JPP Journal of Pharmacy and Pharmacology

JPP 2003, 55: 691–696

Received November 1, 2002
Accepted February 4, 2003
DOI 10.1211/002235702955
ISSN 0022-3573

Astilbin suppresses delayed-type hypersensitivity by inhibiting lymphocyte migration

Yu Cai, Ting Chen and Qiang Xu

Abstract

This study examined the effects of astilbin, a flavanone, on delayed-type hypersensitivity reactions and its mechanisms of action on cell migration. Astilbin significantly inhibited the sheep-red-blood-cell-induced footpad reaction and picryl-chloride-induced ear dermatitis without affecting the organ weights, when administered during the effector phase but not the induction phase. The flavanone also significantly inhibited the migration to gelatin of spleen cells isolated from mice with ear dermatitis in a transwell system. Furthermore, the isolated spleen cells from normal mice were incubated with astilbin in the presence of concanavalin A, or those from mice with ear dermatitis were cultured with astilbin. In the supernatants collected, the activity of matrix metalloproteinases (MMPs) MMP-2 and MMP-9 was remarkably inhibited by astilbin. These results suggest that astilbin may inhibit delayed-type hypersensitivity reactions through selectively suppressing the lymphocyte functions, including cell migration, via down-regulating MMP activity.

Introduction

Cell migration plays a central role in a wide variety of biological phenomena. In the delayed-type hypersensitivity response, for example, lymphocytes migrate into areas of insult where they mediate phagocytic and immune functions. This requires the interaction between lymphocytes and the underlying basement membrane and interstitial matrix (Shimizu & Shaw 1991; Cid et al 1994). Matrix metalloproteinases (MMPs) are a family of zinc- and calcium-dependent endopeptidases, which includes matrilysin, stromelysins, gelatinases, interstitial and neutrophil collagenases, collagenase-3 (MMP-13) and membrane-type MMPs (Westermarck & Kahari 1999). They digest different components of the extracellular matrix during physiologic and pathologic turnover (Tan et al 1996). The gelatinase-type matrix MMPs, MMP-2 and MMP-9, are capable of degrading type IV collagen in the basement membrane and thus are regarded as key enzymes in migration and invasion by several cell types, including macrophages, T cells and some tumour cells (Lee et al 1994; Cuenda et al 1995; Beyaert et al 1996; Suttles et al 1999). Recently, constitutive secretion of MMP-9 and activation-induced secretion of MMP-2 have been shown to favour T-cell migration through a basement membrane in-vitro (Leppert et al 1995a). Inhibition of T cell transmigration by interfering with the expression of these proteinases may represent a useful approach to the treatment of T-cell-mediated inflammation and autoimmune diseases.

Previously, we have examined the effects of various kinds of Chinese herbs on the activation and function of T lymphocytes. The aqueous extracts from several kinds of Chinese herbs selectively inhibited either the induction or the effector phase of delayed-type hypersensitivity (a typical T-cell-mediated immune response), while the steroid agent prednisolone comparatively strongly inhibited both phases (Xu et al 1991, 1993, 1997; Xu & Xu 1993). Among the extracts, that from Rhizoma Smilacis Glabrae significantly inhibited delayed-type-hypersensitivity-related inflammation, including ear contact dermatitis, and the footpad reaction, as well as liver injury induced by picryl chloride or other immunogens, only when given in the effector phase of delayed-type hypersensitivity (Xu et al 1997). Such selective activity is quite different from that of immunosuppressors thus far. As a main principle, astilbin, a flavanone, was isolated

State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, 22 Han Kou Road, Nanjing 210093, China

Yu Cai, Ting Chen, Qiang Xu

Department of Pharmacology for Chinese Materia Medica, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, China

Yu Cai, Qiang Xu

Correspondence: Q. Xu, School of Life Sciences, Nanjing University, 22 Han Kou Road, Nanjing 210093, China. E-mail: molpharm@163.com

Funding: This work was supported by National Natural Science foundation of China (nos 39925041 and 30070876)

from the plant. This compound showed a selective inhibition similar to that of the extract on the delayed-type-hypersensitivity-induced liver injury at the effector phase. Its mechanism was found to involve a significant induction of apoptosis in the liver-infiltrating T lymphocytes (Xu et al 1999) and mitogen-activated Jurkat T cells (Yan & Xu 2001). These results drove us to explore the possibility of treating immunological diseases by selectively inhibiting the activated T lymphocytes. Therefore this study was designed to confirm the selective inhibition of astilbin on delayed-type hypersensitivity reactions and then to examine its mechanisms in the aspect of cell migration.

