Effects of *Chaenomeles speciosa* glucosides on cyclophosphamide potentiated contact hypersensitivity in mice

ZHENG Yong-Qiu, WEI Wei*, WANG Ni-Ping
(*Institute of Clinical Pharmacology, Anhui Medical University, Hefei* 230032, *China*)

Abstract: AIM To investigate the roles of thymus T lymphocytes subsets in contact hypersensitivity (CHS) and effects of glucosides of *Chaenomeles speciosa* (GCS) on this response. **METHODS** CHS model induced by 2, 4-dinitro-I-dinitrofluorobenzene (DNFB) and cyclophosphamide (Cy) potentiated CHS model were used. GCS(60, 120 and 240 $mg \cdot kg^{-1}$) were given intragastrically (ig) once daily for 12 consecutive days. Concanavalin A (Con A)-induced lymphocytes proliferation was observed by MTT assay. CD4/CD8 T lymphocytes subsets were measured by flow cytometry and the levels of Con A-induced cytokines from thymocytes were measured by enzyme-linked immunosorbent asssay (ELISA). **RESULTS** Splenocyte proliferation stimulated by Con A was augmented and $CD4^+$ $CD8^+$ T lymphocytes were increased in thymus of mice with CHS. The balance of $CD4^+$ T_h subsets was changed to favor T_h1 and T_h3 as shown by the increased interleukin-2 (IL-2) and transforming growth factor β_1 ($TGF-\beta_1$) production and the decreased interleukin-4 (IL-4) production. GCS, similar as the control drug actarit (4-acetylaminophenylacetic acid), could elevate the percentage of $CD4^+$ $CD8^-$ T lymphocytes and $CD4^-$ $CD8^-$ T lymphocytes, and reduce the percentage of $CD4^+$ $CD8^+$ T cells in Cy-potentiated CHS mice. GCS inhibited the production of IL-2 and $TGF-\beta_1$, and increased the IL-4 level in cultures of thymocytes from mice with Cy-potentiated CHS. **CONCLUSION** GCS inhibits Cy-potentiated mice CHS response and modulates the balance of CD4/CD8 or helper T cells subsets.

Key words: glucosides; *Chaenomeles speciosa*; contact hypersensitivity; $CD4^+$-positive T lymphocytes; $CD8^+$-positive T lymphocytes; interleukin-2; interleukin-4; transforming growth factor beta

CLC number: R967
Document code: A
Article ID: 1000-3002(2004)06-0415-06

Contact hypersensitivity (CHS) is a T cell-mediated immune response. Erard, *et al*[1] reported that CHS to 2, 4-dinitro-I-dinitrofluorobenzene (DNFB) was decreased after thymectomy in the adult mice and it is related to T cell defect. T lymphocytes from thymus have been divided into $CD4^+$ and $CD8^+$ cells. Studies from gene-deficient mice proved that $CD8^+$ T cells were found to be pivotal for induction of CHS by repeated application of the hapten DNFB, while $CD4^+$ T cells were involved in downregulation of CHS[2, 3]. Contradictory findings have also been reported, implicating $CD4^+$ T cells as effector cells in CHS[4]. Except the two phenotypes, $CD4^+$ and $CD8^+$, there are significant numbers of $CD4^+$ $CD8^+$ double positive lymphocytes existing in individuals suffering from certain disease conditions such as arthritic joints, rejected kidney grafts and certain tumors[5]. Some studies indicate that $CD4^+$ $CD8^+$ cells can secret high levels of IL-2 and gamma-interferon (IFN-γ)[6]. So far, there are few reports about the roles of $CD4^+$ $CD8^+$ in CHS response.

