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Effects of Sijunzi Decoction on Small Intestinal T Lymphocyte Subsets Differentiation in Reserpine Induced Spleen Deficiency Rats

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Abstract: Sijunzi decoction (SJZ), composed of Dangshen (*Codonopsis pilosula* Nannf.), Baizhu (*Atractylodes macrocephala* Koidz.), Fuling (Tuckahoe) and Gancao (*Radix glycyrrhiza*) is well known as a classical Chinese traditional and herbal medicamentum aiming directly at spleen deficiency in Traditional Chinese Medicine (TCM). The present research is to evaluate the regulatory effect of SJZ on small intestinal T lymphocyte subsets differentiation in reserpine-induced spleen deficiency rats. Reserpine induced spleen deficiency rats were orally administrated with SJZ (0.2, 0.6 and 1.0 mL/200 g, body weight) once a day for a period of 5 days. Clinical symptoms including body weight were observed every day, the small intestine damage and recovery by SJZ were detected by electron microscope scanning, small intestine lymphocytes were separated for T lymphocyte subsets detection by flow cytometry and T lymphocyte related cytokine expression was studied using quantitative real time PCR. Results showed that proper dose of SJZ can significantly promote the recovery of body weight and small intestine damage as well as regulate the differentiation of $CD3^+$, $CD3^+CD4^+$ T lymphocyte subsets and the expression of type-1 cytokines (IL-2, IFN-γ and IL10). Besides, a significant $CD4^-$ $CD8^-$ T lymphocyte subset was found in reserpine treated and high dose SJZ administrated rats. Data from this study proved that SJZ possess a significant promoting effect on the amelioration of spleen deficiency, $CD4^+$ T lymphocyte and cytokines related with regulation of T lymphocyte differentiation would be key targets of SJZ on spleen deficiency.

Key words: Sijunzi decoction, T lymphocyte subsets, reserpine induced, spleen deficiency, small intestine

INTRODUCTION

In Traditional Chinese Medicine (TCM), spleen represents a macroscopic concept of digestion, absorption and nutrition metabolism (Yin *et al*., 2004). Spleen deficiency is a common disease from clinical syndrome differentiation based on Zang-Fu theory and generally refers to a series of pathological symptoms, such as body weight loss, watery diarrhea, abdominal distention and depression (Hijikata *et al*., 2008; Cheng, 2005) which were caused by deficiency of spleen-Qi (Qi is the life force that flows through living beings and it is the energy that enables each organ system to do its job) (Batliner, 2004). Many reasons including maladjustment between work and rest, stress, anxiety and some chronic disease, may result in spleen deficiency. Researches on spleen deficiency mainly consisted of two respects. One is establish new spleen deficiency models and another is spleen deficiency treatment. Up to now, lots of methods were developed to establish spleen deficiency models which mainly divide into three kinds, method of starvation and exhausted (Li, 1991), breaking the spleen-Qi with potent Chinese herbal drugs and injection of Qi-inhibiting chemicals (Xu *et al*., 2004; Li *et al*.,

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2009; Xu *et al*., 2003). Reserpine is a drug that is thought to induce gastrointestinal damage via the depression of adrenergic activity with an increase in the cholinergic tone (Yamaguchi *et al*., 1978) and was defined as a Qi-inhibiting chemical in TCM. Experimental use of reserpine as an inductor of Qi inhibiting is widely accepted as a convenient model of spleen deficiency in laboratory (Zhao *et al*., 2010; Jia *et al*., 2006; Mi *et al*., 2005; Lin *et al*., 2010). Traditional Chinese medicine prescriptions are always the first choice for the treatment of spleen deficiency (Gao *et al*., 2009; Zhang *et al*., 2009; Xu *et al*., 1996), common used prescriptions for spleen deficiency including Liujunzi Tang, Yigongsan, Guipi Tang and so on. Most of them were derived from Sijunzi decoction (SJZ) which is composed by Dangshen (*Codonopsis pilosula* Nannf.), Baizhu (*Atractylodes macrocephala* Koidz.), Fuling (Tuckahoe) and Gancao (*Radix glycyrrhiza*) (Liu *et al*., 2005; Dai *et al*., 2006). It is a basic prescription for the cure of spleen deficiency and is also one of the most famous prescriptions recorded by Prescription of Peaceful Benevolent Dispensary (a famous prescription standard that exerted a very significant contribution to the development of TCM in history and also is the first prescription standard that published by the government of the People's Republic of China).

