

Effect of Different Kinds of Ginseng Saponin on the Immunity of Young and Old Mice

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ABSTRACT

Ginseng saponin from different part of Ginseng and 7 kinds of ginsenosides effect on immno-function of young and old mice. There have up-regulation effect *in vivo* and *in vitro* experiments for low function of old mice, but not up-regulation effect at normal immuno-function of young mice. That means “Adaptogens” of Ginseng saponin. Not oppositions effect at Rg1 and Rb1 and has find, that effect relations with molecular weight of ginsenosides.

Introduction

There are a number of different kinds of ginseng: *Eleutherococcus senticosus* (Siberian ginseng), *Panax quinquefolium* (American ginseng), *Panax ginseng* (Chinese or Korean ginseng), and *Panax japonicum* (Japanese ginseng). *Panax ginseng* is the most widely used species. But, there have different types according to manufacturing (Red Ginseng, Whit Ginseng and freeze dry Ginseng), and different part of ginseng (Root, Rhizoma Leaf- stalk and fruit). What is it's different effects ? how can selection of several ginseng and it's manufacture products is useful in clinical?

The two most important ginsenosides are Rb-1(Protopanaxadiol Saponin PDS) and Rg-1. (Protopanaxatriol Saponin PTS). Some reports said: Rb-1 has a stress relieving effect on the body while Rg-1 has a stimulating effect. By Chinese traditional theory, the effect of Red Ginseng which is tonic is Yang and heat (like stimulating effect), the effect of white ginseng and American ginseng which are worm or cool is nourish Yin (like relieving effect).

A large number of Pharmacological studies on *Panax ginseng* and its saponin have shown a variety of effects (1). However, there have been so far poor information about the combinative studies for different kinds and part of ginseng. The body has a homeostatic mechanism to maintain internal environment, and the immune system plays a major role in homeostatic mechanism together with nervous and endocrine system. Medical substances which help maintaining homeostasis were named as “Adaptogens”, and ginseng saponin is on of well- known adaptogens in Chinese traditional medicine.

Two questions are very important: one question is different kinds of ginseng, there have same

effect for “adaptogens” or not. Second question is same kind of ginseng was used by different condition of body, which is same effect or not ? such as young or old , hypotension or hypertension.

In this report, we studied on immuno-regulatory effect of ginseng saponin from different parts of Ginseng (root, fruit and stalk-leaf) and 7 kinds of ginsenosides, and observed *in vivo* and *in vitro* effect of these materials on the immune functions of young and old mice.

Materials and Methods

Mice : C57BL/6 male mice reared in animal facility of Tokyo Metropolitan Institute of Gerontology were used in the present study. Young mice were 7 weeks old and old ones were 25 months of age.

Ginseng preparations: Crude extracts of *Panax ginseng* employed were GSF (extract from ginseng fruit), GSSL (extract from ginseng stalk and leaves) and GSR (extract from ginseng root). They were prepared according to the method by Shibata (3). Seven kinds of ginsenosides were purified by HPLC according to the method of Shibata: Rb1, Re, Rg1, Rg2, Rg3 and Rh1 in the order of decreasing molecular weight. Molecular weights of these ginsenosides were shown in Table 1. It was shown in TLC profiles (Fig.1) that 3 kinds of crude extracts ginseng (GSF,GSSL,GSR) were composed of different kinds and amounts of ginsenosides. The crude extracts were used for both *in vivo* and *in vitro* experiments, and ginsenosides were used only for *in vitro* experiments.