Materials and Methods

Animals

Kunming mice, 6–8 weeks old, half each of female and male, were obtained from Experimental Animal Center of China Pharmaceutical University (Nanjing, China), and maintained with free access to pellet food and water in plastic cages at $21 \pm 2\,^{\circ}C$, and kept on a 12-h light–dark cycle. Animal welfare and experimental procedures were strictly in accordance with the guide for the care and use of laboratory animals (National Research Council of USA, 1996) and the related ethical regulations of our university. All efforts were made to minimize suffering and to reduce the number of mice used.

Drugs and reagents

Astilbin, 3,3′,4′,5,7-pentahydroxyflavanone 3-(6-deoxy-L-mannopyranoside), with more than 92% of purity, was isolated from the rhizome of *Smilacis glabra*, a Liliaceae plant, which was purchased from Nanjing Medicinal Material Co. (Nanjing, China). The other reagents purchased were as follows: sheep red blood cells (SRBC; Jiangning Epidemic Prevention Station, Jiangsu, China); dexamethasone injection (Huaiyin Pharmaceutical Factory, Jiangsu, China); picryl chloride (Nacalai Tesque Inc., Kyoto, Japan); 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2*H*-tetrazolium bromide (MTT; Amresco); acrylamide and bis-acrylamide (Shanghai Shenggong Biotechnical Limited Co. Shanghai, China); gelatin and Coomassie brilliant blue R-250 (Sigma).

SRBC-induced footpad reaction

Mice were sensitized by injecting 1×10^8 cells of SRBC in $40\,\mu L$ of saline into the left hind paw of mice. Five days later, they were challenged by injecting 1×10^9 SRBC in $40\,\mu L$ of saline into the right hind paw. The reaction was evaluated by the increase in hind-paw thickness, right against left, 24 h after the challenge. Paw thickness was measured with the engineer's micrometer (0.001 mm; Mitutoyo Co., Tokyo, Japan).

Picryl-chloride-induced ear contact dermatitis

Kunming mice were sensitized by painting 0.1 mL of 1% picryl chloride in ethanol on the skin of their abdomens where the hair had been shaved. They were then challenged by painting $30\,\mu L$ of 1% picryl chloride in olive oil on the right ear 5 days later. The ear swelling was evaluated by the difference in the thickness between the two ears measured with an engineer's micrometer 24 h after the challenge.

Preparation of splenocyte suspensions

Spleen was aseptically taken from mice, crushed gently and separated into single cells by squeezing in Hank's solution. The cells obtained were passed though a gauze of eight-layers and centrifuged at 1000 rev min^{-1} for 10 min at 4 °C. The pellet was added to 10 mL of sterile Tris-NH_4Cl (pH 7.5) followed by centrifugation to remove erythrocytes. After washing twice with RPMI 1640 (GIBCO BRL) medium supplied with 100 U mL^{-1} of penicillin, 100 U mL^{-1} of streptomycin and 10% fetal calf serum (FCS), they were resuspended in the medium and used for culture.

Gelatin zymography assay

Analysis by zymography on gelatin gel allows detection of enzymatic activity of the secreted collagenases MMP-2 and MMP-9. This was performed as described previously (Hauzenberger et al 1999) with modification. Briefly, spleen cells isolated from mice were suspended in serum-free RPMI 1640 medium at a density of 2×10^7 mL^{-1} and incubated with various concentrations of astilbin at 37 °C in 5% CO_2 for 24 h. The supernatants ($20\,\mu L$) were collected and mixed with $10\,\mu L$ of sample buffer (62.5 mM Tris containing 10% glycerol, 0.00125% bromophenol blue, 12% sodium docecyl sulfate (SDS)) without reducing agent, and they were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in 5% polyacrylamide gels that were copolymerized with 2 mg mL^{-1} of gelatin at 4 °C for 1 h. After electrophoresis, the gels were washed twice in the rinsing buffer (2.5% Triton X-100, 1 mM $CaCl_2$, 1 μM $ZnCl_2$, 0.05% NaN_3) for 1 h at room temperature to remove SDS. Then they were incubated for 36 h at 37 °C in 50 mM Tris buffer containing 5 mM $CaCl_2$, 1 μM $ZnCl_2$ and 0.05% NaN_3. The gels were stained with 0.25% Coomassie brilliant blue R250 for 30 min, and destained for 8 h in a solution of 10% acetic acid and 30% methanol. The proteolytic activity was shown as clear bands (zones of gelatin degradation) against the blue background of stained gelatin. Note that the zymography technique classically shows two bands for MMP-9, corresponding to proMMP-9 (released as inactive proenzyme) and active MMP-9 (after cleavage of regulation domain).