Glucosides of *Chaenomeles speciosa* (GCS) are active constituents extracted from the fructus of *C. speciosa*, which has been recognized as a valuable traditional herb used in treatment for rheumatoid arthritis (RA). Results from our previous studies indicate that GCS can reduce the secondary inflammation in adjuvant arthritis (AA) rats, which is associated with the prevention of ul-

Received date: 2004-01-06 **Accepted date**: 2004-04-27
Foundation item: The project supported by the Research Foundation of Anhui Province (00044411)

Biographies: ZHENG Yong-Qiu(1973 –), female, native of Wuhu, Anhui Province, PhD Candidate, main research field is antiinflammatory pharmacology and immunopharmacology; WEI Wei (1960 –), male, native of Anhui Province, PhD, professor, main research field is antiinflammatory pharmacology immunopharmacology and clinical pharmacology.
* Corresponding author. E-mail: wwei @ ahmu. edu. cn Tel: (0551)5161208, (0551)5161206 Fax: (0551)5161208

trastructural changes of synoviocytes and inhibition of secretion of proimflammatory cytokines[7]. In another study, we reported that GCS also had therapeutic effects on collagen-induced arthritis (CIA)[8]. However, its roles in therapy of T lymphocyte-mediated immune response have not yet been elucidated clearly.

Actarit (4-acetylaminophenylacetic acid), an oral active immunomodulator, suppresses types Ⅲ and Ⅳ allergic reactions in mice[9]. In the present studies, actarit was used as the positive control drug.

1 MATERIALS AND METHODS

1.1 Animals

Male ICR mice (8 – 10 weeks old) used in this study were purchased from Shanghai BK Experimental Animal Center (Grade Ⅱ, Certificate No D-65). All mice were kept on a 12 h dark: 12 h light cycle at constant temperature of 20 – 25℃.

1.2 Drugs and materials

GCS (umber powder, glucosides content > 50%) was provided by the Chemistry Lab of Institute of Clinical Pharmacology of Anhui Medical University. Actarit was obtained from Drug Reseach Institute of Anhui (batch number 20020616; Hefei, China). Cyclophosphamide (Cy) was obtained from Hengri Inc. (batch number 02032121; Jiangsu, China). Dimethyl sulfoxide was obtained from Shanghai Vitriolic Factory. RPMI-1640 medium, fetal calf serum, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), concanavalin A (Con A, stored at −20℃) and 2,4-dinitro-1-dinitrofluorobenzene (DNFB) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). (PE)-conjugated rat monoclonal antibody (mAb) to mouse CD8 and (FITC)-conjugated rat anti-mouse CD4 mAb were from Caltag Laboratories (1849 Bayshore Blvd., Burlingame) and were stored at 2 – 8℃ until use. Mouse interleukin-4 (IL-4) ELISA kit (Jingmei Inc., Beijing, China) and mouse transforming growth factor beta-1 (TGF-β_1) enzyme-linked immunosorbent assay (ELISA) kit (LIFEKEY BioMeditech Inc., New Jersey, USA) were stored at 2 – 8℃.

1.3 Induction of CHS model and potentiated CHS model in mice

The mice were sensitized by applying 50 μL of 1% DNFB in acetone/sesame oil (1∶1, *V*/*V*) to the shaved abdominal area for 2 consecutive days. Four days after the second application, mice were challenged on the inner and the outer surface of the right ear with 10 μL of 0.2% DNFB in the same vehicle. Control mice received 10 μL of acetone/sesame oil (1∶1, *V*/*V*) on the same surface of the right ear. The potentiation of CHS reaction in mice was induced by given an intraperitoneal (ip) injection of Cy at dose of 250 mg·kg^{-1} 3 d before primary sensitization[10].

1.4 Drug treatment

GCS and actarit were dissolved in sterile water containing 0.5% carboxymethylcellulose (CMC-Na) solution. The mice were randomly divided into 7 groups which were given GCS (60, 120, and 240 mg·kg^{-1}) and actarit (120 mg·kg^{-1}) intragastrically once daily from 5 d before primary sensitization and the last dose was given at 12 h after DNFB ear challenge. For the control, CHS model and CHS + Cy model, animals received an equal amount of vehicle at the same time.

1.5 Evaluation of CHS

All mice were sacrificed 24 h after DNFB challenge, and then ear swelling, thymus index and spleen index were measured. Ear pieces were cut from both ears using a perforator of 8 mm diameter at the outer two-thirds of the ear, avoiding skin folds located at the base. The ear pieces removed from each ear were weighed. The difference of ear weight between the pieces from left and right ears was denoted as ear swelling.