Many researches on the use of SJZ were conducted by TCM scholars in recent history. Xu *et al*. (1993) firstly proved that SJZ can enhance the clearance rate of i.v. charcoal particles as well as bone marrow cells inhibited by cyclophosphamide in mice. Later researches demonstrated SJZ possess many other functions such as radio-protective activities (Hsu *et al*., 1996; Chen and Fu, 1996) inhibition of gastric cancer cell growth (Zhao *et al*., 2002), increase the ability of T lymphocyte transformation and the activity of superoxide dismutase and glutathione pemfidase (Cheng *et al*., 2009). Simultaneously, researches on the treatment of spleen deficiency by SJZ found that SJZ could significantly improved the disequilibrium of gastric electrical activity and the microelement in spleen deficiency cattle and goat models (Zheng *et al*., 2007) as well as improving xylose absorption in spleen deficiency rats (Gao *et al*., 2009).

Previous study in the lab discovered SJZ also plays a role in regulating the gastrointestinal hormone secretion in spleen deficiency Beijing ducks (Dong *et al*., 2006; Zhang *et al*., 2006). However, up to now, few reports was found of the SJZ on gastrointestinal immune system and the mechanism of SJZ on reserpine induced spleen deficiency is still unclear. In the present study, to assess the Effects of SJZ on small intestinal T lymphocyte subsets differentiation in Reserpine induced spleen deficiency rats and to further explore the mechanism of SJZ on reserpine induced spleen deficiency, SD rat models were prepared by intraperitoneally injected with reserpine, the ameliorate effects of small intestine damage by SJZ were evaluated according to the clinical situation, microstructure of small intestine, CD molecules differentiation and cytokine expression in the models. The results proved that SJZ decoction possess a significant promoting effect on the amelioration of spleen deficiency, $CD4^+$ T lymphocyte and cytokines related with regulating of T lymphocyte differentiation would be key targets of SJZ decoction on spleen deficiency.

MATERIALS AND METHODS

Chinese crude drugs: *Codonopsis pilosula* Nannf., *Atractylodes macrocephala* Koidz., Tuckahoe and *Radix glycyrrhiza* were bought from Beijing Tongrentang Pharmaceutical Co., Ltd. (Beijing, China); Reserpine was purchased from Bangmin Pharmaceutical Co., Ltd. (Guangdong, China); Goat anti-mouse monoclonal anti-CD3-FITC, anti-CD4-APC and anti-CD8-PE antibodies were purchased from Becton Dickinson (Oxford, UK); Trizol and RevertAid First Strand cDNA Synthesis kit were obtained from Invitrogen (Carlsbad, CA, USA); Brilliant SYBR Green QPCR master mix was brought from Stratagene (La Jolla, California, USA). All other reagents used were of analytical grade.

Animals: Male SD rats weighing 200-220 g were purchased from Beijing Laboratory Animal Center (Beijing, China). The rats were kept in polypropylene cages in an air-conditioned room at 24±1°C with 12 h light cycle and fed pathogen-free food and water. All the procedures were performed in strict accordance with internationally accepted principles and the P.R. China legislation on the use and care of laboratory animals.

Preparation of SJZ: SJZ, a standard yin-yang-enrichment prescription of TCM composed by 60 g of Codonopsis pilosula Nannf, 60 g of Atractylodes macrocephala Koidz., 60 g of Tuckahoe and 30 g of Radix glycyrrhiza was extracted by water boiling method. Briefly, the dried prescription of herbs (210 g) was extracted twice with 10 fold volumes of boiling water (2100 mL) for 2 h each time. The result decoction was filtrated and concentrated to 1 g mL^{-1} and then stored at 4°C before administration. One of main components in the decoction was determined by high-performance liquid chromatography as 141.30 μg mL^{-1} of glycyrrhizin ($C_{42}H_{62}O_{16}$) and the yield of glycyrrhizin is 2.83% that is in accordance with the specifications of the Chinese veterinary pharmacopoeia.