Migration assay

The migration of spleen cells along a gradient of substratum-bound gelatin was assayed in a transwell cell culture

chamber according to the methods previously reported (Rubinstein et al 1994; Leppert et al 1995a; Sheng et al 1997). The filters with a 5.0-μm pore size were pre-coated with 2.5 μg gelatin in a volume of 50 μL in the lower surface as described above. Cells (200 000) in 100 μL RPMI 1640 medium were added to the upper compartment of the transwell cell chamber and incubated at 37 °C for 10 h. After incubation, to count the number of cells that migrated into the lower compartment, the upper compartment of the transwell was removed and MTT solution (5 mg mL^{-1}) 100 μL was added into the lower compartment, for a further 4-h incubation. Then, the plate was centrifuged at 1000 rev min^{-1} for 5 min, and 200 μL of dimethyl sulfoxide (DMSO) was added to dissolve the MTT that was taken into the viable cells, and the optic density was read by an ELISA reader in a 96-well plate. Each assay was performed in duplicate.

Statistics

Results were expressed as mean ± s.d. Statistical analysis was evaluated by one-way analysis of variance followed by post-hoc Bonferroni for multiple comparisons, with the level of significance set at $P < 0.05$.

Results

Effects of astilbin on the footpad swelling induced by SRBC and the ear contact dermatitis to picryl chloride

In the induction phase of the footpad reaction, mice were given astilbin orally dissolved in water and intramuscularly with dexamethasone in saline for 6 days from the SRBC or picryl chloride sensitization, respectively. Compared with the control, astilbin neither influenced the paw nor the ear swelling, while dexamethasone (5 mg kg^{-1}) strongly inhibited both the footpad and the ear reaction (Tables 1 and 2). In the effector phase of the reactions, the drugs were given at 0 (immediately), 5 and 10 h after the SRBC or picryl chloride challenge. Compared with the control, astilbin dose-dependently inhibited both the paw and the ear swelling. The intramuscular injection of dexamethasone also showed a strong inhibitory effect (Tables 1 and 2).

Effect of astilbin on thymus and spleen weights in mice with contact dermatitis to picryl chloride

Drugs were given at 0 (immediately), 5 and 10 h after the picryl chloride challenge. As a result, the thymus and spleen weights in astilbin-treated mice showed an almost similar level to normal. However, dexamethasone strongly reduced the organ weights (Table 3).

Effect of astilbin on the migration of spleen cells isolated from mice with contact dermatitis to picryl chloride

Spleen cells were isolated 6 h after the picryl chloride challenge. Compared with the control, the number of cells that migrated to the medium in the lower compartment of the transwell system was significantly reduced in a dose-dependent manner due to astilbin exposure (Figure 1).

Effect of astilbin on the activity of matrix metalloproteinases in spleen cells isolated from mice with contact dermatitis to picryl chloride

Spleen cells were isolated from mice 6 h after the picryl chloride challenge and incubated with various concentrations of astilbin for 24 h. Supernatant was then collected and subjected to zymography assay. Against the control, astilbin dose-dependently inhibited the activity of both

Table 1 Effect of astilbin and dexamethasone on SRBC-induced footpad reaction in mice.

