Th1/Th2バランスに対する漢方方剤の影響（I）

－Th1/Th2の変化に対する柴胡剤の影響－

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Effect of Kampo medicines on Th1/Th2 balance (I)

－Effect of Kampo formula including Bupleuri radix－

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Key words　Th1/Th2 balance, IL-12, Bupleuri radix.

緒　　言

現代の疾病構造は感染症を中心とした疾患から体質にもとずく疾患へと大きく変化している。当院を受診する患者のうち，アトピー性皮膚炎，気管支喘息，アレルギー性鼻炎といった体質に原因のある疾患であるものは約2割を占めている。

マウスではヘルパーTリンパ球の中にTh1とTh2があることが知られている[1]。Th1細胞はγ-IFN，IL-2を産生し，キラーTリンパ球をサポートすることにより，細胞性免疫を賦活する。一方Th2細胞はIL-4，IL-10を産生し，Bリンパ球からの抗体産生を促進する[2]。ヒトでも同様の現象が観察され，疾患を免疫の状態により，Th1病とTh2病に分けることができる。アトピー性皮膚炎，気管支喘息は細胞性免疫が落ち，IgEが高値であることよりTh2病に属する[3,4]。治療により，IgEが低下していく過程はThバランスがTh2に傾いていたのがTh1に戻っていく過程と考えることができる。

このことから，漢方方剤の薬理作用がこれらTh1/Th2のバランス調節を介して発現する可能性が考えられる。マウスにおいてはC57BL/6マウスではTh1が，またBALB/cマウスではTh2がそれぞれ優位であり，その免疫応答が異なることが報告されている。そこで今回，柴胡剤のTh1/Th2の変化に対する影響について検討し，またC57BL/6マウスとBALB/cマウスに対する反応性の違いについて比較した。

材料および方法

(1)　実験動物：7週齢雄性C57BL/6マウスおよびBALB/cマウスを日本SLCより購入し，実験に供した。

(2)　漢方方剤：小柴胡湯，柴苓湯，柴朴湯および五苓散，半夏厚朴湯を常法に準じて生薬より調製した。構成生薬および量は北里東医研の処方集に拠った。漢方方剤の投与は給水ビンを用いて経口的に2週間自由摂取させた。コントロール群には水を投与した（各群5匹）。

(3)　体重の変化：漢方方剤投与前後の体重の変化を測定した。

(4)　リンパ球幼若化試験：抗CD3抗体刺激後の幼若化活性をアラマーブルー法にて測定した。

(5)　リンパ球サブセットの検討：漢方方剤投与後，それぞれのマウスより脾臓リンパ球を採取し，モノクローナル抗体を用いてCD4/CD8およびCD3/$\gamma\delta$ TCRをフローサイトメーターを用いて測定した。

(6)　サイトカイン産生能の検討：抗CD3抗体刺激後の上清のIFN-γ，IL-4をELISAにて測定した。また，LPS刺激後の上清のIL-12をELISAにて測定した。

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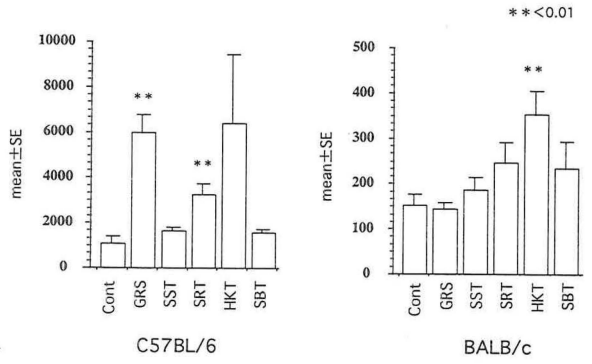

Fig. 1 Effects of Kampo medicines on Th1/Th2 balance. The ratio of IL-4 and IFN-γ secreted by T lymphocytes. Cont: control, GRS: Gorei-san, SST: Sho-saiko-to, SRT: Sairei-to, HKT: Hangekoboku-to, SBT: Saiboku-to.

(7) 統計学的処理：各パラメーターのコントロール群との比較は student's t-test にて行なった。

結　果

(1) C57BL/6 マウス，BALB/c マウスともに投与終了時の体重および体重の変化とも，いずれの方剤においてもコントロール群との間に有意な差は認められなかった。

(2) 脾リンパ球の抗 CD3 抗体による幼若化活性は C57BL/6 マウスにおいて，五苓散，柴苓湯で低下していた。BALB/c マウスではいずれの方剤においても有意な差は認められなかった。