Table 1: Primer sequences for detection of cytokines

Description	Accession number	Primer sequence	Production size (bp)
IFN-γ	NM_138880.2	5'-ACGCCGCGTCTTGGTTTTGC-3'	175
		5'-ACCGTCCTTTTGCCAGTTCCTCC-3'	
IL-2	NM_053836.1	5'-CTCGGAGCTCTGCAGCGTGT-3'	164
		5'-TCCACCACAGTTGCTGGCTCATC-3'	
IL-10	NM_012854.2	5'-CCGAGAGCTGAGGGCTGCCT-3'	183
		5'-CCATGGTTCTCTGCCTGGGGC-3'	
IL-4	NM_201270.1	5'-GGCTTCCAGGGTGCTTCGCAA-3'	150
		5'-GTGGACTCATTCACGGTGCAGC-3'	
β-actin	NM_031144.2	5'-GCGTCCACCCGCGAGTACAA-3'	118
		5'-ACATGCCGGAGCCGTTGTCG-3'	

Experimental model and treatment protocol: The spleen deficiency pattern in rat was induced by the intraperitoneal injection of reserpine. After 1 week' acclimatization, the rat were randomly assigned to reserpine group (30 rats) and normal control group (6 rats). They were intraperitoneally injected with reserpine or saline 0.5 mg kg^{-1} body weight for 1 week. The 6 rats from each group were sacrificed on day 8. The rest of the rats form reserpine group was divided into four groups (6 rats each group): $SJZ_{0.2}$ group, $SJZ_{0.6}$ group, $SJZ_{1.0}$ group and saline group. Rats form SJZ groups were orally treated with different dosages of SJZ decoction (0.2, 0.6 and 1.0 mL of SJZ decoction per 200 g body weight, equivalent to Sijunzi crude drugs 11.5, 34.5, 57.5 g kg^{-1} of body weight) for 5 days. Matched saline group were treated with corresponding volume of saline. All rats from above four groups were sacrificed on day 13.

Observation on the villus of duodenum membrane: The specimens from duodenum (15-25 mm from the pylorus) were cut into blocks measuring 5×3×2 mm and immersed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH = 7.4) for 24 h. The samples were dehydrated in a graded series of alcohol and acetone and transferred into amyl acetate. After critical-point drying, the blocks were mounted on aluminium plates with the peritoneal surface exposed and sputter-coated with gold-paladium. The examination was carried out with a scanning electron microscope (S-3400N, Hitachi, Japan).

Determination of T lymphocyte subsets in small intestine: Rats from each group were sacrificed on day 8 (reserpine group and control group) or day 13 (SJZ treated groups and saline group) for T lymphocyte subsets determination. Single lymphocyte suspension at a concentration of 1×10^6 cells/100 μL was separated from ileum segment and stained with isotype control or triple-stained with anti-CD3-FITC, anti-CD4-APC and anti-CD8-PE monoclonal antibody. Cells stained with isotype-matched monoclonal antibodies were used to determine the level of background fluorescence. The fluorescence intensities, two hundred thousand cells from each sample were measured using the FACSCalibur machine. $CD4^+$, $CD8^+$, $CD4^+CD8^+$ and $CD4^-CD8^-$ subset cells were counted within gated $CD3^+$ T lymphocytes. Results were analyzed using FlowJo Analysis Software (Version 5.7.2, Tree Star, Ashland, Oregon, USA).

Determination of IFN-γ, IL-2, IL-4 and IL-10 expression in small intestine: The pattern of cytokine mRNA expression in duodenum was determined by standard Reverse Transcription-Polymerase Chain Reaction (RT-PCR). In brief, total mRNAs of small intestinal tissues of individual rats were extracted using Trizol. cDNA was synthesized from 1 μg total RNA using RevertAid First Strand cDNA Synthesis kit following their instructions of Invitrogen. Brilliant SYBR Green QPCR master mix was used for RT-PCR and PCR was run for 40 cycles containing 95°C for 1 min, 56°C for 1 min and 72°C for 1 min. Expression levels were determined using the Relative Threshold Cycle (C_t) Method as described by the manufacturer (Stratagene). The sequences of the sense and antisense primers used for amplification are shown in Table 1.

Statistical analysis: Data were expressed as mean±SD. Statistical analysis was performed using an independent sample t-test available in the SPSS Software (Version12.0, Chicago, USA) where a $p<0.05$ was considered significant.