Group	Dose (mg kg^{-1})	No. of mice	Footpad swelling (μm)	Inhibition (%)
Induction phase				
Control	–	8	510.1 ± 78.2	–
Astilbin	2.5	8	477.8 ± 129.3	6.35
	5	8	482.4 ± 115.2	5.44
	10	8	497.0 ± 116.1	2.57
Dexamethasone	5	8	342.6 ± 79.2*	32.84
Effector phase				
Control	–	9	307.0 ± 27.1	–
Astilbin	2.5	8	254.0 ± 45.7	17.26
	5	8	220.0 ± 61.1*	28.34
	10	8	187.3 ± 73.3*	39.01
Dexamethasone	5	9	64.2 ± 40.9*	79.08

Footpad reaction was induced in mice with SRBC. Astilbin and dexamethasone were given orally and intramuscularly, respectively, for 6 days from the day of SRBC sensitization (induction phase), or 3 times (at 0, 5 and 10 h) after SRBC challenge (effector phase). *$P < 0.01$ vs control.

Table 2 Effect of astilbin and dexamethasone on picryl-chloride-induced ear contact dermatitis in mice.

Group	Dose (mg kg^{-1})	No. of mice	Ear swelling (μm)	Inhibition (%)
Induction phase				
Control	–	8	90.1 ± 20.3	–
Astilbin	2.5	8	89.0 ± 23.4	1.3
	5	8	86.7 ± 21.5	3.7
	10	8	83.7 ± 13.8	7.2
Dexamethasone	5	8	20.9 ± 4.5**	76.8
Effector phase				
Control	–	8	100.1 ± 33.9	–
Astilbin	2.5	8	89.1 ± 45.4	11.0
	5	8	67.0 ± 20.0	33.1
	10	8	61.6 ± 13.0*	38.5
Dexamethasone	5	8	22.0 ± 4.8**	78.0

Contact dermatitis was induced in the ear of mice with picryl chloride. Astilbin and dexamethasone were given orally and intramuscularly, respectively, for 6 days from the day of sensitization (induction phase), or 3 times (at 0, 5 and 10 h) after picryl chloride challenge (effector phase). *$P < 0.05$, **$P < 0.01$ vs control.

Table 3 Effect of astilbin and dexamethasone on the weights of thymus and spleen in mice with ear dermatitis to picryl chloride.

Group	Dose (mg kg^{-1})	Body weight (g)	Immune organ weight (mg)		Organ weight/body weight (mg g^{-1})	
			Thymus	Spleen	Thymus	Spleen
Naive	–	24.3 ± 1.6	61.1 ± 12.6	160.1 ± 90.6	2.5 ± 0.5	6.4 ± 3.2
Control	–	24.3 ± 1.8	62.5 ± 11.2	117.8 ± 29.6	2.6 ± 0.5	4.8 ± 1.0
Astilbin	2.5	24.8 ± 2.4	62.1 ± 20.2	150.4 ± 44.1	2.5 ± 0.8	6.2 ± 2.2
	5	24.5 ± 0.9	62.0 ± 23.2	151.2 ± 32.1	2.5 ± 0.9	6.2 ± 1.3
	10	24.5 ± 1.1	72.0 ± 20.2	158.2 ± 51.7	2.9 ± 0.8	6.4 ± 1.7
Dexamethasone	5	24.5 ± 2.3	28.2 ± 8.2*	67.6 ± 16.0*	1.2 ± 0.3*	2.8 ± 0.7*

Contact dermatitis was induced in the ear of mice with picryl chloride. Astilbin and dexamethasone were given orally and intramuscularly, respectively, 3 times (at 0, 5 and 10 h) after picryl chloride challenge (effector phase). On the seventh day, the mice were weighed and their thymus and spleen were taken out and weighed. Naive: group of naive mice; Control: the mice that were induced with ear dermatitis and administered orally with water instead of drug. *$P < 0.01$ vs control.

MMP-2 and MMP-9 in the cells from mice with picryl-chloride-induced dermatitis (Figure 2).

Effect of astilbin on the activity of matrix metalloproteinases in spleen cells activated by Con A in-vitro

Spleen cells isolated from naive mice were incubated with 20 μg mL^{-1} of concanavalin A (Con A) and various concentrations of astilbin for 24 h. As compared with control, the production of both MMP-2 and MMP-9 in the supernatant collected was remarkably inhibited by astilbin (Figure 3).

