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RESEARCH ARTICLE

The effect of MS14 on Th2 cytokines pattern in Balb/C mice

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Abstract

The tendency of immune response toward either Th1 or Th2 cytokine pattern can cause a number of pathologic conditions. Multiple sclerosis is postulated to be a Th1-type cell-mediated autoimmune disease. MS14—an Iranian natural product—seems to possess anti-inflammatory properties. Thus, we studied the effect of orally administered MS14 on Th2 cytokines (IL-5 and IL-10) in normal Balb/C mice (100 mg/kg; 5 days). The result indicated that activated splenocytes of MS14 group produced significantly more IL-5 and IL-10 (3–4 times in comparison with control group mice). MS14 could upregulate Th2 cytokine and thereby it may possess immunoregulatory properties probably useful in treatment of some diseases.

Keywords: *Immunomodulator; interleukin-5 (IL-5); interleukin-10 (IL-10); MS14; TH2 cytokines*

Introduction

In both murine models and human studies, T-helper lymphocytes have been found to express at least two distinct cytokine profiles. Interferon-γ (IFN-γ) and interleukin-12 (IL-12) are major cytokines produced by Th1 cells, contributing to cell-mediated inflammatory immune responses; Th2 cytokines include IL-4, IL-5, IL-6, IL-10, and IL-13 that can deviate the immune response toward the allergic reaction and mediating the humoral responses. The Th1/Th2 classification has been useful in relating the overall patterns of cytokine production to clinical outcomes in some pathological states. It has been demonstrated that the Th1/Th2 cell response is shifted to a predominantly Th1 cell response during most autoimmune diseases, whereas an overwhelming Th2 response elicits allergic disorders.[1,2] For example, the important role of cytokines, especially Th1 cytokines in the pathogenesis of multiple sclerosis (MS) has been demonstrated.[3] Some researchers believe these cytokines, including IL-2, IL-12, and IFN-γ, are among risk factors of MS.[3] IL-10 had been grouped as Th2-type cytokine, but it is now considered as regulatory cytokine, that is, the important mediator of T-regulatory cells that could regulate and suppress effector T cells.[4]

Numerous risk factors have been identified that can impair host defense, predisposing to serious diseases. Widespread efforts are made to identify immunomodulatory agents to combat infections as prophylactic or therapeutic regimens, or to enhance host immune mechanisms.[5] Complementary and alternative medicine approaches are increasingly being considered by patients, and their use has grown substantially during the past 10 years in Western countries.[6] One class of immune modulators is known as herbal medicines. Herbal medicines have been used since ancient times for treatment of a range of diseases.[7] Plant-derived extracts have been historically considered as an important remedy for maintaining health, enhancing overall immune status, and prevention and treatment of chronic diseases.[8] MS14 is a natural (herbal-marine) product, contained 90% penaeus latisculatus (king prawn), 5% Apium graveolens (Umbelliferae), and 5% Hypericum perforatum L. (St. John s Wort).[9] It has been reported to

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possess anti-inflammatory effects with no toxicity even in very high doses.[10]

In the present study, we have investigated the effects of orally administration of MS14 on Th2 cytokine production of splenocytes in Balb/C mice.

Materials and methods

Mice and MS14

Eight-weeks-old female Balb/c mice were obtained from Animal Lab of Shahed University, and were maintained in temperature- and humidity-controlled, pathogen-free conditions.

MS14 powder was produced by Pharmacology department of Shahed University. The MS14 powder was dissolved in sterile normal saline and 100 µL was administered orally (100 mg/kg) using feeding tube for 5 days. The mice in control group were administered the same volume of normal saline orally the same as test group.

Cell culture

Mice were anesthetized by diethyl ether (Lab scan-Ireland) and the spleens were removed aseptically. Splenocytes were prepared by disrupting the spleen with a syringe using medium (Roswell Park Memorial Institute (RPMI) 1640-Invitrogen GIBCO). After a 10-min centrifugation at 800 *g*, the spleen cell suspension was washed twice with RPMI 1640. The resulting cell pellet were counted using a hemocytometer and 2×10^5 Cells were plated in 96-well plates in 0.2 mL RPMI medium 1640 supplemented with 10% fetal bovine serum (Invitrogen GIBCO). The mitogen Concanavalin A (Con A) were added at final concentration of 12.5 ng/mL where needed.