RESULTS

Changes of signs and body weights: Rat from reserpine group showed poor appetite, loose stools, lassitude, grouping and hypotrichotrophy since day 3. This condition was relaxed to a certain extent in SJZ administrated group and saline group after reserpine injection was ceased. Rat from $SJZ_{0.2}$ group and $SJZ_{0.6}$ group showed a better appetite compared with other groups after SJZ has been continuously administrated for 3 days. No significant difference of other clinical symptoms has been observed among SJZ groups and

saline group. The effect of reserpine on the body weight is shown in Fig. 1. Compared with control group, rats injected with reserpine showed a significant body weight loss from days 5-7. SJZ groups and saline group trend to recover body weight after reserpine ceased to inject (day 7), however, a significant difference was observed between SJZ treated groups and saline group since day 11 which demonstrated administration of SJZ could accelerate the tendency of body weights recovery. No significant differences were observed between the $SJZ_{0.2}$, $SJZ_{0.6}$ and $SJZ_{1.0}$ groups.

Effect of SJZ on the internal structure of the small intestine: Electron microscope pictures were shown in Fig. 2, the structure of duodenum villus in control group is normal with well-arranged intestinal villus and proper interspace (Fig. 2A), individual villus presents a thick body as an oblonga cylinder (Fig. 2A). The 7 days after injection with reserpine, the integrity of the duodenum was destroyed, villus were shown random disposition, thin body (Fig. 2B) and incomplete construction with absence of massive of cellula columno epithelial is in villus top (Fig. 2B). Rats from $SJZ_{0.2}$ and $SJZ_{0.6}$ groups showed a relative complete villus top, less absence of cellula columno epithelial is and many minor new-born cellula columno epithelial is (arrow a, Fig 2C and D), besides, a significant difference between these two groups is more fibrinous exudates can be observed in $SJZ_{0.6}$ group. The villus of rats in $SJZ_{1.0}$ group remaining seriously damaged, cellula columnoepithelialis of the villus exposed its basilar part (arrow b, Fig. 2E) around the denudate lamina propria (arrow c, Fig. 2E). The structure of duodenum is unsharp in saline group, most of the villuses were covered by massive of disaggregated products and it is difficult to distinguish individual villus (Fig. 2F).

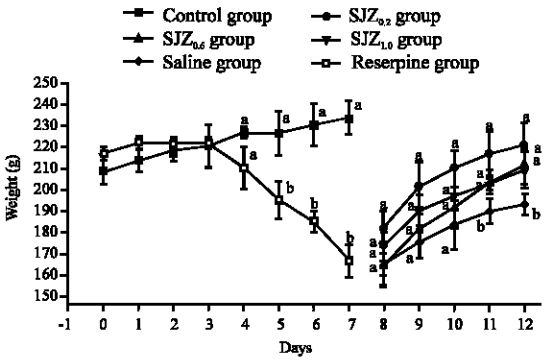

Fig. 1: Changes of the body weight in the rats from different groups. Letters (a, b) denote significant differences ($p<0.05$)

Changes on T lymphocyte subsets in small intestine: Presentation of $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD3^+CD4^-CD8^-$ T lymphocyte subsets in rat small intestine and the difference between different groups were shown in Fig. 3a-e. In normal control group, $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$ T lymphocyte account for a proportion of 63.58, 56.86 and 20.21%, separately (Fig. 3a-c). A very small population of $CD4^+CD8^+$ and $CD4^-CD8^-$ T lymphocytes <2% were detected. In reserpine treated group, the rats of $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ T lymphocytes were significantly decreased to a lower proportion of 47.21, 15.92 and 3.18%, separately (Fig. 3a-c). $CD4^-CD8^-$ T lymphocytes, few was expressed in normal control group, increased significantly (from 1.98-4.56%) and constructed a conspicuous T subpopulation (Fig. 3d). In SJZ or saline treated groups, $CD3^+$ and $CD3^+CD4^+$ T lymphocytes of $SJZ_{0.2}$ group were significantly higher than that of $SJZ_{0.6}$, $SJZ_{1.0}$ and saline