Discussion

This study first examined the inhibition of astilbin on delayed-type hypersensitivity to SRBC or picryl chloride in mice. The flavanone significantly inhibited ear dermatitis when administered during the effector phase but not the induction phase of the delayed-type hypersensitivity, while the steroidal agent dexamethasone strongly inhibited the reaction in both phases (Tables 1, 2). These results further confirmed previous findings that the compound significantly improved the delayed-type-hypersensitivity-mediated liver injury only in the effector phase (Xu et al 1999) and selectively caused the apoptosis of activated Jurkat T cells (Yan & Xu 2001). The findings also indicate that astilbin may have a different immunosuppressive activity from steroids and other immunosuppressants, such as dexamethasone and ciclosporin (cyclosporin A). In our unpublished work, we have compared the effects of astilbin with ciclosporin on the production of interferon-γ in picryl chloride-induced contact dermatitis. While ciclosporin produced significant inhibition in both the induction phase and the effector phase, astilbin reduced the interferon-γ production selectively in the effector phase.

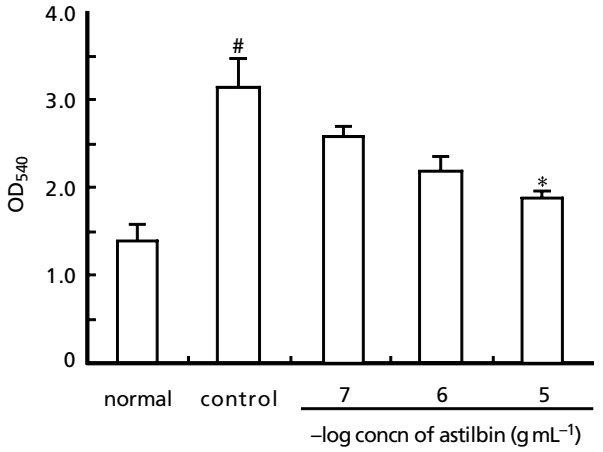

Figure 1 Effect of astilbin on the migration of spleen cells isolated from mice with ear dermatitis induced by picryl chloride. Spleen cells were isolated from mice 6 h after picryl chloride challenge. The filters with a 5.0-μm pore size in the transwell system were pre-coated with 2.5 μg gelatin in a volume of 50 μL in the lower surface. The 2×10^5 spleen cells seeded in the upper compartment of transwell cell chamber were incubated at 37 °C for 10 h in the presence or absence of astilbin, in total, 1 mL in the lower compartment. Then, the number of cells which had migrated to the lower compartment was determined as described in Materials and Methods. Each column represents the mean ± s.d. of three independent experiments and each experiment includes triplicate sets. #$P < 0.05$ vs naïve mice; *$P < 0.05$ vs control mice.

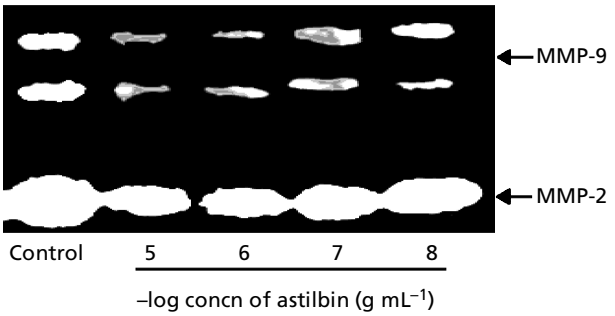

Figure 2 Effect of astilbin on the activity of matrix metalloproteinases (MMPs) produced by spleen cells isolated from mice with ear dermatitis to picryl chloride. Spleen cells were isolated 6 h after picryl chloride challenge and incubated at 37 °C for 24 h with astilbin. Then, the supernatants were collected and subjected to zymography assay. The figure shown here is a representative of two experiments with the same results.

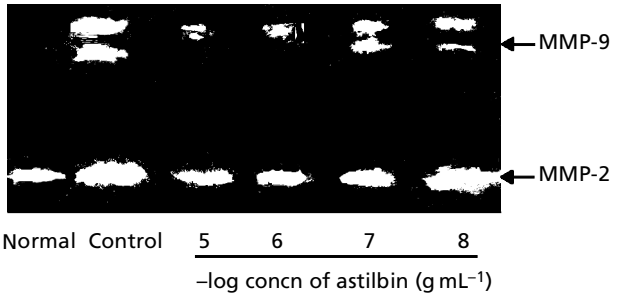

Figure 3 Effect of astilbin on the activity of matrix metalloproteinases (MMPs) produced by mice spleen cells activated by Con A. Spleen cells were isolated and cultured for 24 h with astilbin in the presence of 20 μg mL^{-1} of Con A at 37 °C. The supernatants were collected and subjected to zymography assay.