After incubation at 37°C in a 5% CO_2 atmosphere for 48 h, the culture plate was centrifuged at 800 *g* for 10 min and the supernatants were collected and stored at –70°C for cytokine assay.

Cytokine assays

The concentrations of IL-5, IL-10, in the supernatant were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using an ELISA kit (Biosource, Switzerland). Briefly, flat-bottom 96-well plates were coated overnight at 4°C with anti-IL-5 or -IL-10 mAbs. The primary mAbs were discarded and the plates were blocked with Assay Diluent (Biosource, Switzerland) for 1 h at room temperature. The plates were washed three times with wash buffer (0.05% Tween 20 in phosphate buffered saline) and blotted on a paper towel. The supernatants and standard samples were added and the plates were incubated for 2 h at room temperature. The supernatant was discarded and the wells were washed five times with wash buffer. IL-5 or IL-10 detecting second antibody was added and incubated for 30 min at room temperature.

After washing, tetra methyl benzidine substrate solution (Biosource, Switzerland) was added. The color was allowed to develop for 30 min in the dark before the reaction was quenched with a stop solution (1.8 N H_2SO_4). The plates were read at 450 nm and the sample concentrations were determined with the help of a standard curve.

Statistical analysis

Data are presented as mean and SEM. Comparisons between drug group and control group were performed with Student's *t*-test. $P<0.05$ was considered statistically significant.

Results

IL-10 production of activated splenocytes is augmented with MS14 administration

Splenocytes were incubated for 48 h at the presence or absences of con A, then their supernatant were collected and IL-10 concentration measured with ELISA method. Data shows (Figure 1) that there is no significant difference between IL-10 production of nonactivated splenocytes in MS14 (100 mg/kg) and control group. However at presence of con A mitogen (12.5 ng/mL final concentration) as an activator, splenocytes of MS14 group produce significantly more IL-10 (about three times) in comparison with control group (585.9 ± 172.1 and 193.9 ± 26.2 pg/mL, respectively, $P<0.04$).

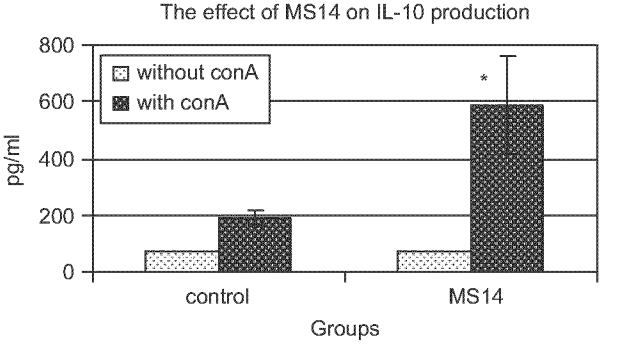

Figure 1. The effect of oral administration of MS14 on IL-10 production of mouse spleen cell culture. The splenocytes were cultured at 2×10^5 cell/well and IL-10 concentration measured by ELISA. All data represent as mean ± SEM.

MS14 uptake increases IL-5 production of activated splenocytes

As shown in Figure 2, nonactivated splenocytes of MS14 group produces less IL-5 comparing with control group (77.8±6.5 and 97.5±4.6 pg/mL respectively, $P<0.02$), but at the presence of con A mitogen (12.5 ng/mL final concentration) a significant increase of IL-5 production is observed (Figure 2). IL-5 concentration in supernatants of activated splenocytes (with con A) in MS14 group is about four times more than control group (241.7±31.8 and 956.24±146.1 pg/mL, respectively, $P<0.00001$).

Discussion

Regarding the use of MS14 in treatment of MS[11] it should be noted that MS is a chronic inflammatory disease of the central nervous system postulated to be a T cell-mediated autoimmune disease. Many studies have shown correlations between disease progression in MS and the Th1 cytokines IFN-γ, IL-2, TNF-α, and IL-12.[3] Proinflammatory cytokines are thought to be crucial for the initiation and amplification of inflammatory brain lesions and direct myelin damage in MS.[12] In experimental autoimmune encephalomyelitis (animal model of MS) Auto reactive T cells directed against myelin antigens produce high levels of Th1/proinflammatory cytokines IFN-γ, TNF-α, and IL-17, and resistance to, or recovery from the disease is mediated through Th2/regulatory T (Tr) cells producing cytokines IL-4, IL-5, IL-10, among others.[13]

In the present study, we observed that IL-5 production of nonstimulated splenocytes from MS14 treated mice (100 mg/kg; 5 days) is slightly decreased (no significant change in IL-10 production). However, production of both IL-5 and IL-10 is augmented significantly (3–4 times of control group) at the presence of stimulator (Con A).