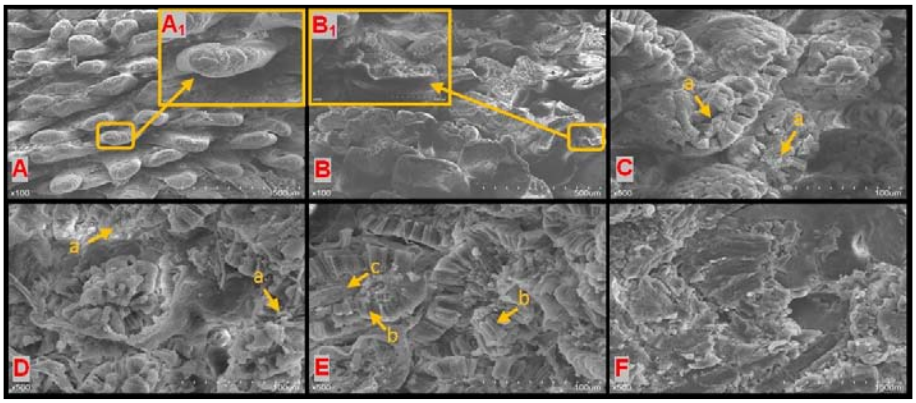

Fig. 2: Scanning electron microscope images of the small intestine. A and A_1) control group, B and B_1) reserpine group, C) SJZ0.2 group, D) SJZ0.6 group, E) SJZ1.0 group, F) Saline group where A1, B1, C, D, E and F magnified 500 times, A and B magnified 100 times. Similar results were obtained in three rats from each group