Indeed, ciclosporin and its derivatives usually lack target selectivity. For example, they not only disrupt the activation of the peripheral T-lymphocyte pool but also block the maturation of thymocytes within the thymus and change the thymic microenvironment (Damoiseaux et al 1997; Milicevic & Milicevic 1998). The selective inhibition by astilbin in the special stage of the disease or on the special state of T lymphocytes may imply its low incidence of side effects and be very important for the treatment of immunologically related diseases, such as rheumatic arthritis, hepatitis, etc. In fact, compared with the strong inhibition by dexamethasone on the weights of spleen and thymus, astilbin maintained the organ weights in the normal range (Table 3).

Considering the role of T lymphocytes in the delayed-type hypersensitivity reaction, we used the transwell system to evaluate the effects of astilbin on the ability of spleen cells isolated from mice with ear dermatitis to transmigrate through basement membrane. As a result, astilbin produced a dose-dependent inhibition (Figure 1). Furthermore, we examined the in-vitro effect of astilbin on the activity of MMP-2 and MMP-9. This is because gelatinase A and B have been reported to be involved in transmigration of T cells through basement membrane both in-vitro and in-vivo, and this process was specifically blocked by the inhibitor of matrix MMPs (Leppert et al 1995a, b). We found, in the experiment, that the compound could inhibit the activity of both MMP-2 and MMP-9 produced by spleen cells activated by Con A or isolated from mice with ear dermatitis (Figures 2 and 3). These results (Figures 1–3) suggested that the inhibition of lymphocyte migration by astilbin is related to the inhibition of MMP activity. Additional experiments are also needed to examine the effects of astilbin on other factors, such as chemokines and integrins, which are related to lymphocyte migration besides MMP. To further confirm and elucidate the effects of astilbin, however, we investigated the in-vivo and in-vitro effects of astilbin on inflammation, cell proliferation and migration. Astilbin did not influence the acute and chronic inflammation induced by xylene, carrageenan and cotton pellet, etc., neutrophil infiltration and T cell proliferation to Con A (data not shown). The data, together with the previous findings on activated T lymphocytes (Xu et al 1999; Yan & Xu 2001), suggested again that astilbin mainly targets T lymphocytes rather than other cell types and inhibits their function rather than activation.

In conclusion, astilbin may be an efficient agent for treating delayed-type-hypersensitivity-related diseases through selectively suppressing T cell functions including cell migration. Down-regulation of MMP activity in the lymphocytes may be one of the main mechanisms.

References

Beyaert, R., Cuenda, A., Vanden, B. W., Plaisance, S., Lee, J. C., Haegeman, G., Cohen, P., Fiers, W. (1996) The p38/RK mitogen-activated protein kinase pathway regulates interleukin-6 synthesis in response to tumour necrosis factor. *EMBO J.* **15**: 1914–1923

Cid, M. C., Esparza, J., Juan, M., Miralles, A., Ordi, J., Vilella, R., Urbano-Marquez, A., Gaya, A., Vives, J., Yague, J. (1994) Signaling through CD50 (ICAM-3) stimulates T lymphocyte binding to human umbilical vein endothelial cells and extracellular matrix proteins via an increase in beta 1 and beta 2 integrin function. *Eur. J. Immunol.* **24**: 1377–1382

Cuenda, A., Rouse J., Doza, Y. N., Meier, R., Cohen, P., Gallagher, T. F., Young, P. R., Lee, J. C. (1995) SB 203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1. *FEBS Lett.* **364**: 229–233

Damoiseaux, J. G., Beijleveld, L. J., van Breda Vriesman, P. J. (1997) Multiple effects of cyclosporin A on the thymus in relation to T-cell development and autoimmunity. *Clin. Immunol. Immunopathol.* **82**: 197–202