Table 1. Ginsenosides and molecular weight

Type	KINDS	MOLECULAR WEIGHT
PDS	Rb1	1108
	Rb2	1078
	Rd	964
PTS	Re	946
	Rg1	800
	Rg2	784
	Rg3	784
PTS	Rh1	638

In vivo experiment:

Crude ginseng extracts (GSF) 6 mg were dissolved in DDW(6mg/ml) and daily given to mice by gastric tube at 0.01/g body weight for 7 days. One day after the last administration, mice were sacrificed and their spleen cell suspensions were used for the immunological assessment. Number of spleen T cells (Thy-1+ cells), T cell subsets (L3T4+ cells and Lyt-2+ cells) and B cells (surface immunoglobulin+ cells) were assessed by a flow cytometer (FCM-1, JASCO), spleen cell suspen-

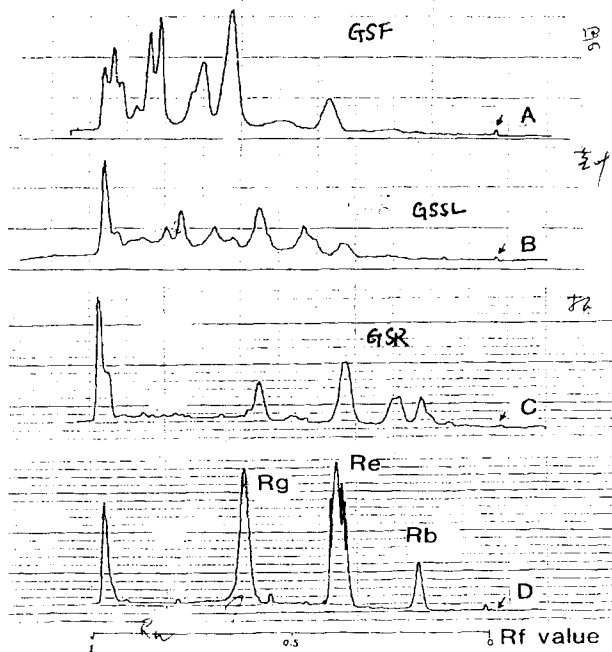


Figure- 1

Table-2: In vivo effect of GSF on the immunity of young and old male C57BL/6 mice

	young (7 weeks)		old (25 months)	
	control (n=5)	ginseng (n=5)	control (n=5)	ginseng (n=6)
body weight (g)	23.12	23.22	36.62	36.02
spleen (mg)	61.32	67.80	86.18	92.05
spleenic cell number (x10 ⁷)	9.87	9.92	11.6	10.1
Thy12 ⁺ (%)	37.99	35.11	24.23*	29.35*
number (x10 ⁷)	3.73	3.47	2.82	2.96
L3T4 ⁺ (CD4) (%)	22.74	21.89	19.88	24.12 [†]
number (x10 ⁷)	2.24	2.16	2.39	2.41
Lyc2 ⁺ (CD8) (%)	16.56	16.22	8.40	8.57
number (x10 ⁷)	1.63	1.60	0.982	0.821
CD4/CD8 ratio	1.37	1.35	2.73	3.35
IgG ⁺ (%)	40.90	42.26	57.77	55.18
number (x10 ⁷)	4.03	4.19	5.67	5.56
T/B ratio	0.930	0.831	0.430	0.537
PHA (cpm)	21,292	15,553	9,996	2,850
ConA	55,539	55,001	4,762	3,420
LPS	14,255	9,996	20,073**	12,217**

* : p < 0.05 ** : p < 0.01

sions were also used for the activities of mitogenic responses as described below.

In vitro experiment:

Crude ginseng extracts (GSR, GSF and GSSL) and seven kinds of ginsenosides were dissolved in RPMI 1640 at 0.6mg/ml, and serially diluted at 10 fold. Fifty μ l of the serially diluted solutions were mixed with 5×10^5 spleen cells in RPMI-1604 containing 10% FBS and optimum doses of PHA(2.5 μ g/ml) or LPS (2.5 μ g/ml) in 96 wells microplate. For experiment of proliferation in HELF 2BS cells were in RPMI-1640 containing 10% FBS with different concentrations of crude ginseng extracts and seven kinds of ginsenosides . The 96 wells plates were cultured in CO₂ incubator 68 hours of spleen cells ³H- thymidine was added 2 hours before the harvesting. HELF 2BS cells were cultured in CO₂ incubator 7 days and cell numbers were determined.