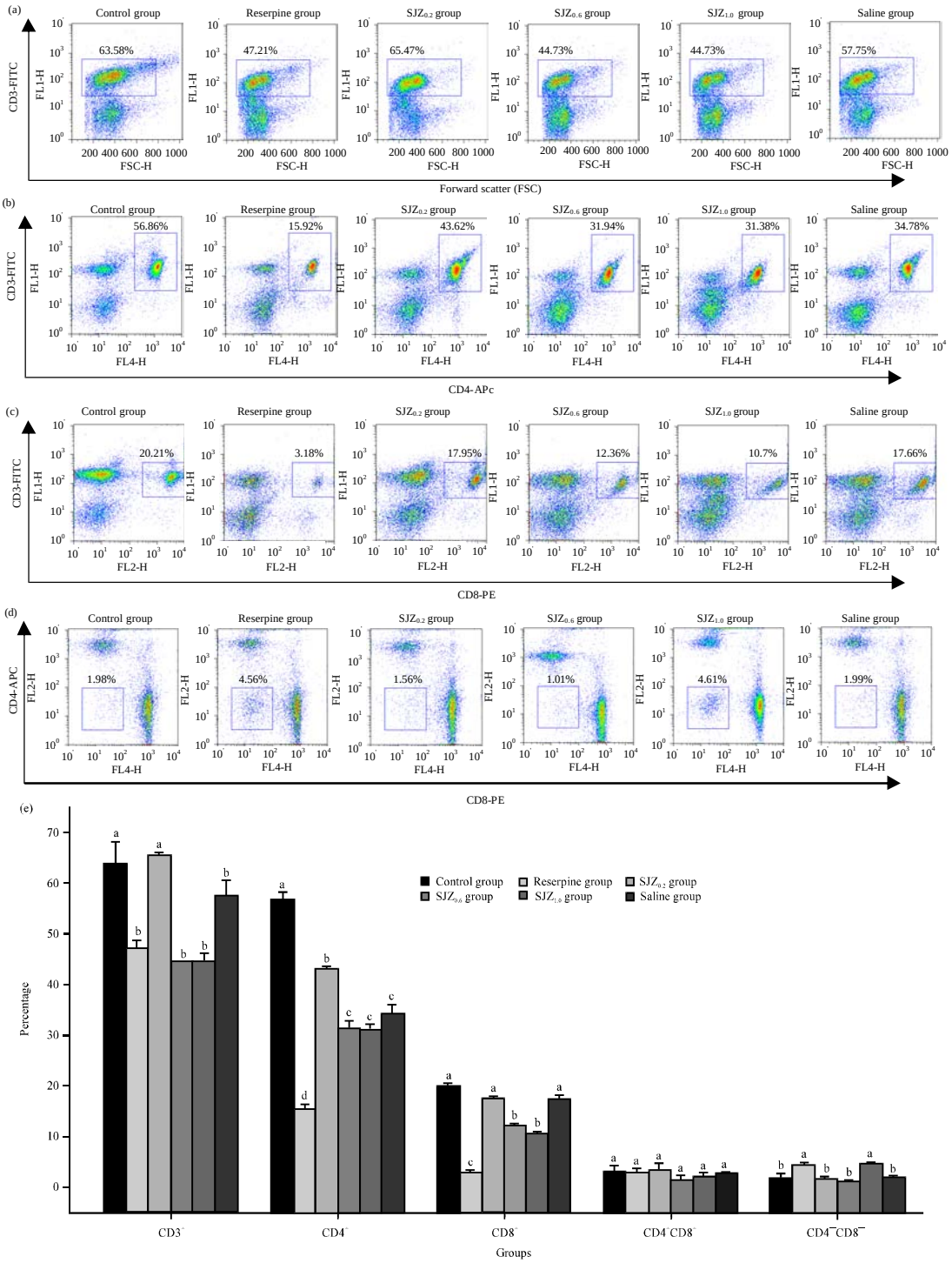

Fig. 3: Flow cytometric analysis of rat T cell subsections in small intestine. Cells in a-d) were gated on $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells and $CD3^+CD4^-CD8^-$ T cells, separately. Similar results were obtained in three independent experiments. e) The T cell subsection changing between different groups. Data at the same time point with different letters (a-c) differed significantly ($p<0.05$)

group. $CD3^+CD8^+$ T lymphocytes of $SJZ_{0.6}$ and $SJZ_{1.0}$ group were significantly lower than that of $SJZ_{0.2}$ and saline group but no significant difference were observed between $SJZ_{0.6}$ group and saline group. $CD4^-CD8^-$

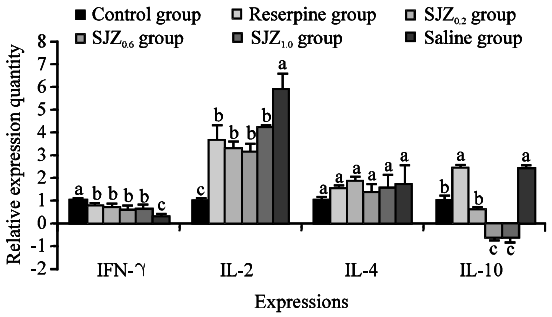

Fig. 4: Effect of SJZ on IL-2, IL-4, IL-10 and IFN-γ expression in small intestine. Data at the same time point with different letters (a-d) differed significantly ($p<0.05$)

T lymphocytes were up regulated in $SJZ_{1.0}$ group and significantly higher than other SJZ or saline treated groups. Besides, no significant variation of $CD4^+CD8^+$ T lymphocytes was detected in all groups (Fig. 4).

Changes on IL-2, IL-4, IL-10 and IFN-γ expression in small intestine: Compared with control group, expression of IL-2 and IL-10 were significantly enhanced while IFN-γ was significantly depressed in reserpine treated group. This tendency kept on through the following treatment stage, especially IL-2 reached to a significant higher level and IFN-γ reached to a significant lower level in saline group. However, in SJZ decoction administrated groups, the expression of IL-2 and IL-10 were significantly down regulated while IFN-γ was significantly up-regulated compared with saline group. $SJZ_{0.6}$ and $SJZ_{1.0}$ groups showed a significant down regulation of IL-10 compared with all other groups. No significant differences were observed of IL-4 expression among all groups.

DISCUSSION

Although, the TCM is widely utilized clinically as well as the Western medicine, differences do exist between those two kinds of medicine, especially in the development process and evaluation of pharmacodynamics. The development process of Western medicine always begins with research on mechanism and ends with clinical verification. The influencing on biological effect of a certain target of the Western medicine is usually regarded as a standard in evaluation of pharmacodynamics in Western medicine. However, most of the new TCM due to the compatibility of drugs, complex ingredients and multi-targets are not from laboratory but directly from the distilling of the daily clinical practice (Zhou *et al*., 2010) and hard to indentify its mechanism of pharmacologic action. To choose the most effective formula by clinical practice or clinical experiment of several different prescriptions is always the first step to develop a new TCM. Further investigation of the pharmacological properties and mechanism is always a posterior step. The history of SJZ is a typical development process of TCM. This classical formula was sieved from a great quantity of TCM specifically for curing stomach intestine diseases by long-term clinical practice and its clinical effect has been proved by using in humanity and animals for hundreds of years history.

