Influence of Flavonoid of Astragalus Membranaceus's Stem and Leaf on the Function of Cell Mediated Immunity in Mice*

JIAO Yan(焦 艳), WEN Jie(闻 杰), YU Xiao2hong(于晓红), and ZHANG De2shan(张德山)

ABSTRACT Objective: To investigate the immune regulation of flavonoid of Astragalus membranaceus's stem and leaf(FAM2sl). Methods: Changes of total T cell count and subsets in mice were determined by mono2clonal antibody assay before and after treatment with FAM2sl, and the lymphokine activated killer cell (LAK) activity was tested simultaneously by isotope label method. Results: FAM2sl could promote the proliferation of lymphocytes induced by ConA, raise the total T cell count and regulate the T cell subsets disturbance, and ele2vate the LAK activity induced by recombinant interleukin22 (rIL22). Conclusion: FAM2sl possesses effects of immune stimulation and immune regulation in treating immunosuppressive mice. This study provides experi2mental evidence for clinical application of FAM2sl.

KEY WORDS flavonoid in Astragalus membranaceus's stem and leaf, T lymphocyte, lymphokine activated killer cell, immune regulation

Astragalus membranaceus is one of the major tonics often used in traditional Chinese medicine. The root of Astragalus mongholicus Bunge and Astragalus membranaceus (Fisch) Bunge used to be approved for clinical use, while the stem and leaf of them were thrown away. Many studies have shown that the root of Astragalus membranaceus may definitely regulate immune system[1-3] through its chem2ical components, pharmacological activities and clinical practice. Recently, a lot of chemical ingredients in the stem and leaf of Astragalus membranaceus have been reported to have the same functions as those of the root[4,5]. A comparative study of the root and the stem and leaf has got consistent results in regard to the chemical ingredients and pharmacological para2meters[5]. But there has been little literature on their pharmacological effect on immunity. It has been demonstrated by our previous stud2ies that the flavonoid of Astragalus membrana2ceus's stem and leaf (FAM2sl) might increase the weight of immune organs, the number of antibody forming cells, and phagocytosis of mono2nuclear macrophages[6] in immunosup2pressive model induced by hydrocortisone (HC). In the present study, the effects of FAM2sl on the function of cell2mediated im2munity in HC model mice were further ex2plored by taking the indexes of T cell subsets, proliferous activity of lymphocytes and activity of lymphokine activated killer cell (LAK) as endpoints.

METHODS

Drugs and Reagents

FAM2sl, provided by the Department of Plant Chemistry, Heilongjiang University of TCM, was dissolved (2.5 mgPml) in double distilled water before usage. HC was pur2chased from the Third Pharmaceutical Compa2ny of Harbin (Batch No. 8903181). Mono2clone antibodies against mouse ($Thy2.1^+$, $L_3t_4^+$, Lyt_2^+) were obtained from the Depar2tment of Immunology, Beijing Medical Uni2versity. Recombinant interleukin22 (rIL22) was made in Switzerland (Batch No. 20MD4YD). Tritiated thymidine (^{3}H2TdR) was made in Atomic Energy Institute, Chinese Academy of Sciences.

Animals

Kunming mice were provided by Experimental

* This study was supported by National Natural Sciences Foundation of China

Department of Microbiology and Immunology, Heilongjiang University of TCM, Harbin(150040)
Correspondence to: Dr. JIAO Yan, Department of Microbiolo2gy and Immunology, Jiangxi College of TCM, Nanchang (330006)
Tel: 0791- 6836403

CJIM 2001;7(2)B117- 120

Animal Center, Heilongjiang University of TCM. BALBPc mice were obtained from Har2 bin Veterinary Institute, Chinese Academy of Agricultural Sciences. All the mice were 6- 8 weeks old, weighing 18 ? 2 g, half male and half female.