(3) CD4/CD8 の検討では，C57BL/6 マウスで，五苓散と柴苓湯が CD4 のポピュレーションを，柴苓湯が CD8 のポピュレーションを減少させたが，CD4/CD8 の比では，C57BL/6 マウスおよび BALB/c マウスともに変化を認めなかった。CD3/$\gamma\delta$ TCR の検討では，CD3 陽性細胞中，$\gamma\delta$ TCR 陰性細胞を $\alpha\beta$ 細胞として検討した。C57BL/6 マウスでは柴苓湯が，CD3 細胞を減少させたが，$\gamma\delta/\alpha\beta$ 陽性細胞比では，C57BL/6 マウスおよび BALB/c マウスともに変化は認められなかった。

(4) サイトカインの産生能は，C57BL/6 マウスでは五苓散により Th2 細胞より分泌される IL-4 が低下していた。また，IFN-γ は五苓散，柴苓湯で増加していた。BALB/c マウスでは，半夏厚朴湯が IL-4 を低下させ，IFN-γ を増加させたが，C57BL/6 マウスと異なり，五苓散，柴苓湯ではサイトカインの有意な変化は認められなかった。IFN-γ と IL-4 の比でみると，C57BL/6 マウスでは五苓散，柴苓湯で IFN-γ と IL-4 の比が増大していた。BALB/c マウスでは半夏厚朴湯で IFN-γ と IL-4 の比が増大していた。IL-12 の産生能では C57BL/6 マウスでは五苓散，柴苓湯で IL-12 の分泌が亢進していた。また，BALB/c マウスでは半夏厚朴湯で IL-12 の分泌が亢進していた。

考察および結論

漢方方剤のアレルギー性疾患に対する作用機序として，生体内の免疫バランスを変えうることが考えられる。C57BL/6 マウスでは五苓散，柴苓湯で Th1/Th2 バランスが Th1 にシフトしうることを示した。また，BALB/c マウスでは半夏厚朴湯で Th1/Th2 バランスが Th1 にシフトした。Th1 細胞はマクロファージからの IL-12 により活性化されることが知られている[5]。今回の検討で IL-12 の産生能は，C57BL/6 マウスでは五苓散，柴苓湯投与群で亢進しており，BALB/c マウスでは半夏厚朴湯投与群で亢進していた。このことは，これら漢方薬の作用機序として，マクロファージからの IL-12 産生促進が考えられる。

またマウスの系統により，検討した漢方方剤に対する反応性が異なり，遺伝的背景によって，免疫応答に相違のあることが示された。

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Th1/Th2 バランスに対する漢方方剤の影響 (III)

-補剤の投与期間による影響-

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Effects of Kampo medicines on Th1/Th2 balance (III)

-Influence of the duration of administration-

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Key words Th1/Th2 balance, Juzentaiho-to, Hochuekki-to, Ninjin-yoei-to,

Introduction

We have reported that Kampo medicines can alter the Th1/Th2 balance (1, 2). However, each Kampo formula had different effects in different mouse strains. Furthermore, Kampo formulas with tonic action and the Kampo formulas which contain Bupleuri radix perform in different ways, i.e. Kampo formulas with Bupleuri radix changed the Th1/Th2 balance in a rather short period of time. While, Kampo formulas with tonic action did not affect the Th1/Th2 balance in the short term. In Oriental medicine, Kampo formulas with a tonic action tend to be administered for rather long periods. Therefore, we administered Kampo formulas with a tonic action for different lengths of time to examine the influence of the duration of the administration period.

Materials and Methods

Kampo formulas: Juzentaiho-to (JTT), Ninjin-yoei-to (NYT) and Hochuekki-to (HET) formulations and quantities were prepared based on prescriptions of Oriental Medicine Research Center of the Kitasato. SKT and JTT were decocted using conventional methods.

Mice and treatments: BALB/c male mice were purchased from Japan SLC Inc. (Shizuoka, Japan) and kept in a specific pathogen-free environment. There were 7 mice in each group. Seven week old mice were administered JTT, NYT or HET for 4 weeks via their water bottles. Control mice drank water only. The mean intake of Kampo medicine was 2.5 ml which is about 20-fold higher than human doses per body weight basis.

Lymphocyte subpopulation: Spleens were removed and single cell suspensions were prepared. Cells were incubated with fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse CD8a (Ly-2) monoclonal antibody, or hamster anti-mouse CD3e monoclonal antibody together with R-phycoerythrin (PE)-conjugated rat anti-mouse CD4 (L3T4) monoclonal antibody or rat anti-mouse CD19 monoclonal antibody respectively. After incubation, the cells were washed 3 times with cold PBS and analyzed using an EPICS Elite flow cytometer (Coulter Cytometry Co, Hialeah, FL, USA).