With the development of science and technology, TCM scholars began focusing their attentions on the mechanism and new functions of SJZ in recent years. Up to now, research proved that SJZ could inhibit gastric cancer cell growth via induction the cell apoptosis and expression of *p53* and *bcl-2* genes (Zhao *et al*., 2002), improve the immunomodulatory effects on D-galactose-induced aging mice by increasing the ability of T lymphocyte transformation and increased the activity of superoxide dismutaseand glutathione pemfidase in serum (Cheng *et al*., 2009) as well as enhance immune function of organism by strengthening spleen and tonifying Qi (Zhang *et al*., 1999). However, few researches with regard to the recovery effect of SJZ on spleen deficiency from the immunology point of view were carried out and the mechanism of SJZ on spleen deficiency is still unclear.

It is well known that the epithelial cells of the gastrointestinal tract are continuously exposed to various toxic stimuli that may cause mucosal injury (Martinez *et al*., 2004). During injury, it is customarily the intestinal villi that get hurt at the very beginning, so the injury degree of intestinal villi is always regards as a standard to evaluate the damage. In the present research, electron microscope scanning on the small intestinal villi proved that the microstructural integrity of small intestine in rats of SJZ treated groups is obviously better than that in control group (mainly personification in the absence of intestinal villi epithelial cells). And among all SJZ treated group, $SJZ_{0.2}$ and $SJZ_{0.6}$ group have a more clear structure of intestine villi and less absent of epithelial cells. These results suggested that SJZ decoction at a proper dose can significantly enhance the recovery of small intestine damage in reserpine-induced spleen deficiency.

The cellular immune response is critical to the host defense system against infection by accelerating the clearance of pathogens and secreting many cytokines for the regulation of the immune response. T lymphocyte mediated immunity is usually regarded as a basic index when evaluating the cellular immune state in different organs. In this research, small intestinal T lymphocyte subsets differentiations were detected by flow cytometry. The expression of CD3 in reserpine treated group (47.21%)

and $SJZ_{0.2}$ group (65.47%) proved that $SJZ_{0.2}$ can enhance CD3 molecule's expression in reserpine induced spleen deficiency. Most of the T lymphocytes express CD3 molecule and the expression of CD3 molecule always represent the differentiation state of T lymphocyte to a certain extent, so this result also indicated a role of SJZ in regulating T lymphocyte differentiation. Helper T lymphocyte (Th-cell, $CD3^+CD4^+$ subset) and cytotoxic T lymphocyte (Tc-cell, $CD3^+CD8^+$ subset) are two major T subsets defined by their selective surface expression of CD4 or CD8. Th-cells are activate both humoral immune responses and cellular responses and Tc-cells show a major cytotoxic activity against cells infected with intracellular microbes and against tumor cells (Chaplin, 2010).

Both effector Th-cells and Tc-cells can be subdivided into two cell subsets separately, termed as Th1, Th2 and Tc1, Tc2, according to differences in their corresponding cytokine expression profiles. Th1 and Tc1 produce mainly IL-2 and IFN-γ (type-1 cytokines) whereas Th2 and Tc2 produces IL-4 and IL-10 (type-2 cytokines). Both type-1 and type-2 cytokines play an important role in regulating T lymphocyte differentiation. To evaluate T lymphocyte mediated immunity correctly, the differentiation state of two major T lymphocyte subsets and the expression of type-1 and type-2 cytokines were detected in this research. Result showed that SJZ can significantly influence the differentiation of Th cells but much less obvious to Tc cells. Simultaneously, the expressions of type-1 cytokines and one of type-2 cytokines (IL10) were significantly regulated in SJZ group.