Assessment of T Lymphocyte and Its Subsets

The Kunming mice were randomly divid2 ed into the model control group, FAM2sl treated group, and the normal control group (9 in each group). The mice in the model and FAM2sl groups received intramuscular injec2 tion of HC (0.1 mlPd, equivalent to 0.5 mgPd HC each mouse), and normal saline was given to the mice in the normal control group at equal volume for 6 consecutive days. From the fifth day on, each mouse in FAM2sl group re2 ceived injection of FAM2sl 0.2 ml per day per2 itoneally, while mice in the other two groups got normal saline in equal volume for 5 consec2 utive days. Six hours after the final injection, the mice were killed and their spleens were taken out. Percentages of $Thy21^+$, $L_3t_4^+$, Lyt_2^+ as well as ratio of $L_3t_4^+PLyt_2^+$ were de2 termined referring to the cytotoxic method of T2lymphocyte specific antigen determination prepared by the Department of Immunology, Beijing Medical University.

Assay of Proliferation of Lymphocytes in Spleen

Normal BALBPc mice were killed and their spleens were taken out to determine the proliferous activity according to the method re2 ported in literature[7].

Assessment of LAK Cell Activity

Spleens were obtained in the way men2 tioned above from normal BALBPc mice, and then the LAK cell activity was tested by ^3H2 TdR post labeling target cell method[8].

RESULTS

Effects of FAM2sl on T Cell and Its Subsets

Table 1 showed the percentage of Thy21, $L_3t_4^+$, Lyt_2^+ and ratio of $L_3t_4^+PLyt_2^+$ in mice of different groups. These results indicat2 ed that FAM2sl might significantly increase the total T2lymphocyte count, the percentage of $L_3t_4^+$, Lyt_2^+ and $L_3t_4^+PLyt_2^+$ ratio in im2 mune suppressed mice, making these parame2 ters close to those of the normal group.

Table 1. Influence of FAM2sl on T Cell and Its Subsets (n= 9, x ? s)

Group	$Thy21^+$ (%)	$L_3t_4^+$ (%)	Lyt_2^+ (%)	$L_3t_4^+PLyt_2^+$
FAM2sl	36.38? 4.39*	25.10? 2.20*	12.17? 1.46*	2.10? 0.41*
Model	20.83? 3.49v	10.90? 2.20v	10.00? 1.73	1.13? 0.39v
Normal	39.94? 3.53	27.00? 1.94	12.39? 1.58	2.21? 0.29

Notes: * P< 0.01, compared with the model control; v P< 0.01, compared with normal control

Effects of FAM2sl on Proliferation of Lympho2 cyte in Spleen

See Table 2. FAM2sl alone could slightly stimulate transformation of spleen cell at the level of 31.3- 250.0 LgPml, and the most ef2 ficient level was 250.0 LgPml. Stimulation In2 dex (SI) was markedly increased when FAM2 sl was added to 5 LgPml ConA, and the best effect was obtained at the dosage of 250.0 LgP ml.

Table 2. Effects of FAM2sl on Proliferation of Lymphocyte in Mice (x ? s, n= 8)

Group	Proliferation without ConA		Proliferation with ConA	
	(cpm)	SI	(cpm)	SI
FAM2sl (LgPml)				
3.9	894? 117	1.02	20525? 6541	22.43
7.8	902? 136	1.03	22408? 6452	24.58
15.6	964? 163	1.10	23224? 6824	25.51
31.3	1095? 331	1.25	23151? 6043	25.43
62.5	1579? 1070	1.80	26534? 5341	29.29
125.0	2269? 1720*	2.59	30889? 11342v	35.26
250.0	3450? 1916**	3.94	34824? 11030vv	39.75
500.0	954? 173	1.09	19646? 5273	22.43
Blank	876? 80	1.00	-	-
ConA (5LgPml)	-	-	20549? 7421	22.46

Notes: * P< 0.05, ** P< 0.01, compared with blank control group; v P< 0.05, vv P< 0.01, compared with ConA group

Effects of FAM2sl on Activity of LAK Cell

See Table 3. More efficient effect was got in stimulating the LAK cell by the combination of FAM2sl at the range of 3.9- 250 LgPml with rIL22 (10 uPml) than by rIL22 (10 uPml) alone. The cytotoxic index (CI) reached its peak when the level of FAM2sl was 250 LgP ml, above which the CI tended to decline.