Results and Discussion

1. *In vivo* effect of crude ginseng extract GSF:

As shown in Table 2, statistically significant effect was observed only in the percentage of splenic

T cells of old mice, not of young mice, although the absolute number was almost comparable between control and experimental groups. In terms of ratio, not absolute number, a trend of increase was also observed in L3T4+ cells in the spleen as well as in the ration of L3T4/lyt-2 (CD4/CD8) of old mice. The T/B ratio is increased at old mice also.

Although statistically not significant, a trend of decrease was observed in PHA response of both young and old mice. ConA response was almost comparable between control and experimental groups, both in young and old mice. A trend of decrease was also observed in LPS response of both young and old mice.

2. *In vitro* effect of crude ginseng extracts.

2.1 In Young spleen cells : showed GSR and GSSL suppressive effect on PHA responses and showed a slightly suppressive effect of GSF. In LPS responses is very slightly(Fig. 2).

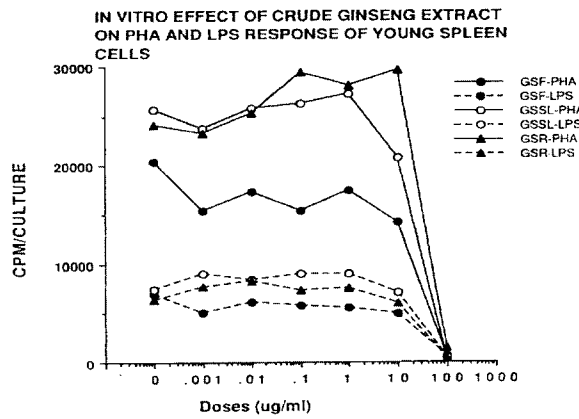


Figure-2

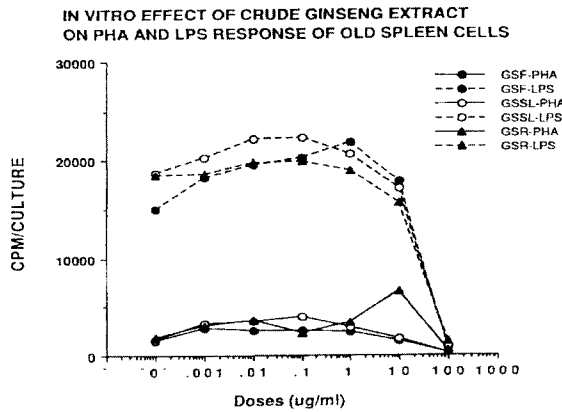


Figure-3

2.2 In Old spleen cells: showed GSR,GSSL and GSF suppressive effect on LPS responses , and no suppressive effect on PHA responses(Fig.3).

3. In Spleen cells , in vitro effect of ginsenosides Rb1, Rd, Re, Rg1, Rg2, Rg3 and Rh1.

3.1 PHA response of Young spleen cells: ginsenosides Rg1, Rg1 and Rge on PHA responses is over 1 and lower 2 times of level, another is lower 1. But, Rg3 was over 2 times at 100 μ g/ml. (Fig.4)