1.6 Cell preparations

After being removed from sacrificed mice and passed through 200-mesh stainless-steel screen, the splenocytes and thymocytes were washed twice in D-Hanks solution supplemented with 10% heat-inactive fetal calf serum by centrifugation.

Between the two washings, red blood cells (RBC) were lysed with 0.83% NH_4Cl. The number of cells was counted and adjusted to 1×10^9 L^{-1} cells.

1.7 Con A-induced splenocytes proliferation

In 96-well plates, splenocytes (1×10^5 cells per well) were cultured in triplicate with Con A(1 μg per well) in RPMI-1640 medium(pH = 7.0) containing 10% heat-inactive fetal calf serum and 100 kU·L^{-1} penicillin, 100 g·L^{-1} streptomycin, 2 mmol·L^{-1} *L*-glutamine, 0.5 μmol·L^{-1} 2-mercaptoethanol and 25 mmol·L^{-1} HEPES in 24-well plates. After being cultured for 72 h at 37℃ in a humidified atmosphere of 5% CO_2, proliferation of splenocytes were measured by the MTT colorimetric method[11].

1.8 Measurement of cytokine production by thymocytes

Thymocytes (1×10^5 cells per well) from each sample was cultured with Con A(1 μg per well) in RPMI-1640 medium(pH = 7.0) containing 10% heat-inactive fetal calf serum and 100 kU·L^{-1} penicillin, 100 g·L^{-1} streptomycin, 2 mmol·L^{-1} *L*-glutamine, 0.5 μmol·L^{-1} 2-mercaptoethanol and 25 mmol·L^{-1} HEPES in 24-well plates, then culture supernatants were collected 48 h later by centrifugation and frozen at −20℃ until assay for cytokines.

The levels of IL-4 and TGF-β_1 in culture supernatants were determined by the ELISA kits. The activity of IL-2 in the same supernatants was measured by testing its ability to support the proliferation of Con A stimulated splenocytes which was detected by colorimetric assay with MTT.

For cytokine assays, splenocytes suspension from a new ICR mice were adjusted to 1×10^9 L^{-1} cells. Then triplicate cultures of the splenocytes (1×10^5 cells per well) were incubated with Con A (1 μg per well) and the supernatants which contained cytokines (100 μL per well) in a flat bottomed 96-well plate with the same culture condition as mentioned in 1.7. Fourth-eight hours later, proliferation of splenocytes were measured by the MTT colorimetric method[11]. The absorbance (*A*) at 490 nm of each well was regard as the activity of IL-2 in the supernatants of thymphocytes culture.

1.9 Thymocyte subsets measurement by flow cytometry

For each sample, 5 μL thymocyte suspension (1×10^9 L^{-1}, 5×10^3 cells) was mixed with 10 μL PE-conjugated rat monoclonal antibody (mAb) to mouse CD8 and 10 μL FITC-conjugated rat antimouse CD4 mAb. 30 min later each data from 3000 volume-gated viable cells were collected by flow cytometry.

1.10 Statistical analysis

Data are expressed as $\bar{x} \pm s$. Differences between groups were examined using ANOVA followed by Newman-Keals *t* test.

2 RESULTS

2.1 Effects of GCS on ear swelling, spleen index, thymus index and Con A-induced splenocytes proliferation

Ear swelling, thymus index and spleen index were enhanced in CHS mice as compared with normal control. Con A-induced proliferation of splenocytes was also significantly enhanced. It was demonstrated that an intraperitoneal injection of Cy at dose of 250 mg·kg^{-1} 3 d before primary sensitization could induce a higher degree of CHS reaction including ear swelling, thymus index, spleen index and Con A-induced proliferation of splenocytes. GCS (120, 240 mg·kg^{-1}) could significantly inhibit the Cy-enhanced CHS reaction. The efficacy of GCS 120 mg·kg^{-1} was similar to that of actarit 120 mg·kg^{-1}. GCS 60 mg·kg^{-1} also significantly inhibited the ear swelling and the spleen index (Tab 1).