This finding indicated that the LAK activity stimulated by combination of FAM2sl and rIL2 2 was dose2dependent, while LAK cell activity could not be stimulated by using FAM2sl alone, suggesting that no stimulating effect could be obtained by FAM2sl only.

Table 3. Effects of FAM2sl on Activity of LAK Cell in Mice (n= 8)

Group	LAK Cell Activity (cpm, x? s)	CI
FAM2sl+ rIL22(10uPml)		
3.9(LgPml)	2422? 328	0.31
7.8	2358? 288	0.33
15.6	2352? 214	0.33
31.3	2317? 192*	0.34
62.5	2282? 194*	0.35
125.0	2176? 264**	0.38
250.0	1720? 468**	0.51
500.0	2632? 369	0.25
rIL22(10uPml)	2492? 115	0.29

Notes: * P< 0.05, ** P< 0.01, compared with rIL22 group

DISCUSSION

The present study showed that there were marked decrease in the total number of T cell and percentage of $L_3t_4^+$ as well as imbalance of $L_3t_4^+PLyt_2^+$ in HC2induced immune sup2 pressed model mice, while the decrease of Lyt_2^+ was not obviously seen. After treatment with FAM2sl, T cell and $L_3t_4^+$ cell increased, which in turn corrected the $L_3t_4^+PLyt_2^+$ im2 balance in immunosuppressive mice. These findings indicated that FAM2sl possesses the effects of immune stimulation and immune regulation.

In the lymphocyte proliferation test, FAM2sl stimulated proliferation of lymphocyte in mouse's spleen, and SI reached its peak at the dosage of 250 LgPml, but it turned to de2 crease when the dosage rose to over 250 LgPml and showed an inhibitory effect when the dos2 age reached 500 LgPml. When combined with ConA (5LgPml, the suboptimal dose defined in this experiment), FAM2sl might remark2 ably increase SI in the form of concentration2 dependence. The synergism reached its peak when FAM2sl was given at the dosage within 125- 250 LgPml, above which the synergism decreased. These results showed that there ex2 isted a dose2effect relation in the effect of FAM2sl stimulating lymphocyte proliferation. The reason why FAM2sl in higher dose inhib2 its the proliferation remains to be further stud2 ied.

Experiment also showed that FAM2sl alone cannot stimulate the activity of LAK cell. When FAM2sl was added to rIL22, the CI of LAK was increased more than that when rIL22 was given alone. These findings indicat2 ed that FAM2sl might be used in combination with rIL22 to stimulate the activity of LAK cell with reduced clinical dose and less side2ef2 fects. This result suggested that FAM2sl might stimulate immune response against tu2 mor in immunosuppressive body.

In TCM clinical practice, patients with Deficiency Syndrome were often complicated with decreased cell immunity and imbalanced T cell subsets. Therefore, they are susceptible to infection or tumor. Astragalus membrana2 ceus may stimulate immune system and in2 crease defense mechanism against tumor. This study demonstrated that FAM2sl might stimu2 late and regulate immune system like other tonics frequently used in TCM clinically, espe2 cially the root of Astragalus membrana2 ceus[1, 9].

In summary, the present study confirms that as Astragalus membranaceus polysaccha2 ride, FAM2sl is an effective ingredient in As2 tragalus membranaceus, and provides the ex2 perimental evidence for clinical application of FAM2sl.

