect on the migration of CXCL12-induced migration of BMcDCs, but berberine, isofraxidin and curcumin inhibited the migration.
However, selective pDC migration inhibitors oxymatrine and astragaloside IV did not ameliorate the symptoms of the spontaneous systemic lupus erythematosus model mice.

研究分野：漢方薬理学

キーワード：樹状細胞　遊走　漢方薬　探索研究　創薬研究　自己免疫疾患

１．研究開始当初の背景

Dendritic cells (DCs) play a pivotal role in maintaining immunological homeostasis. DCs are known as the high ability of antigen presenting cells, and even the cytokines secreting cells. To regulate the immune responses, DCs can migrate to the inflammatory site and lymph nodes depend on the stimulation of chemokines. Therefore, it is assumed that the migration of DCs contributes to the pathogenesis of immune diseases.

DCs are defined as the conventional dendritic cells (cDCs) and the plasmacytoid dendritic cells (pDCs). Especially, pDCs are known as type 1 IFN (IFN I) secreting cells. pDCs play an important role in defense against virual infections by IFN I secretion. Meanwhile, excessive IFN I is involved in autoimmune diseases, such as systemic lupus erythematosus (SLE). Although the involvement of pDCs in autoimmune diseases have been reported, there are few reports about the regulation of the migration of pDCs and no drug available with selective inhibitory activity on pDC functions.

２．研究の目的

The aim of this study is to duscover lead compounds that regulate the DC migration in immune diseases.

３．研究の方法

Therefore, we screened 80 constituents of traditional Japanese (Kampo) formulae which are frequently used in Japan using bone marrow-derived dendritic cells (BMDC) for inhibitory activities on DC migration and then investigated the effects of screened compounds in a murine autoimmune disease model.

pDCs and cDCs were derived from bone marrow (BM) cells of BALB/c mice with Flt3 ligand and GM-CSF, respectively. The screening was performed using an EZ-TAXIScan chemotaxis device. To assess chemotaxis, BMpDCs was stimulated by chemokine CCL21, whereas BMcDCs was stimulated by chemokine CXCL12. Time-lapse images of cell migration during chemotaxis were observed directly with an optically accessible horizontal chemotaxis apparatus EZ-TAXIScan. The number of migrating BMDCs was analyzed by TAXIScan Analyzer 2. The number of migrating BMDCs was automatically counted by TAXIScan Analyzer 2 for every 30 seconds. Based on these data, the velocity and the direction of BMDC migration were calculated by TAXIScan Analyzer 2 for every 30 seconds. The mean velocity and the mean direction were calculated from the all data of the measurement period.

Table I. List of 80 chemical compounds that were examined for their Hsp70 inducing ability.

1. Aconitine	17. Bufotalin	33. Epihesperidin	49. Gomisin N	65. Paeoniflorin
2. Albiflorin	18. Capillarisin	34. Ergosterol	50. Hesperidin	66. Paeonol
3. Alisol A	19. Capsaicin	35. beta-Eudesmol	51. Hirsutine	67. Palmatine chloride
4. Alisol B	20. Catalpol	36. (E)-Ferulic acid	52. Honokiol	68. (S)-Perillaldehyde
5. Alkannin	21. (E)-Cinnamic acid	37. Geniposide	53. Hypaconitine	69. Puerarin
6. Amygdalin	22. Cinobufagin	38. Geniposidic acid	54. Icariin	70. Rhynchophylline
7. Arbutin	23. Cinobufotalin	39. Gentiopicroside	55. Isofraxidine	71. Saikosaponin a
8. Astragaloside IV	24. Coptisine chloride	40. [6]-Gingerol	56. (Z)-Ligustilide	72. Saikosaponin b2
9. Atractylenolide III	25. Corydaline	41. Gimsenoside Rb1	57. Limonin	73. Saikosaponin c
10. Aucubin	26. Curcumin	42. Ginsenoside Rc	58. Loganin	74. Schizandrin
11. Baicalein	27. Dehydrocorydaline nitrate	43. Ginsenoside Rd	59. Magnolol	75. Sennoside A
12. Baicalin	28. Dehydrocostuslactone	44. Ginsenoside Re	60. Mesaconitine	76. Shikonin
13. Barbaloin	29. Dihydrocapsaicin	45. Ginsenoside Rg1	61. Naringin	77. [6]-Shogaol
14. Berberine chloride	30. Dimethylesculetin	46. Glabridin	62. Nodakenin	78. Sinomenine
15. Bergenin	31. Eleutheroside B	47. Glycyrrhizic acid	63. Osthole	79. Swertiamarin
16. Bufalin	32. (-)-Epigallocatechin gallate	48. Gomisin A	64. Oxymatrine	80. Wogonin

４．研究成果

The compounds were examined for their effects on CCL21-stimulated migration in BMpDCs. Ten compounds obviously decreased the number of the migratory cells and seven compounds increased the number of the migratory cells. Thus, I selected astragaloside IV, berberine, curcumin, isofraxidin and oxymatrine which markedly exerted inhibitory effects on BMpDC migration and further investigated effects of five compounds on cDC. These compounds did not induce the apoptosis in BMpDCs.

