

B

1, 2, 3
 1 . 1 . 2 . 3 . 1*

Characteristics of B cell proliferation by polysaccharide fraction of *Paeonia japonica* miyabe

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Background : *Paeonia japonica* Miyabe is a medicinal plant which has been widely used as a component of blood-building decoctions (Chinese medicinal concept: Bu-Xie). The immunopharmacological characteristics of the extract of *Paeonia japonica* (PJ) were investigated. **Methods** : The effects of fractions of PJ extract on lymphocyte proliferation were measured by H³-thymidine incorporation assay. The proliferated lymphocyte subsets were analyzed in flow cytometry. The subset cell populations of spleen cells were separated by magnetic cell separation system, and their proliferation by the extract were investigated. The effect of the extract on antibody production was determined in mice challenged with sheep red blood cells (SRBC) using hemolytic plaque forming cell assay. **Results** : Spleen cells were proliferated by water extract of PJ. Polysaccharide fraction (PJ-P) of the extract was most active in the proliferation. It was found in flow cytometry that the lymphocyte subset proliferated by PJ-P was B cell population. Among the separated subset cell populations, T cell-depleted cell population and macrophage-depleted cell population were most proliferated by PJ-P. However, positively selected populations of B cells and T cells were not proliferated by PJ-P. These results indicate that B cell proliferation by PJ-P may require the assistance of macrophages or T cells. These results suggest that firstly PJ-P may stimulate macrophages or T cells, and then B cells are activated. The number of antibody-secreting cells was increased by administration of PJ-P in mice immunized with SRBC as a T-dependent antigen. **Conclusion** : These results suggest that macrophages and accessory cells are directly activated by PJ-P and then helper T cells and B cells are indirectly activated. As the results, immune responses might be coordinately improved. In conclusion, PJ-P, a polysaccharide of *P. japonica*, may be a characteristic immunostimulator, which is analogous to polysaccharides such as lentinan, PSK and ginsan.

Key Words: *Paeonia japonica*, immunomodulator, splenocyte, FACS, MACS

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, 1960 paeo-
 niflorin (27). Egger (28)
 paeoniflorin
 가 (29)
 (30)
 가 .
 (1-3).
 (4).
 1970
 Bacillus Calmette-Guerin (BCG)
 (2). , 1980
 cytokine, lymphokine, growth factor C57BL/6 mouse (SPF)
 (1). cytokine 가 22 ± 2 , 가 55 60%
 12 ,
 cytokine
 가 cytokine
 cytokine 2.
 (Paeoniae Radix; root of *Paeonia japonica*
 Miyabe)
 10 2
 (5-11). (PJ-T)
 lentinan (12-14), schizofilan (15), poly-
 saccharide K(PSK)(16,17) ginsan (18-21) 100% ethanol 4 가 가 4 24
 (PJ-P)
 , polysaccharide , ethanol (PJ-E)
 , limulus amoebocyte lysate
 , Lee (22) (E-TOXATE, sigma) test lipopoly-
 saccharide (LPS)가 (31).
 3.
 (23-26). ,
 Rowry 2N NaOH
 가 100 가 lowry
 가 가 UV-
 Ranunculaceae Paeonia folin 가

spectrophotometer 660nm
 (32). phenol-H₂SO₄
 가 가 가
 UV-spectrophotometer 480nm
 (33). metahydroxydiphenyl
 Na₂B₄O₇/H₂SO₄ 가 10
 0 5 가 m-hydroxydiphenyl
 가 UV-spectrophotometer
 520nm (34).
 4.
 70% ethanol
 Hank's balanced salt solution (HBSS; GIBCO)
 petri-dish , 5% FBS-HBSS가 petri-dish
 ,
 1
 1500 rpm 10
 cell pellet ACK
 buffer (Tris-NH₄Cl) 1 FBS
 10ml 가 HBSS 2
 trypan blue
 10% fetal bovine serum 1%
 penicilline-streptomycine RPMI
 37 , 5% CO₂
 5. (proliferation)
³H-thymidine uptake
 96-well flat bottomed microplate (Corning)
 well 2 × 10⁵
 LPS ConA
 가 2 , 3 , 4 , 5
 3 well 1.5
 μCi ³H-thymidine 가 4
 cell harvester (Inotech) glass fiber filter strip
 filter paper
 scintillation vial scintillation cocktail 3 Ml
 -scintillation counter ³H-thymidine incorpora-
 tion cpm