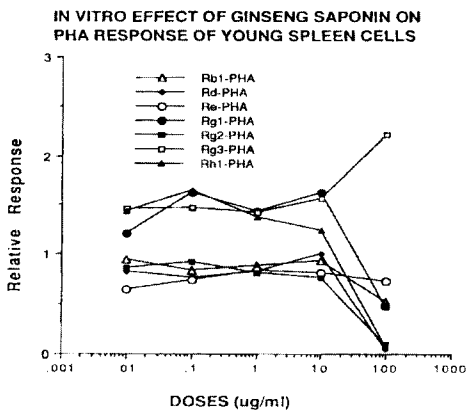


Figure-4

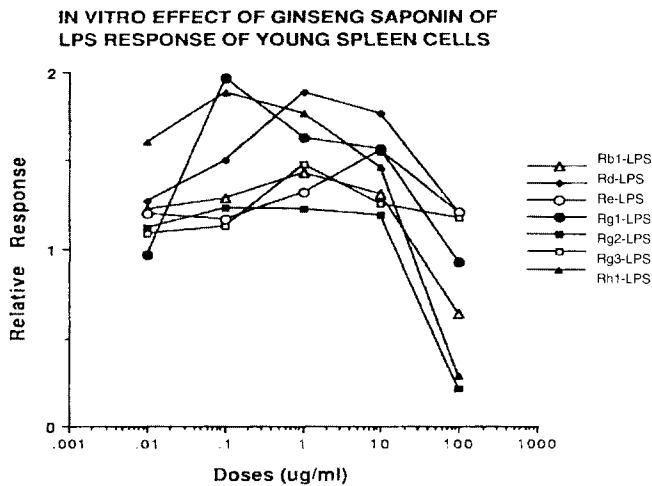


Figure-5

3.2 LPS response of Young spleen cells is lower 2 times. of level of all kinds ginsenosides (Fig.5).

3.3 PHA response of Old spleen cells: Rd and Rg was over 2 times of level at 0.1 μ g/ml concentration (Fig. 6).

3.4 LPS responses of Old spleen cells was lower than 1.5 times of level (Fig.7).

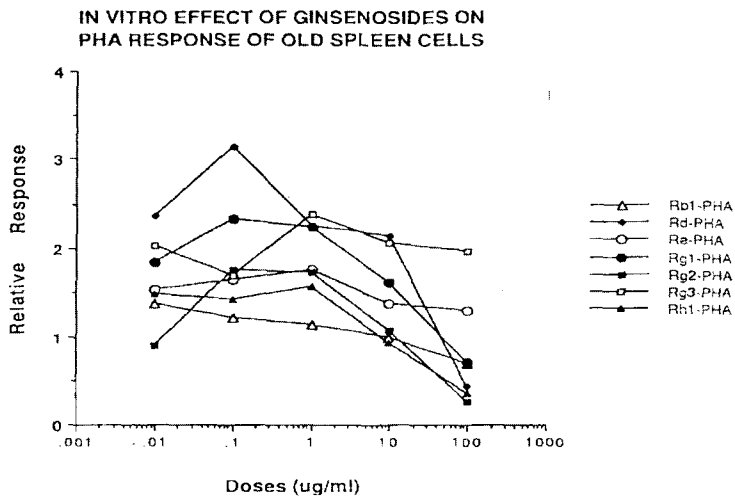


Figure-6

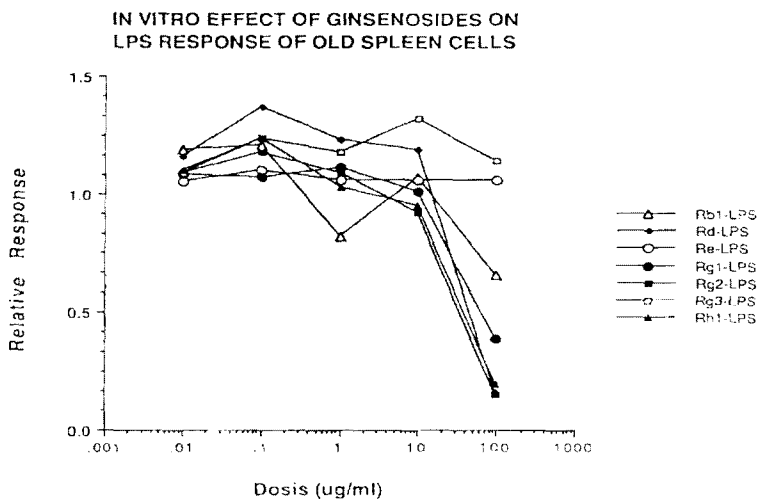


Figure-7

4. Effect of SGR, RGF and GSSL on the rate of proliferation in HELF 2BS cells. All of the three kinds saponin can proliferation in HELF 2BS cells. And they have an equivalent effect point of doses, over this doses, the SGR will going to high top and over 80% rate of proliferation , ant SGF and GSSL will go down and we can see at the same point of doses (doses at 10-100 $\mu\text{g/ml}$) is oppositional (Fig 8).