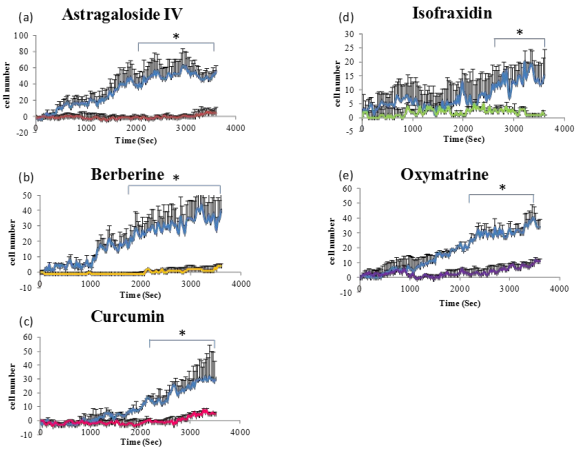

Figure Five compounds completely decreased the number of migrating BMpDCs.
The effects of compounds on the BMpDC migration. (a) Astragaloside IV-treated migration of BMpDCs. (b) Berberine-treated migration of BMpDCs. (c) Curcumin-treated migration of BMpDCs. (d) Isofraxidin-treated migration of BMpDCs. (e) Oxymatrine-treated migration of BMpDCs. Data are expressed as mean ± SE (*P < 0.05, n = 3).

Oxymatrine and astragaloside IV had no effect on CXCL12-induced migration of BMcDCs. Berberine, isofraxidin and curcumin inhibited the migration of BMcDCs.

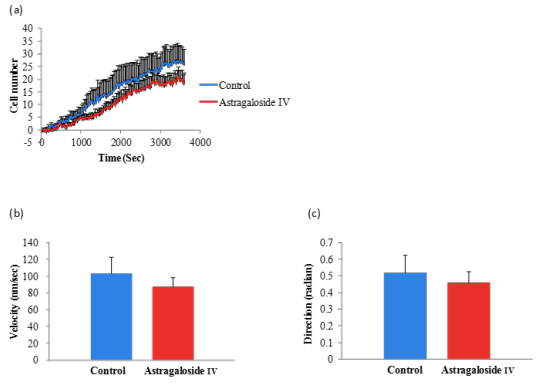

Figure Astragaloside IV had no effect on the migration of BMcDCs.
The migration of 1 μM astragaloside IV-treated BMcDCs was induced by CXCL12. (a) The number of the migration of astragaloside IV-treated BMcDCs. (b) The mean velocity of astragaloside IV-treated migrating BMcDCs. (c) The mean direction of astragaloside IV-treated migrating BMcDCs. All data are represented as mean ± SE (n = 5).

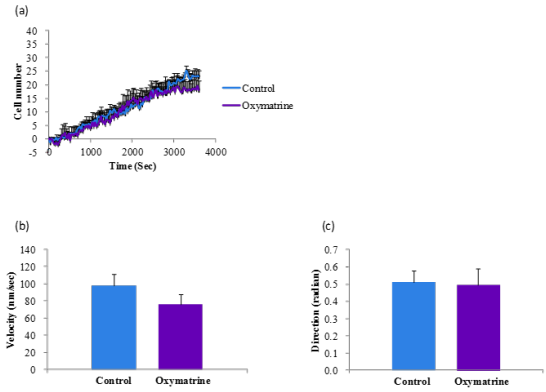

Figure Oxymatrine had no effect on the migration of BMcDCs.
The migration of 1 μM oxymatrine-treated BMcDCs was induced by CXCL12. (a) The number of the migration of oxymatrine-treated BMcDCs. (b) The mean velocity of oxymatrine-treated migrating BMcDCs. (c) The mean direction of oxymatrine-treated migrating BMcDCs. All data are represented as mean ± SE (n = 5).