6. subset (Fluorescence-
 activated cell sorter; FACS)
 2 × 10⁵/well ,
 PJ-P 100 μg/ml 가 3 , 4
 , 5 subset FACS
 . FACS
 trypan blue
 , FACS medium
 tube 10⁶
 Fc RIII block CD16/CD32 antibody (Par-
 mingen) 가 ice bath 5
 tube
 가 ice bath 40
 B
 anti-IgM antibody, T anti-Thy 1.2
 antibody, helper T anti-CD4 antibody,
 cytotoxic T anti-CD8 antibody
 , Pharmingen FITC-conjugated
 antibody . FACS medium 2
 FACStar (CULTER, USA)
 7. MACS subset PJ-P
 PJ-P 가
 subset subset
 magnetic cell sorter (MACS: Miltenyi
 Biotec, Germany) subset
 100mm dish
 2 dish
 . T CD90 micro-
 beads, B CD19 microbeads
 . microbeads ice-bath
 30 T B
 depletion column 25AS column
 , T B positive
 column 25RS column .
 trypan blue
 . well 2 × 10⁵
 PJ-P 100 μg/ml 가 2 , 3 , 4 , 5

³H-thymidine incorporation cpm

8. Hemolytic plaque forming cell assay (35)

C57BL/6 sheep red blood cell(SRBC) 1× 10⁹ cells/0.2 Mℓ

PJ-P 100 mg/kg B.W /0.2 Mℓ

SRBC 4, 7 × 10⁶ cells/Mℓ

ice-bath SRBC 3

RPMI 1640 20%가

ice-bath

0.5% agarose-RPMI

SRBC, 0.5% agarose 100 μℓ, 100 μℓ, 1,600 μℓ 가 petri- dish (Corning)

incubator 2 37 5% CO₂

complement) 1:70 GPC (Guinea pig petri-dish 600 μℓ 2

plaque PFC/spleen

cpm

PJ-P PJ-T 2.3

Fig. 2 PJ-T 3

PJ-P 4

PJ-P

B mitogen

LPS 2, T mitogen ConA

2 3

3. PJ - P subset

PJ-P subset

Table . Chemical composition of the extract of *Paeonia japonica* Miyabe

Component	Content (%)	
	Water extract	Crude polysaccharide
Protein	20.7	19.8
Total sugar	46.3	70.6
Uronic acid	2.2	15.1

1.

uronic acid table

PJ-T가 46.3% PJ-P가 70.6%

20.7% 19.8%

uronic acid 2.2% 15.1%

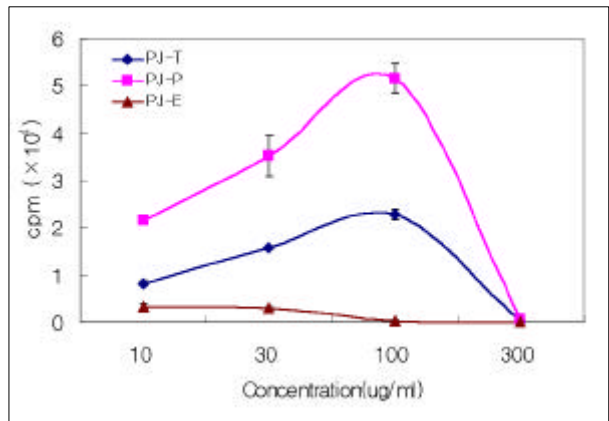


Fig. 1. Effects of fractions of *P. japonica* extract on proliferation of splenic lymphocytes *in vitro*. Spleen cells (2 × 10⁵/well) were cultured with fraction of the extract on 96-well flat bottomed plates for 3 days. After culture, the degree of lymphocyte proliferation was measured by the incorporation of ³H-thymidine after a 4-hr pulsing with 1.5 μCi ³H-TdR. The data present the mean values ± standard deviation of three experiments. PJ-T, total water extract of *P. japonica*; PJ-P, polysaccharide fraction of the extract; PJ-E, ethanol fraction of the extract.

2.