REFERENCES

1. GENG CS, XING ST, ZHOU JH, et al. Study on mech2 nism of Astragalus membranaceus stimulating antibody pro2 duction in T cell defect mice. Shanghai J Immunology 1985; 5(2)B69- 72.
2. MAO XJ, WANG JZ, WANG FL. Advances in immuno2 logical studies of Astragalus membranaceus and Radix Hedysari. J Lanzhou Medical College 1988; (1)B67- 71.
3. WANG WY, CHANG JL, GAO J, et al. Experimental study on effects of Astragalus membranaceus on cytokine production in aged organism. Chinese J Immunology 1995; 11(5)B167- 169.
4. MA YL, TIAN ZK, YUAN CS. Studies on chemical in2

CJIM 2001;7(2)B117-120

gredients in Astragalus membranaceus's stem and leaf. J Shengyang Pharmacol College 1991;(2)B121-123,136.
5. LU SH, ZHU YZ, WU SJ. Study on flavonoids in Astragalus membranaceus Bage van mongolicus stem and leaf. Chinese Traditional and Herbal Drugs 1990;21(6)B249-250,265.
6. JIAO Y, WEN J, ZHANG DS. Effects of flavonoid in Astragalus membranaceus's stem and leaf on immune function in mice. Informations on TCM 1997;14(5)B44.
7. GUAN HZ, KUANG YD, QING HL, et al. Comparison of 4 methods of assessing interleukin22(IL22). Shanghai J Immunology 1986;6(5)B298-301.
8. MA ZZ, DING RR, SHENG T, et al. Detection of activity of LAK cell in mice by ^{3}H2TdR incorporation. Chinese J Immunology 1989;5(1)B20-21,28.
9. SHENG YQ, NA AH, YANG ZJ, et al. Comparison of 12 species of Astragalus membranaceus on immune function in mice. Chinese J Immunology 1989;5(2)B119-121.

(Received December 10, 2000)

Study on Diagnosis and Treatment of Infectious Multiple Organ Dysfunction Syndrome by Integrated Traditional Chinese and Western Medicine

ZHANG Shu2wen(张淑文), REN Ai2ming(任爱民), ZHANG Li2xia(张丽霞),
WANG Hong(王　红), BI Ming2hua(毕铭华), LI Ang(李　昂),
CI Xiu2li(次秀丽), WANG Yan(王　彦), QI Wen2jie(齐文杰) and WANG Bao2en(王宝恩)

Beijing Friendship Hospital, Beijing (100050)

Objective: To explore the diagnostic and therapeutic approach of integrated traditional Chinese and western medicine (TCM2WM) on infectious multiple organs dysfunction syndrome-multiple system and organ failure (MODS-MSOF) for elevating the successful rate of rescuing the patients. Methods: Diagnosis with western medicine and Syndrome Differentiation of TCM in 225 in2patients of acute infectious disease complicated with MODS-MSOF were conducted, and TCM treatment, based on western medical compre2hensive treatment, was given to observe the effect and explore the mechanism of the TCM2WM therapy. Results: Up to the end of 1998, 161 cases of the 225 cases were successfully cured and 64 died, the mortality being 28.4%. Among them, 58 out of 140 cases of MSOF died, the mortality was accounted for 41.4%. In 106 cases conformed to the diagnostic criteria of MSOF proposed by Professor Knaus WA, USA, 52 cases were cured successfully and 54 died, the mortality being 50.94%. Conclusion: TCM2WM treatment could elevate the therapeutic effect in treating MODS, the mechanism might be through improving the hemodynamic and hemorrheologic condition of patients to relieve nail2fold microcirculation disorder; influencing the levels of cytokine and inflammatory mediator, so as to alleviate the systemic inflammatory reaction, it might also abate the inhibited condition of gastrointestinal motility, alleviate the intestinal flora imbalance, prevent intestinal bacteria and endotoxin malposition, and protect cells from peroxidation.

Original article on CJIM (Chin) 2001;21(2)