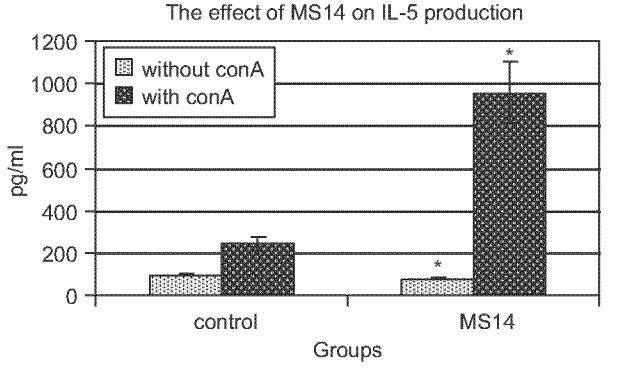

Figure 2. The effect of oral administration of MS14 on IL-5 production of mouse spleen cell culture. The splenocytes were cultured at 2×10^5 cell/well and IL-5 concentration measured by ELISA. All data are presented as mean ± SEM.

It has been shown that proinflammatory and anti-inflammatory cytokines correlate with disease activity in MS. Regulatory cytokines such as IL-4, IL-5, IL-10, and IL-13 may play a role in the resolution of relapses, but in some situations they are associated with exacerbation of autoimmune disease. It is likely that both inflammatory and regulatory processes occur simultaneously in MS. This may explain why proinflammatory and regulatory cytokines are often upregulated simultaneously in MS.[12]

IL-10 is a pleiotropic cytokine produced by monocytes, macrophages, B cells, and Th2 cells, which can inhibit a broad array of immune and inflammatory responses by inhibition from production of several cytokines, including IL-1 and TNF-α. Because of its anti-inflammatory properties, IL-10 is thought to be atheroprotective and have antiatherogenic potential.[14,15] Van and colleagues suggested that IL-10 plays an important role in the control of disease progression of MS.[2] Imitola et al.[15] shown levels of IL-10 were significantly lower in SP patients compared with RR patients. On the other hand, Giuliani et al. founded that IL-10 mRNA levels were reduced in peripheral blood mononuclear cells from MS patients with active relapsing or secondary progressive disease,[16] thus IL-10 involve in decline of MS disease.

IL-5 is mainly produced by TH2 cells and mast cells, seems to be the primary cytokine involved *in vivo* in the production, differentiation, maturation, and activation of eosinophils in the bone marrow and their release into the blood.[17–19] Wiesemann et al.[20] suggested that MS patients treated with Glatiramer acetate increased serum levels of IL-5 and IL-13 and there is a significant correlation between serum IL-5 and clinical response. Comabella et al.[21] have shown in patients treated only with cyclophosphamide/methylprednisolone decreased IFN-γ and increased IL-4, IL-5, and TGF-β expression.

According to Naseri et al.[11] MS14 does not have any undesired side effect on clinical symptoms of MS patients. At the other hand, MS14 causes decrease of IFN-γ and IL-2 production of activated splenic lymphocytes of Balb/C mice (unpublished data). MS14 has also significantly diminishes TNF-α and IL-1 β production of mice peritoneal macrophages (unpublished data) as well, but surprisingly in experimental model of Candida sepsis, MS14 not only do not suppress normal immune response but also helps protection of the mice against sepsis by increasing the number of macrophages.[22] Regarding the anti-inflammatory effects of MS14 and prompting of regulatory cytokine like IL-10 and IL-5 it can be postulated that MS14 as an orally, herbal, immunomodulator may be able to change cytokines pattern toward Th2, and its benefit for autoimmune disease in which Th1 cytokines are pathologic or inflammatory disease should be further considered.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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