2.2 Effects of GCS on T lymphocyte subset balance in thymus

As compared to normal control, the percentage of $CD4^+CD8^+$ T lymphocytes in CHS mice thymus increased and $CD4^-CD8^-$ T lymphocytes decreased correspondingly. There was a significantly increased percentage of $CD4^+CD8^+$ T lymphocytes and decreased $CD4^-CD8^-$ and $CD4^+CD8^-$ T lymphocytes in Cy-potentiated CHS mouse

thymus compared with CHS model. GCS (240 mg·kg^{-1}) could elevate the percentage of $CD4^+CD8^-$ T lymphocytes cells. GCS (120, 240 mg·kg^{-1}) could elevate the $CD4^-CD8^-$ T lymphocytes and reduce the percentage of $CD4^+CD8^+$ T lymphocytes in potentiated CHS mouse thymus. Actarit had the same effects on $CD4^+CD8^+$ and $CD4^+CD8^-$ T lymphocytes as GCS, but it had no effect on $CD4^-CD8^-$ T lymphocytes. GCS and actarit all had no effect on $CD4^-CD8^+$ T lymphocytes(Tab 2).

2.3 Effect of GCS on the level of cytokine production

In Cy-potentiated CHS mice, not only an increase in IL-2 and TGF-β_1 but also a decrease in IL-4 production by thymocytes as compared with normal mice were observed in CHS mice. GCS (120, 240 mg·kg^{-1}) inhibited IL-2 and TGF-β_1 level and increased the IL-4 level in thymocyte cultures from potentiated CHS mice. GCS at dose of 60 mg·kg^{-1} also reduced the TGF-β_1 level but had no effect on IL-2 and IL-4 level(Tab 3).

Tab 1. Influence of glucosides of *Chaenomeles speciosa* (GCS) on ear swelling, thymus index, spleen index and Con A induced splenocytes proliferation in potentiated mice contact hypersensitivity (CHS) reaction

Group	Dose/ mg·kg^{-1}	Ear weight/ mg	Thymus index/ mg·g^{-1} bw	Spleen index/ mg·g^{-1} bw	Splenocytes proliferation /$A_{490\ nm}$
Normal	–	3.7 ± 2.9	1.7 ± 0.3	4.1 ± 1.2	0.24 ± 0.10
CHS	–	24.3 ± 4.9**	2.2 ± 0.5**	7.2 ± 1.2**	0.33 ± 0.12*
CHS + Cy	–	33.2 ± 3.9$^{\#\#}$	3.1 ± 0.4$^{\#}$	11.1 ± 1.3$^{\#\#}$	0.48 ± 0.12$^{\#\#}$
CHS + Cy + Actarit	120	24.2 ± 3.5$^{\Delta\Delta}$	1.9 ± 0.8$^{\Delta\Delta}$	9.0 ± 2.2$^{\Delta}$	0.32 ± 0.15$^{\Delta}$
CHS + Cy + GCS	60	26.0 ± 7.0$^{\Delta}$	2.6 ± 0.8	7.6 ± 1.8$^{\Delta\Delta}$	0.43 ± 0.14
	120	23.3 ± 6.9$^{\Delta\Delta}$	2.0 ± 0.5$^{\Delta\Delta}$	8.4 ± 2.2$^{\Delta\Delta}$	0.34 ± 0.09$^{\Delta\Delta}$
	240	22.7 ± 5.9$^{\Delta\Delta}$	1.8 ± 0.6$^{\Delta\Delta}$	6.8 ± 1.5$^{\Delta\Delta}$	0.38 ± 0.11$^{\Delta}$

GCS, or vehicle were given ig once daily for 12 consecutive days. CHS model was induced by (2,4-dinitro-I-dinitrofluorobenzene, DNFB) and was potentiated by Cy 250 mg·kg^{-1} ip 3 d before primary sensitization. In 96-well plates, proliferation of splenocytes (1×10^5 cells/well) with Con A(1 μg/well) were measured by the MTT colorimetric method after 72 h culture. $\bar{x} \pm s$, $n = 10$. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with normal group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with CHS group; $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$, compared with CHS + Cy group.