Furthermore, we evaluated selective pDC migration inhibitors oxymatrine and astragaloside IV in the spontaneous SLE model mouse (BXSB/MpJ mouse). It has been reported that pDCs is closely involved in the onset of SLE. Recently, the depletion of pDCs in the early stage of disease can ameliorate the symptom of SLE in BXSB/MpJ mice. However, these treatment did not ameliorate the symptoms of SLE model mouse.

pDCs are thought to play a pivotal role, especially in autoimmune diseases, such as SLE, rheumatoid arthritis, multiple sclerosis, psoriasis and inflammatory bowel disease. DCs from the blood of SLE patients show higher migration rate to CCL21 compared with DC from the healthy volunteers. Furthermore, the depletion of pDCs exhibits ameliorating effects on immune diseases. The depletion of the infiltrated pDCs in MRL/lpr mouse ameliorated the renal diseases. The depletion of infiltrated pDCs suppresses cutaneous lesions caused by the tape stripping in NZB/W F1 mouse. In the BXSB/MpJ mouse which spontaneously develops SLE, pDC accumulate at the damaged sites in spleen and kidney. The depletion of pDCs at the initial stage of SLE in BXSB/MpJ mouse attenuates the symptoms of SLE. Thus, the migration of pDCs to the lymphoid tissues and the accumulation of pDCs in the lymphoid tissues are considered to be the major pathogeneses in exacerbating the autoimmune diseases. Therefore, the depletion of pDCs in the initial stage of the autoimmune diseases is critical for the treatment of the diseases.
These reports indicate that pDCs may be a novel therapeutic target for SLE treatment. In this study, I investigated the effect of the inhibition of pDC migration at the initial stage of SLE model (BXSB/MpJ mouse). In contrast to the previous data with the depletion of pDCs, oxymatrine and astragaloside IV did not exhibit the therapeutic effect on the spontaneous SLE model, which may be due to a continuous inhibition of pDC migration.
Oxymatrine is a constituent of Sophora root. Oral administration of sophora root extraction down-regulates the anti-dsDNA autoantibody, alleviates the histological expression in the kidney and decreases the glomerular IgG deposits in NZB/W F1 mouse model. A possible explanation for

this discrepancy is the difference of the models, effects of other constituents of Sophora root, the difference in the pharmacokinetics of oxymatrine between the compound administration and the crude extract administration etc., but further investigation is needed. Furthermore, in the murine dextran sulfate sodium-induced colitis model, oxymatrine treatment with 200mg/kg attenuates the inflammation. Oxymatrine suppresses the LPS-induced activation of NF-κB in the colitis model. Pharmacokinetics and metabolism of oxymatrine in vivo is different in each model. Thus, the improvement of the experiment design is required for the evaluation of oxymatrine in immune disease models.
It has been reported that Astragaloside IV has ameliorating effects on a rheumatoid arthritis model. The underlying mechanism appears to be the suppression of splenocyte proliferation. However, astragaloside IV did not exhibit the therapeutic effect on the spontaneous SLE model. Thus, further study is needed for the investigation of astragaloside IV in immune disease models.

In conclusion, I found selective pDC migration inhibitors and pDC & cDC migration dual inhibitors from Kampo medicines. The therapeutic treatment with the regulation of DC migration is still under investigation. However, the present findings may provide useful tools for the elucidation of the pathophysiological role of the DC migration in immune diseases and useful information for the development of therapeutic agents against immune diseases, particularly autoimmune diseases.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計 11 件）
以下、全て査読有り

⑴ Yamamoto T., Kodama T., Lee J., Utsunomiya N., Hayashi S., Sakamoto H., Kuramoto H., Kadowaki M.: Anti-allergic role of cholinergic neuronal pathway via α7 nicotinic ACh receptors on mucosal mast cells in a murine food allergy model. PLoS One. 9: e85888, 2014. DOI: 10.1371/journal.pone.0085888.

⑵Li P., Zhao QL., Wu LH., Jawaid P., Jiao YF., Kadowaki M., Kondo T.: Isofraxidin, a potent reactive oxygen species (ROS) scavenger, protects human leukemia cells from radiation-induced apoptosis via ROS/mitochondria pathway in p53-independent manner. Apoptosis. 19: 1043-1053, 2014. DOI: 10.1007/s10495-014-0984-1

⑶Kim JH., Yamamoto T., Lee J., Yashiro T., Hamada T., Hayashi S., Kadowaki M.: CGRP, a neurotransmitter of enteric sensory neurons, contributes to the development of food allergy due to the augmentation of microtubule reorganization in mucosal mast cells. Biomedical Research. 35: 285-293, 2014. DOI: 10.2220/biomedres.35.285