Most researches reported that the effect of ginseng was up-regulation of immune function (2,4).

Fig 8. Effect of Ginseng saponin from different parts on proliferation of HELF 2BS

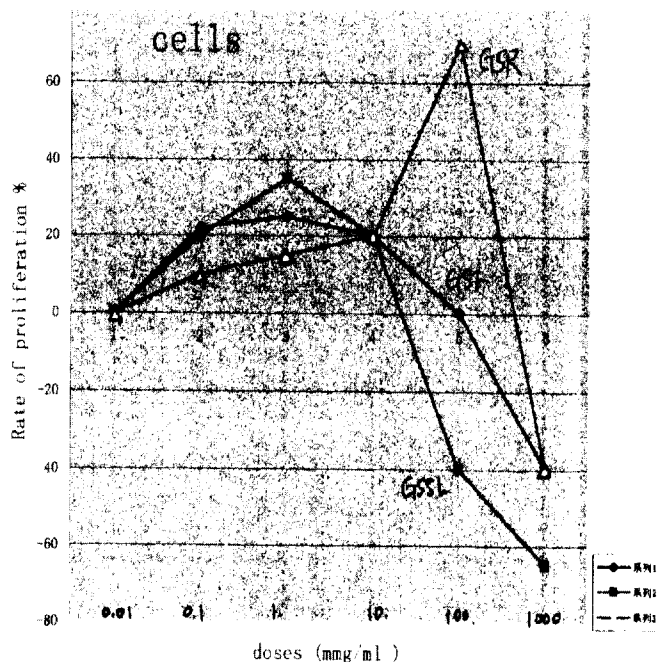
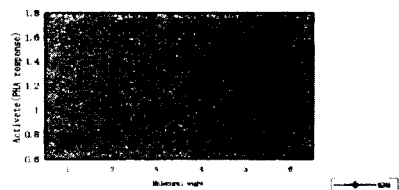


Fig 9. Linear regression analysis between activity and molecular weight of Ginsenoside



To the contrary, Chong (5) reported that ginseng could reduce the response of lymphocytes to PHA-P.

In our experiment, *in vivo*, ginseng saponin can up-regulation of immune-function at old mice, that is lower condition of immune function of old age mice. But at young mice, that is normal condition of immune function, the ginseng saponin have not any up-regulation of immune -function. That is means “Adaptiton” or “Adaptogens” for ginseng .

What is the mechanism of the “Adaptogens” or double side regulations effect ? Some reports said : There have two kinds of different effects of ginsenoside, such as Rb1 (and group Protopanaxadiol

Table 3. Linear regression analysis between immunity activity on mice and molecular of Ginsenoside (Stimulational index of PHA on Spleen cells of mice)

Doses of GS	Young mice (7weeks)		Old mice (25 month)	
	Y= a + bX	r	Y = a+bX	r
0.01 µg/ml	2.35 - 1.28X	- 0.89	3.34 - 1.78X	- 0.97
0.1	2.35 - 1.28X	- 0.98	3.96 - 2.47X	- 0.79
1.0	2.78 - 1.07X	- 0.97	5.05 - 3.54X	- 0.99
10.0	3.08 - 1.93X	- 0.97	4.03 - 2.75X	- 0.93

Molecular weight : Rb1 = 1080 Rg1 = 800 Rg3 = 784 Rh1 = 638

Saponin PDS) have down-regulation effect and Rg1 (and group Protopanaxatriol Saponin PTS) has up-regulation effect. In this experiment, we find : important factor is the condition of organs , if it is old (Low function), ginseng can up - regulation, or no effect on normal condition. All of the different kind was regulation at same effect, especial *in vivo* can change it's structure in gastric and intestine tube (6,8) but may have different doses at same effect, and effects power may relations with it's molecular weight of ginsenosides (Fig. 9 and Tab. 3)

All of saponin from different part of Ginseng such as GSR, GSF and RSSL. They have proliferation effect on HELF 2BS cells, but the effect is different at doses. At some range they may have opposition effect at same doses. (7)

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