Fig.1 3 PJ-P

100 μg/Mℓ 51,616 cpm

45

PJ-T 22,773 cpm PJ-P

PJ-E

10 μg/Mℓ 30 μg/Mℓ

2 4 , 100 μg/Mℓ

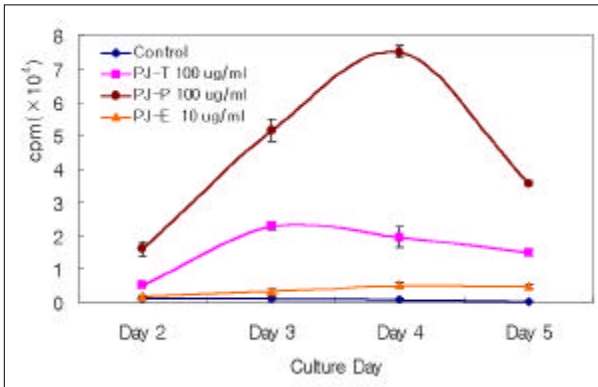


Fig. 2. Time course of the proliferation of splenic lymphocytes by the fractions of *P. japonica* extract. Spleen cells (2×10^5 /well) were cultured with fraction of the extract at a optimal concentration. On day 2, 3 and 4 after culture, the degree of lymphocyte proliferation was measured by the incorporation of ³H-thymidine after a 4-hr pulsing with 1.5 μ Ci ³H-TdR. The data present the mean values \pm standard deviation of three experiments. The cpm of LPS was 139213, 48734, 18570 on day 2, 3, 4, respectively. The cpm of ConA was 149178, 121190, 16214 on day 2, 3, 4, respectively. PJ-T, total water extract of *P. japonica*; PJ-P, polysaccharide fraction of the extract; PJ-E, ethanol fraction of the extract.

100 μ g/ml PJ-P 가 3, 4, 5
 FACS (Table). Table II
 IgM, Thy 1.2 가
 60% 35% , 3
 74% 25% .
 PJ-P 가 3
 IgM 가 82.6%, Thy 1.2 가 10.6%
 , 4 86%, 13.8% , 5
 90% 9.2% . PJ-P
 B
 , B mitogen LPS 가 3
 , IgM 가 88% Thy 1.2
 9% . T mitogen ConA
 가 IgM 가 20%
 Thy 1.2 가 84% .
 4. PJ - P subset
 PJ-P subset
 subset 가
 MACS subset

Table . Flow cytometric analysis of lymphocyte subsets of splenocytes cultured with PJ-P

Culture day	Sample treatments	Conc. (μ g/ml)	Percent of lymphocyte subsets			
			IgM	Thy 1.2	CD4	CD8
Day 0			60.4	35.2	20.8	14.4
Day 3	medium		74.2	25.2	12.6	10.8
	PJ-P	100	82.6	10.6	7.4	3.5
	LPS	60	87.7	8.5	NT	NT
	ConA	5	20.0	83.7	14.3	64.6
Day 4	PJ-P	100	86.0	13.8	7.2	3.6
Day 5	PJ-P	100	90.0	9.2	6.7	4.8

NT, not tested.

Table . Flow cytometric conformation of the cell populations separated from spleen cells by MACS

Separation of lymphocyte subsets from spleen cells	Percent of lymphocyte subsets	
	IgM	Thy 1.2(CD90)
Depletion of T cells	89.2	5.9
Depletion of B cells	3.8	91.2
Positive selection of T cells	1.7	NT
Positive selection of B cells	99.0	NT

NT, not tested.

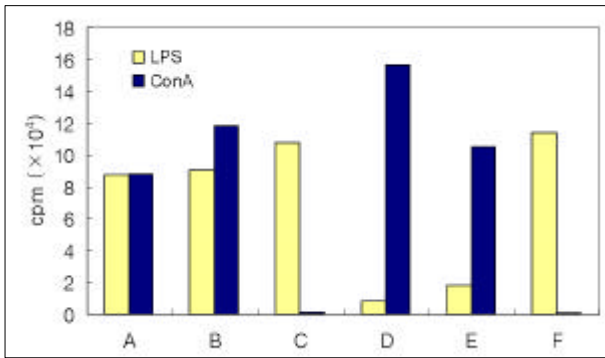


Fig. 3. Confirmation of the proliferation of lymphocyte subsets by LPS and ConA. LPS and ConA was given to six kinds of cell preparations such as total cell population (A), macrophage-depleted cell population (B), T cell-depleted cell population (C), B cell-depleted cell population (D), positively selected T cell population (E), and positively selected B cell population (F) by MACS. After 2 days of culture, the degree of lymphocyte proliferation was measured by the incorporation of ³H-TdR after a 4-hr pulsing with 2 μCi ³H-TdR.