⑷Wu LH., Li P., Zhao QL., Piao JL., Jiao YF., Kadowaki M., Kondo T.: Arbutin, an intracellular hydroxyl radical scavenger, protects radiation-induced apoptosis in human lymphoma U937 cells. Apoptosis. 19: 1654-1663, 2014. DOI: 10.1007/s10495-014-1032-x

⑸Hayashi S., Hamada T., Zaidi SF., Oshiro M., Lee J., Yamamoto T., Ishii Y., Sasahara M., Kadowaki M.: Nicotine suppresses acute colitis and colonic tumorigenesis associated with chronic colitis in mice. Americal Journal of Physiology Gastrointestinal and Liver Physiology. 307: G968-978, 2014. DOI: 10.1152/ajpgi.00346.2013

⑹ Wang X., Hayashi S., Umezaki M., Yamamoto T., Kageyama-Yahara N., Kondo T., Kadowaki M.: Shikonin, a constituent of Lithospermum erythrorhizon exhibits anti-allergic effects by suppressing orphan nuclear receptor Nr4a family gene expression as a new prototype of calcineurin inhibitors in mast cells. Chemico-Biological Interactions. 224: 117-127, 2014. DOI: 10.1016/j.cbi.2014.10.021

⑺Piao JL., Cui ZG., Furusawa Y., Ahmed K., Rehman MU., Tabuchi Y., Kadowaki M., Kondo T.: The molecular mechanisms and gene expression profiling for shikonin-induced apoptotic and necroptotic cell death in U937 cells. Chemico-Biological Interactions. 205:119-127, 2013. DOI: 10.1016/j.cbi.2013.06.011.

⑻ Oda S., Uchida K., Wang X., Lee J., Shimada Y., Tominaga M., Kadowaki M.: TRPM2 contributes to antigen-stimulated Ca^{2+} influx in mucosal mast cells. Pflügers Archiv-European Journal of Physiology. 465: 1023-1030, 2013. DOI: 10.1007/s00424-013-1219-y

⑼ Lee J, Yamamoto T, Hayashi S, Kuramoto H, Kadowaki M.: Enhancement of CGRP sensory afferent innervation in the gut during the development of food allergy in an experimental murine model. Biochem Biophys Res Commun. 430: 895-900, 2013. 10.1016/j.bbrc.2012.12.058.

⑽ Emam H., Zhao QL., Furusawa Y., Refaat A., Ahmed K., Kadowaki M., Kondo T.: Apoptotic cell death by the novel natural compound, cinobufotalin. Chemico-Biological Interactions. 199:154-60, 2012. DOI: 10.1016/j.cbi.2012.07.005.

⑾Wang X., Kageyama-Yahara N., Hayashi S., Yamamoto T., Kadowaki M.: Sphingosine kinase-1-dependent and -independent inhibitory effects of zanthoxyli fructus to attenuate the activation of mucosal mast cells and ameliorate food allergies in mice. Evidence-Based Complementary and Alternative Medicine. 862743, 2012. DOI: 10.1155/2012/862743.

〔学会発表〕（計 4 件）

⑴Yamamoto T., Matsunami E., Kanauchi Y., Kadowaki M.: Migration of plasmacytoid DC is inhibited though JAK2-caspase-3 pathway activated by α7 nicotinic acetylcholine receptors. Keystone Symposia, 2013, 3. 3-8, Keystone, U.S.A.

⑵Yamamoto T., Matsunami E., Kanauchi Y., Kadowaki M.: Activation of the Cholinergic Anti-Inflammatory Pathway Through Alpha-7 Nicotinic Acetylcholine Receptors Improves Symptoms in Murine Oxazolone-Induced Ulcerative Colitis by Inhibiting Migration of Plasmacytoid Dendritic Cells. Digestive Disease Week, 2013, 5. 18-21, Orlando, U.S.A.

⑶**山本武、金内優也、林周作、門脇真：α7 ニコチン受容体による JAK2 –カスパーゼの活性化を介した形質細胞様樹状細胞遊走抑制作用. 第 14 回日本神経消化器病学会 2012, 年 11 月 9 日　大阪.**

⑷**門脇真：腸管免疫性疾患に対する和漢薬からのアプローチ. 第 15 回天然薬物研究方法論アカデミー　2012 年 8 月 18 日　千葉.**

〔その他〕

ホームページ

http://www.inm.u-toyama.ac.jp/jp/departments/06_gp.html

６．研究組織

⑴研究代表者

門脇　真（KADOWAKI Makoto）
富山大学・和漢医薬学総合研究所・教授
研究者番号：20305709