Cytokine analysis: Spleen cells were cultured in RPMI1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units of penicillin and 100 μg of streptomycin. Cells were stimulated with anti-CD3 monoclonal antibody

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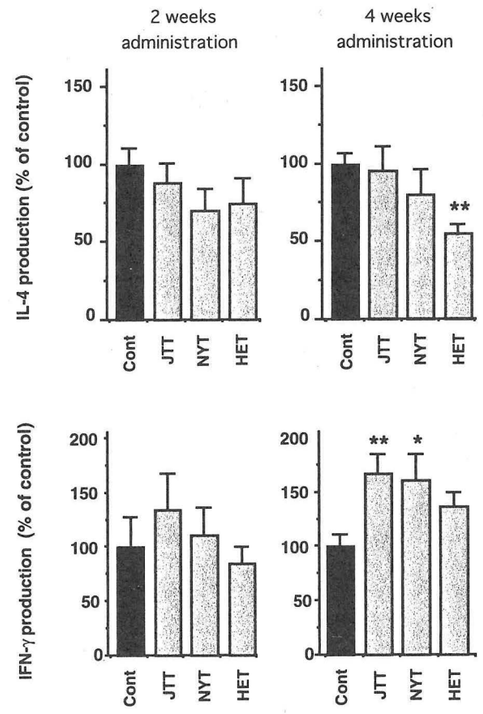

Fig.1 Effect of Kampo administration on IFN-γ (A) and IL-4 secretion by splenocytes. Each Kampo fomulation was administered to mice via their drinking water for 2 or 4 weeks. Spleen cells were stimulated with soluble anti-CD3 mAb. The culture supernatants were harvested and IL-4 and IFN-γ levels were determined by ELISA. *p <0.05, **p <0.01.
Cont: control, JTT: Juzentaiho-to, NYT: Ninjin-yoei-to, HET: Hochuekki-to.

to detect IL-4 and IFN-γ, or LPS to detect IL-12. Supernatant was collected and the cytokine concentrations determined by ELISA.

Statistics and data analysis: The data were analyzed using Fisher's PLSD test. A p value of <0.05 was accepted as statistically significant.

Results:

Effects of the respective Kampo formulas on splenocyte subpopulations:

Two weeks administration of NYT or HET significantly increased CD8 positive cells without influencing $CD4^+$ cells. Thus, the ratios of $CD4^+$ and $CD8^+$ cells in both groups were decreased. A significant decrease in $CD4^+$ cells was observed in both groups with 2 more weeks of administration (total of 4 weeks). NYT also decreased $CD8^+$ cells. The ratios of CD4 and CD8 positive cells were not changed. Two week administration of JTT did not change $CD4^+$ or $CD8^+$ cells. However, the ratios of $CD4^+$ and $CD8^+$ cells were significantly decreased after 4 weeks since the number of $CD4^+$ cells had decreased.

Effects of the Kampo formulas on cytokine secretion from splenocytes:

IFN-γ productions by cultured spleen cells did not change in any group after 2 weeks of administration. After 4 weeks of administration, significant up-regulated release of IFN-γ was observed with JTT and NYT, but not HET (Fig.1A). IL-4 secretion was not affected by any Kampo formula after 2 weeks of treatment. After 4 weeks, HET-treated mice had lower IL-4 levels than control mice (Fig 1B).

Discussion

The results obtained in this study were similar to those presented last year, i.e. different immunological responses were observed in different mouse strains. The Kampo formulas used have tonic actions and they were administered for a rather long period of time from a clinical standpoint. This study has shown that Kampo formulas such as JTT, NYT and HET should be administered for rather long period of time in order to affect the Th1/Th2 balance.

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Th1/Th2バランスに対する漢方方剤の影響 (IV)

-週令による相違についての検討-

金光弼、渡辺賢治、中田勉、三田一樹、加藤裕之、鳥居塚和生、花輪壽彦

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Th1/Th2 バランスに対する漢方方剤の影響 (IV)
-週令による相違についての検討-

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Effect of Kampo medicines on Th1/Th2 balance (IV)
-The influence of age differences-

Guangbi Jin, Kenji Watanabe, Tsutomu Nakada, Kazuki Santa,

Hiroyuki Kato, Kazuo Toriizuka and Toshihiko Hanawa

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Key words Th1/Th2 balance, Juzentaiho-to, Saikokeishi-to, Age

Introduction

Many reports have found that Kampo medicines are effective for treating patients with atopic dermatitis (1, 2). It is thought that Kampo medicines work by affecting the Th1/Th2 balance (3,4), and that their effects depend on age of the patient (5). We have shown that atopic dermatitis is easier to improve in infants than in their mothers. To determine the influence of age on changes in the Th1/Th2 balance, we examined mice of different ages to study the immunological changes associated with Kampo medicines.

Materials and Methods

Kampo formulas: Saikokeishi-to (SKT) and Juzentaiho-to (JTT) formulations and quantities were prepared based on prescriptions of Oriental Medicine Research Center of the Kitasato. SKT and JTT were decocted by usual methods.

