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

KADOWAKI, MAKOTO

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.

Research field: Traditional Chinese medicine

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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 I IFN (IFN-I) secreting cells. pDCs play an important role in defense against viral 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 discover 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.

| 10. Astragaloside IV | 27. Epigallocatechin gallate | 10. Intracellular | 58. Euphorbia | 74. Salvia persica |

4．研究結果
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 oxytetracycline 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) Oxytetracycline-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.

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は下線）

[雑誌論文]（計11件）
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