Tab 2. Influence of GCS on the balance of T lymphocyte subsets in mice thymus

Group	Dose/ mg·kg^{-1}	$CD4^+CD8^+$/ %	$CD4^+CD8^-$/ %	$CD4^-CD8^+$/ %	$CD4^-CD8^-$/ %
Normal	–	73.4 ± 3.9	17.5 ± 3.3	4.9 ± 1.0	4.4 ± 0.4
CHS	–	81.3 ± 0.9*	14.1 ± 1.2	3.7 ± 1.3	1.7 ± 0.5**
CHS + Cy	–	88.2 ± 0.8$^{\#}$	7.6 ± 0.7$^{\#\#}$	3.2 ± 0.6	0.9 ± 0.1$^{\#}$
CHS + Cy + Actarit	120	81.9 ± 1.0$^{\Delta}$	13.0 ± 0.6$^{\Delta}$	3.7 ± 0.1	1.2 ± 0.04
CHS + Cy + GCS	60	82.8 ± 3.5	11.1 ± 2.2	4.7 ± 2.3	1.1 ± 0.2
	120	80.2 ± 3.1$^{\Delta}$	14.5 ± 3.7	4.2 ± 1.0	1.5 ± 0.2$^{\Delta}$
	240	72.7 ± 5.9$^{\Delta}$	18.0 ± 4.0$^{\Delta}$	5.1 ± 0.9	4.3 ± 1.2$^{\Delta}$

For each sample, 5×10^3 thymocytes were removed from thymocytes suspension (1×10^9 L^{-1}) and were mixed with 10 μL PE-conjugated rat anti-mouse CD8 mAb and 10 μL FITC-conjugated rat anti-mouse CD4 mAb, 30 min later each data from 3000 volume-gated viable cells were collected by flow cytometry. $\bar{x} \pm s$, $n = 5$. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with normal group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with CHS group; $^{\Delta}P < 0.05$, compared with CHS + Cy group.

Tab 3.　Influence of GCS on the level of cytokine production

Group	Dose/ $mg \cdot kg^{-1}$	IL-2/ $A_{490\ nm}$	TGF-β_1/ $mg \cdot L^{-1}$	IL-4/ $ng \cdot L^{-1}$
Normal	–	0.28 ± 0.03	9.2 ± 0.4	34.0 ± 2.6
CHS	–	$0.53 \pm 0.05^{**}$	$11.8 \pm 0.9^{**}$	$26.1 \pm 1.4^{**}$
CHS + Cy	–	0.62 ± 0.06	$13.4 \pm 0.8^{\#}$	$29.0 \pm 1.2^{\#}$
CHS + Cy + Actarit	120	$0.46 \pm 0.07^{\Delta\Delta}$	$9.9 \pm 1.3^{\Delta\Delta}$	$35.8 \pm 3.5^{\Delta\Delta}$
CHS + Cy + GCS	60	0.53 ± 0.10	$10.3 \pm 2.0^{\Delta}$	32.6 ± 4.5
	120	$0.41 \pm 0.06^{\Delta\Delta}$	$9.6 \pm 1.7^{\Delta\Delta}$	$35.2 \pm 3.8^{\Delta}$
	240	$0.42 \pm 0.06^{\Delta\Delta}$	$9.6 \pm 1.7^{\Delta\Delta}$	$37.8 \pm 4.7^{\Delta\Delta}$

Thymocytes (1×10^5 cells/well) with Con A(1 μg/well) in RPMI-1640 medium (pH = 7.0) were cultured for 48 h. The levels of IL-4 and TGF-β_1 in culture supernatants were determined by the ELISA kits. The activity of IL-2 in the same supernatants was measured by testing its ability to support the proliferation of Con A-induced mice splenocytes. $\bar{x} \pm s$, $n = 10$. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with normal group; $^{\#}P < 0.05$, compared with CHS group; $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$, compared with CHS + Cy group.

3　DISCUSSION

Thymus and spleen play important roles in CHS response. The CHS response to DNFB can be decreased by thymectomy in adult mice or by suppressing IL-2 and IFN-γ production of splenocytes in mice[12]. In present studies, it was found that in CHS mice ear swelling was induced, thymus index, spleen index and splenocyte proliferation were increased. In mice with Cy-potentiated CHS, the above response became exacerbated. GCS had an inhibitory effect on the potentiated response in Cy-potentiated CHS. It implicates that GCS can modulate T-cells-mediated CHS response by influencing the function of thymus and spleen.