According to these result, researchers concluded that the effect of SJZ on T lymphocyte mediate immunity in reserpine induced spleen deficiency rats mainly depending on its regulation effect on Th-cell and type-1 cytokines. With regard to the down regulation of IL10 in SJZ administrated groups, it is uncertain whether it was result from a direct effecting of SJZ. It is now known that IL10 not only has a key effect on the suppression of Th1-cell responses but also suppress the differentiation of Th2 cells (Hawrylowicz and O'Garra, 2005). Down regulation of IL10 may indicate an enhancement of cell mediated immunity. Although, IL-10 was originally isolated from Th2 cells, the expression of IL10 is not specific to Th2 or Tc2 cells but instead that it is a much more broadly expressed cytokine (Saraiva and O'Garra, 2010). Except for T lymphocyte, many other cells of the of the innate immune system, including dendritic cells, macrophages, mast cells, NK cells, eosinophils and neutrophils (Moore *et al*., 2001). Besides, IL10 is an anti-inflammatory cytokine with an important role in preventing inflammatory, the expression of IL10 often reflect the level of inflammation. Accordingly, high-level expressions of IL10, the damage of the intestinal villus (results from scanning electron microscope images) in saline group and a low-level expression of IL10, the relative complete construction of intestinal villus made we believe that IL10 may an important target, through which the inflammation degree could be significantly down-regulated by SJZ. To sum up, the mechanism of SJZ on spleen deficiency has an important relation with small intestinal T lymphocyte differentiation activity. $CD4^+$ T subset and related cytokines expression (IL2, IFN-γ and IL10) would be the main targets regulated by SJZ in spleen deficiency rats.

Apart from effector T-cell subsets, accumulating evidence has demonstrated that regulatory T-cell (Treg) subsets also play an important role in the maintenance of immunologic self-tolerance and down-regulating various immune responses (Chen *et al*., 2004). The most classic Treg cell includes $CD4^+CD25^+FOXP3^+$ T-cell (Pillai *et al*., 2007), γδ+T cell and NK cell (Von Bubnoff *et al*., 2010; Lu *et al*., 2007). $CD4^-CD8^-$ T lymphocyte is a novel subset with regulating effect on eliminating the undesired effector clone via Fas/FasL mediated apoptosis (Fisher *et al*., 2005). Matured $CD4^-CD8^-$ Treg cells are a subset of T lymphocytes that present less in the periphery (1-3% of small intestinal T lymphocytes in this research) and educe depressant effect on effector T lymphocyte. Recent research found that $CD4^-CD8^-$ T lymphocyte will express significantly in parasitic infection, heterogenic transplantation and some other immunological diseases (Nagib *et al*., 2007; Ling *et al*., 2007; Zhang *et al*., 2000; Priatel *et al*., 2011).

However, there is no information on the occurrence of these cells in intestinal damage and recovery. In this research, researchers find that $CD4^-CD8^-$ T lymphocyte subset participated in intestine damage of spleen deficiency rats and SJZ exerted an effect on the expression of this subset. Results demonstrated that the influence SJZ on cell immunity is not only limited to major T lymphocyte subsets but also on smaller subsets with specific regulating function just like $CD4^-CD8^-$ which may play a significant role in spleen deficiency rats. Beside, taking into account the damage and recovery situation of the small intestine in reserpine treated group and $SJZ_{1.0}$ group detected by electron microscope scanning, researchers prefer to believe that high expression of $CD4^-CD8^-$ T subset in reserpine treated group (4.56%) and $SJZ_{1.0}$ group (4.61%) may imply this subset exerted an adverse regulating effect on T lymphocyte mediated immune response and is harmful for the amelioration of reserpine induced spleen deficiency. Chinese medical philosophy is characterized by its emphasis on the restoration and maintenance of

balance (Wang *et al*., 2007). The effect of SJZ on spleen deficiency rats including the amelioration of small intestine damage, recovery of T lymphocyte differentiation and cytokines expression proved SJZ is an effective TCM prescription and the pharmacologic mechanism of SJZ was coincidence with the theory of Chinese medical philosophy.

CONCLUSION

SJZ exerts regulating effects on the recovery process of reserpine induced spleen deficiency mainly by regulating $CD4^+$ T subset and cytokines related with T lymphocyte differentiation. $CD4^-CD8^-$ T subset participated in the reserpine induced spleen deficiency and the amelioration process regulated by SJZ but this subset may act as a suppressor on the amelioration of spleen deficiency.

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