MACS 가
FACS ,
depletion 90%, positive selection
99% (Table). ,
B mitogen LPS T mitogen ConA
2 (Fig. 3),
가 , positive
selection T LPS
LPS가 T (5)
B 가
PJ-P 가 (Fig.
4), ,
T 2 5
3가
T 가 . B 3

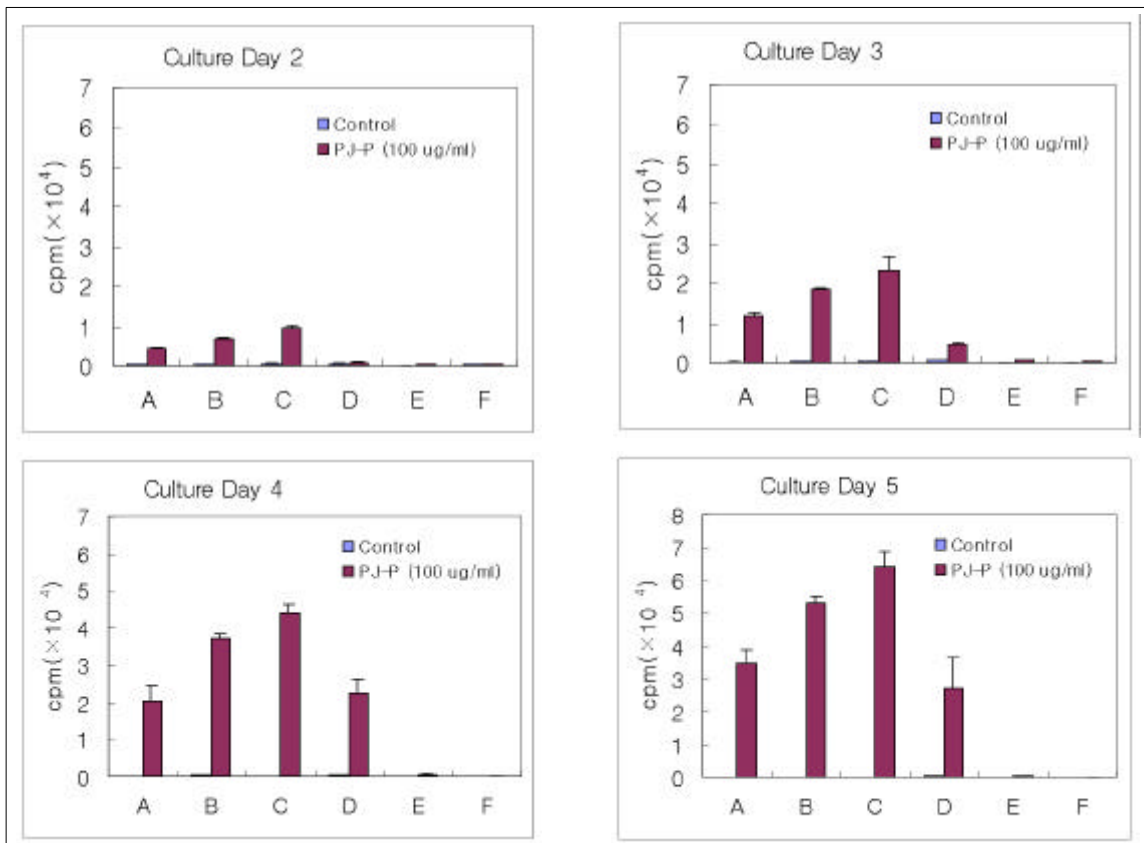


Fig. 4. The proliferation pattern of lymphocyte subsets by PJ-P. PJ-P(100 μg/ml) was given to six kinds of cell preparations such as total cell population(A), macrophage-depleted cell population(B), T cell-depleted cell population(C), B cell-depleted cell population(D), positively selected T cell population(E), and positively selected B cell population(F). On day 2, 3, 4 and 5 after incubation, the degree of the lymphocyte proliferation was measured by the incorporation of ³H-thymidine into the cells.

5
 , T B positive selection (10) B mitogen LPS B
 PJ-P 가 5 (Fig. 3)
 , PJ-P B
 LPS
 5. PJ - P
 PJ-P B lentinan (12-14), schizofilan (15), PSK (16,
 T 17), ginsan (18-21) (3)
 PJ-P B 가 , PJ-P
 T B
 SRBC PJ-P T
 Fig. 5 , SRBC cytokine B
 PJ-P 가 ,
 (100 mg/kg B.W.) SRBC cytokine 가
 가 2.7 가 , PJ-P가 T-dependent antigen
 PJ-P T 가 , PJ-P T
 B
 PJ-P
 PJ-P 가
 B , PJ-P PJ-P
 (18-21)
 lentinan (12-14)
 PJ-P가 B subset
 가 MACS
 subset
 PJ-P B
 B T
 , B 가 B T
 4 PJ-P가 T
 cytokines B 가

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