Contrary to the conventional viewpoint, we found that $CD4^+$ $CD8^+$ phenotype increased in thymus of CHS mice and Cy could further increased it. It implicates that CHS reaction is related to the high percentage of $CD4^+$ $CD8^+$ phenotype in thymus. The results that GCS reduced the higher percentage of $CD4^+$ $CD8^+$ T lymphocytes and elevated the lower percentage of $CD4^-$ $CD8^-$ T lymphocytes in potentiated CHS mouse thymus indicates that GCS can influence the function of thymus by modulating the balance of CD4/CD8 T lymphocyte subsets in thymus.

Cytokines such as IL-1α, TNF-α, IL-2, and IFN-γ are considered as the promoters of CHS[13]. In present studies, the increase of IL-2 production accompanying with the elevation of $CD4^+$ $CD8^+$ percentage implicates that the role of $CD4^+$ $CD8^+$ lymphocyte in CHS pathogenesis may be relative to the production of cytokines which promoted CHS response. Asada, *et al*[14] indicated that T_h2 type cytokine IL-4 was produced in murine skin during the elicitation phase of CHS and was an important down-modulator of inflammation. IL-4 could weaken CHS by regulating local production of proinflammatory cytokines.

In our studies, we detected the cytokine production in thymocytes of CHS mice and found that IL-2 and TGF-β_1 production was increased while IL-4 production was decreased. In the culture supernatants of thymocytes from Cy-potentiated CHS model, the imbalance of cytokines was exacerbated. GCS could resume the balance of cytokine by decreasing IL-2 activity and TGF-β_1 level and increasing IL-4 level in the culture supernatants of thymocytes from the normal and the potentiated CHS mice. The results indicated that GCS could modulated T-cell mediated response by resuming the cytokine production by thymocytes.

In conclusion, GCS inhibits CHS response in mice by modulating the function of central immune system and the balance of T cell subsets.

Acknowledgements: A special thanks to Dr. ZHAI Zhi-Min and the Central Laboratory of Anhui Provincial Hospital for their assistance in preparation of flow cytometry

and analysis of correlative results.

4 REFERENCES:

[1] Erard D, Charreire J, Auffredou MT, Galanaud P, Bach JF. Regulation of contact sensitivity to DNFB in the mouse: effects of adult thymectomy and thymic factor[J]. *J Immunol*, 1979, **123**(4):1573 – 1576.

[2] Gocioski BL, Tigerlaar RF. Role of $CD4^+$ and $CD8^+$ in murine contact sensitivity revealed by *in vivo* monoclonal antibody depletion[J]. *J Immunol*, 1990, **144**(5):4121 – 4128.

[3] Bour H, Peyron E, Gaucherand M, Garrigue JL, Desvignes C, Kaiserlian D, *et al*. Major histocompatibility complex class Ⅰ-restricted $CD8^+$ T cells and class Ⅱ-restricted $CD4^+$ T cells, respectively, mediate and regulate contact sensitivity to dinitrofluorobenzene[J]. *Eur J Immunol*, 1995, **25**(11):3006 – 3010.

[4] Gautam SC, Matriano JA, Chikkala NF, Edinger MG, Tubbs R. L3T4 $CD4^+$ cells that mediate contact sensitivity to trinitrochlorobenzene express I – A determinants[J]. *Cell Immunol*, 1991, **135**(1):27 – 41.

[5] Zuckermann FA. Extrathymic CD4/CD8 double positive T cells[J]. *Veterin Immunol Immunopathol*, 1999, **72**(1 – 2):55 – 66.

[6] Patel SS, Wacholtz MC, Duby AD, Thiele DL, Lipsky PE. Analysis of the functional capabilities of $CD3^+$ $CD4^-$ $CD8^-$ and $CD3^+$ $CD4^+$ $CD8^+$ human T cell clones[J]. *J Immunol*, 1989, **143**(4):1108 – 1117.

[7] Dai M, Wei W, Shen YX, Zheng YQ. Glucosides of *Chaenomeles speciosa* remit rat adjuvant arthritis by inhibiting synoviocytes activities[J]. *Acta Pharmacol Sin*(中国药理学报), 2003, **24**(11):1161 – 1166.