Mice and treatments: BALB/c female mice purchased from Japan SLC Inc., (Shizuoka, Japan) were kept in a specific pathogen-free environment. Neonatal, 8 week and 25 week old mice were used. Eight and 25 week old mice were given SKT and JTT via their drinking water for 4 weeks. Neonatal mice ingested SKT and JTT through breast milk from birth until weaning and then directly in their drinking water until 4 weeks old. Control mice were given drinking water only. The mean intake of Kampo was 2.5 ml which is about 20-fold higher than human doses on a per body weight basis.

Lymphocyte subpopulation: Spleens were removed and single cell suspensions were prepared. Cells were incubated with fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse CD8a (Ly-2) monoclonal antibody, or hamster anti-mouse CD3e monoclonal antibody together with R-phycoerythrin (PE)-conjugated rat anti-mouse CD4 (L3T4) monoclonal antibody or rat anti-mouse CD19 monoclonal antibody respectively. After incubation, cells were washed 3 times with cold PBS and analyzed using EPICS Elite flow cytometer (Coulter Cytometry Co, Hialeah, FL, USA).

Cytokine analysis: Spleen cells were cultured in RPMI1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units of penicillin and 100 μg of streptomycin. Cells were stimulated with anti-CD3 monoclonal antibody in order to detect IL-4 and IFN-γ, or LPS to detect IL-12. Supernatant was collected and cytokine concentrations in the supernatant detected by ELISA.

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Statistics and data analysis: The data were analyzed using Fisher' PLSD test. A p value of < 0.05 was accepted as statistically significant.

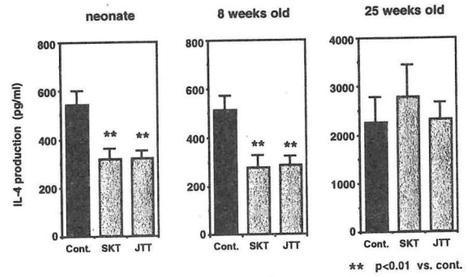

Fig.1 Effects of SKT or JTT administration on IFN-γ production by splenocytes in BALB/c mice of different ages. Cont: control, SKT: Saikokeishi-to, JTT: Juzentaiho-to

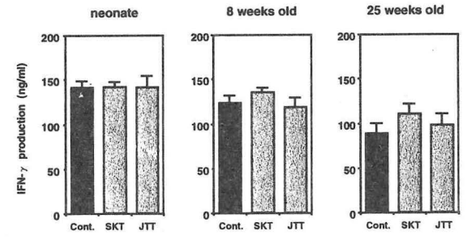

Fig.2 Effects of SKT or JTT administration on IL-4 production by splenocytes in BALB/c mice of different ages.

Results

Effects of SKT and JTT on spleen lymphocyte subpopulations in different aged mice.

Spleen lymphocyte subpopulations were determined after 4 week administration of SKT and JTT. In neonatal mice, the percentages of $CD4^+CD8^-$ cells and $CD4^-CD8^+$ cells were significantly decreased in the SKT group compared to the control group. Meanwhile, the percentage of $CD3^+CD19^-$ cells decreased significantly and the percentage of $CD3^-CD19^+$ cells increased significantly in the SKT group compared to the control group. As a result, there were no significant differences in the spleen lymphocyte subpopulations in the JTT group. The effects of SKT and JTT on spleen lymphocyte subpopulations in 8 week and 25 week old BALB/c mice were also examined. There were no significant differences in the spleen lymphocyte subpopulations in 25 week old mice administered SKT or JTT for 4 weeks. In 8 weeks old mice however, the percentage of $CD4^+CD8^-$ was significantly decreased in the SKT group.

These results indicate that JTT did not affect CD4/CD8 or CD3/CD19 subsets in BALB/c mice of different ages, while SKT had different effects in mice of different ages. SKT reduced the T lymphocyte population and increased the B lymphocyte population in neonatal mice, decreased T helper cells in 8 week old mice and had no effects in 25 week old mice.

Effects of SKT and JTT on cytokine secretions from spleen cells in mice of different ages.

IL-4 production in the SKT and JTT groups was significantly reduced ($p<0.01$) in neonatal and 8 week old mice compared to the control mice (Fig.1a). While there were no significant differences in IFN-γ production in mice of different ages (Fig.1b) in both the SKT and JTT groups. SKT reduced IL-12 production in neonatal mice, while JTT did not. IL-12 production was not changed in 8 week and 25 week old mice in the JTT and SKT groups.

Discussion

The results of this study indicate that SKT and JTT reduce the secretion of IL-4 from spleen cells in neonatal and 8 weeks old mice, but do not affect IFN-γ production resulting in up-regulation of the Th1/Th2 balance to in the Th1 profile. On the other hand, SKT and JTT did not affect cytokine production in 25 week old mice. Thus, the findings of this study support the clinical observation that Kampo medicine can change the immunological constitution in children more easily than in adults.

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