Hauzenberger, D., Bergstrom, S. E., Klominek, J., Sundqvist, K. G. (1999) Spectrum of extracellular matrix degrading enzymes in normal and malignant T lymphocytes. *Anticancer Res.* **19**: 1945–1952

Lee, J. C., Laydon, J. T., McDonnell, P. C., Gallagher, T. F., Kumar, S., Green, D., McNulty, D., Blumenthal, M. J., Heys, J. R., Landvatter, S. W. (1994) A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* **372**: 739–746

Leppert, D., Waubant, E., Galardy, R., Bunnett, N. W., Hauser, S. L. (1995a) T cell gelatinases mediate basement membrane transmigration in vitro. *J. Immunol.* **154**: 4379–4389

Leppert, D., Hauser, S. L., Kishiyama, J. L., An, S., Zeng, L., Goetzl, E. J., (1995b) Stimulation of matrix metalloproteinase-dependent migration of T cells by eicosanoids. *FASEB J.* **9**: 1473–1481

Milicevic, N. M., Milicevic, Z. (1998) Cyclosporin A-induced changes of the thymic microenvironment. A review of morphological studies. *Histol. Histopathol.* **13**: 1183–1196

Rubinstein, E., Le, N. F., Billard, M., Prenant, M., Boucheix, C. (1994) CD9 antigen is an accessory subunit of the VLA integrin complexes. *Eur. J. Immunol.* **24**: 3005–3013

Sheng, Y., Yoshimura, M., Inoue, S., Oritani, K., Nishiura, T., Yoshida, H., Ogawa, M., Okajima, Y., Matsuzawa, Y., Taniguchi, N. (1997) Remodeling of glycoconjuates on CD44 enhances cell adhesion to hyaluronate, tumor growth and metastasis in B16 melanoma cells expressing β-1,4-N-acetylglucosaminyltransferase III. *Int. J. Cancer* **73**: 850–858

Shimizu, Y., Shaw, S. (1991) Lymphocyte interactions with extracellular matrix lymphocyte integrins. *FASEB J.* **5**: 2292–2299

Suttles, J., Milhorn, D. M., Miller, R. W., Poe, J. C., Wahl, L. M., Stout, R. D. (1999) CD40 signaling of monocyte inflammatory cytokine synthesis through an ERK1/2-dependent pathway. A target of interleukin (IL)-4 and IL-10 anti-inflammatory action. *J. Biol. Chem.* **274**: 5835–5842

Tan, Y., Rouse, J., Zhang, A., Cariati, S., Cohen, P., Comb, M. J. (1996) FGF and stress regulate CREB and ATF-1 via a pathway involving p38 MAP kinase and MAPKAP kinase-2. *EMBO J.* **15**: 4629–4642

Westermarck, J., Kahari, V. M. (1999) Regulation of matrix metalloproteinase expression in tumor invasion. *FASEB J.* **13**: 781–792

Xu, Q., Xu, L. (1993) Inhibition of delayed type hypersensitivity reactions by Radix Gentianae. *Pharmacol. Clin. Chin. Materia Medica* **9**: 25–26

Xu, Q., Zhao, H., Hang, B. (1991) Inhibition of delayed type hypersensitivity reactions by Fructus Tribuli. *J. Chin. Pharm. Univ.* **22**: 12–16

Xu, Q., Wang, R., Xu, L., Jiang, J. (1993) Effects of Rhizoma Smilacis Glabrae on cellular and humoral immune responses. *Chin. J. Immunol.* **9**: 39–42

Xu, Q., Yuan, K., Lu, J., Wang, R., Wu, F. (1997) A new strategy for regulating the immunological liver injury – effectiveness of DTH-inhibiting agents on DTH-induced liver injury to picryl chloride. *Pharmacol. Res.* **36**: 401–409

Xu, Q., Wu, F., Cao, J., Chen, T., Jiang, J., Saiki, I., Koda, A. (1999) Astilbin selectively induces dysfunction of liver-infiltrating cells – novel protection from liver damage. *Eur. J. Pharmacol.* **377**: 93–100

Yan, R., Xu, Q. (2001) Astilbin selectively facilitates the apoptosis of interleukin-2-dependent phytohemagglutinin-activated Jurkat cells. *Pharmacol. Res.* **44**: 135–139