[8] Chen Q, Wei W. Effects and mechanisms of glucosides of *Chaenomeles speciosa* on collagen-induced arthritis in rats[J]. *Int Immunopharmacol*, 2003, **3**(4):593 – 608.

[9] Nakagawa Y, Ogawa T, Umezu K, Shibata Y, Nishimura T. Characterization of suppressor cells activated by 4-acetylaminophenylacetic acid (MS-932) in delayed-type hypersensitivity[J]. *Int J Immunother*, 1990, **6**(1):149 – 156.

[10] Corsini AC, Bellucci SB, Costa MG. A simple method of evaluating delayed type hypersensitivity in mice[J]. *J Immunol Methods*, 1979, **30**(2):195 – 199.

[11] Russell CA, Vindelov LL. Optimization and comparison of the MTT assay and the 3H-TdR assay for the detection of IL-2 in helper T cell precursor assays[J]. *J Immunol Methods*, 1998, **217**(1 – 2):165 – 175.

[12] Exton1 MS, Elfers1 A, Jeong WY, Bull DF, Westermann J, Schedlowskil M. Conditioned suppression of contact sensitivity is independent of sympathetic splenic innervation[J]. *Am J Physiol Regul Integr Comp Physiol*, 2000, **279**(4):1310 – 1315.

[13] Hoefakker S, Caubo M, van't Erve EH, Roggeveen MJ, Boersma WJ, Joost T, *et al*. *In vivo* cytokine profiles in allergic and irritant contact dermatitis[J]. *Contact Dermatitis*, 1995, **33**(1):258 – 266.

[14] Asada H, Linton J, Katz SI. Cytokine gene expression during the elicitation phase of contact sensitivity: regulation by endogenous IL-4[J]. *J Invest Dermatol*, 1997, **108**(1):406 – 411.

木瓜苷对环磷酰胺增强的小鼠接触性超敏反应的影响

郑咏秋, 魏　伟, 汪倪萍

(安徽医科大学临床药理研究所, 安徽 合肥　230032)

摘要: 目的　探讨胸腺在环磷酰胺(Cy)增强的小鼠接触性超敏反应(CHS)中的作用及木瓜苷(GCS)对胸腺 T 淋巴细胞亚型的影响。**方法**　采用了 2,4-二硝基氟苯(DNFB)诱导小鼠 CHS 模型及 Cy 诱导小鼠增强 CHS 模型,检测 Con A 诱导的小鼠脾脏 T 淋巴细胞增殖、胸腺 T 淋巴细胞亚型和 Con A 诱导的胸腺 T 淋巴细胞培养上清中 TGF-β_1,IL-4 和 IL-2 水平。**结果**　小鼠 CHS 模型中,Con A 诱导的脾淋巴细胞增殖增强,$CD4^+$ $CD8^+$ 双阳性胸腺 T 淋巴细胞比例增加,胸腺细胞产生的 T_h1 和 T_h3 型细胞因子 IL-2 和 TGF-β_1 水平增高而 T_h2 型细胞因子 IL-4 水平降低。DNFB 初次致敏前 3 d 腹腔注射 Cy (250 mg·kg^{-1})可以增强 CHS 反应。GCS(120 和 240 mg·kg^{-1})连续灌胃 12 d 可以提升 Cy 增强的小鼠 CHS 胸腺 T 淋巴细胞中 $CD4^-$ $CD8^-$ 和 $CD4^+$ $CD8^-$ 细胞比例,降低 $CD4^+$ $CD8^+$ 细胞比例;并提高胸腺淋巴细胞培养上清中 IL-4 水平,降低 IL-2 和 TGF-β_1 水平。**结论**　GCS 对 Cy 增强的小鼠 CHS 有明显抑制作用;可有效调节小鼠胸腺 CD4/CD8 和 T_h 淋巴细胞亚群及细胞因子产生平衡。

关键词: 木瓜苷; 接触性超敏反应; $CD4^+$-T 淋巴细胞; $CD8^+$-T 淋巴细胞; 白细胞介素-2; 白细胞介素-4; 转化生长因子 β

基金项目: 安徽省自然科学基金资助项目(00044411)

(本文编辑　乔　虹)