Mechanisms of Antitumor Activity of Aqueous Extracts from Chinese Herbs: Their Immunopharmacological Properties

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Accepted December 9, 1988

Abstract—Previously we reported the antitumor activity of 4 kinds of Chinese herbs: *A. capillaris*, *S. doederleinii*, *A. macrocephala* and *S. subprostrata*. Evidence has been also presented that *A. capillaris* shows the activity mainly through a direct tumoricidal action and the others display the activity through the enhancement of tumor-immune response. The present results are as follows: *A. capillaris* did not affect Meth A tumor-neutralizing activity (Winn's assay) in spleen cells of BALB/c mice bearing the primary tumor. The other herbs enhanced the activity on days 10 and 15 and prevented the decay of the activity on day 20 after the Meth A tumor transplantation. However, none of them enhanced the local graft versus host reaction (GvHR) induced in CBF₁ mice by spleen cells of BALB/c mice. *A. capillaris* hardly affected the humoral antibody formation against sheep red blood cells in mice. On the contrary, the other 3 herbs enhanced the formation. Blast transformation of murine lymphocytes with PHA-P or LPS was suppressed by 10⁻⁵ to 10⁻⁴ g/ml of *A. capillaris*. *A. macrocephala* enhanced the response by LPS. It should be noted that *A. capillaris* showed no immunosuppressive action in spite of having a direct tumoricidal action in vitro. Further investigations will be required on the latter 3 herbs to clarify the relationship between their humoral immunity-stimulating activity and their tumor immunity-enhancing activity.

It has been well-documented that BALB/c mice immunologically respond to Meth A tumor. Namely, these mice can resist the growth of a subcutaneously implanted Meth A tumor if they are treated with immunostimulating agents such as LPS (1, 2) and *Corynebacterium parvum* (3). In the previous paper (4) using the syngeneic system of Meth A tumor and BALB/c mice, we demonstrated the antitumor activity of 4 kinds of Chinese herbs: *A. capillaris*, *S. doederleinii*, *A. macrocephala* and *S. subprostrata*. *A. capillaris* significantly inhibited the growth of Meth A tumor even in BALB/c-nu/nu mice, while the others did not. It was also shown that *A. capillaris* had cytotoxic activity against Meth A tumor and L-929 cells in an in vitro study. Further investigation (5) regarding the antitumor mechanisms of these herbs in-

dicated that all of them enhanced the Meth A tumor-induced delayed type hypersensitivity reaction (Meth A-DTH), although they did not affect the picryl chloride-induced delayed type hypersensitivity reaction (PC-DTH). These observations suggest that *A. capillaris* shows the antitumor activity by a mechanism mainly based on a direct tumoricidal action, although this herb includes some components that can enhance Meth A-DTH. The other 3 herbs display the activity through the enhancement of T cell-mediated immunity, particularly tumor specific delayed type hypersensitivity.

In the present paper, to reconfirm the effect of these Chinese herbs on tumor immunity, we intended to examine whether these herbs enhance the tumor-neutralizing activity in spleen cells of mice bearing Meth

A tumor. In addition, we studied the effects of these herbs on some immune responses other than the tumor-immune response to clarify their immunopharmacological properties.

Materials and Methods

1. Drugs: Chinese herbs employed in this study were as follows: *Artemisia capillaris* THUNB., *Selaginella doederleinii* HIERON., *Atractylodes macrocephala* KOIDZ. and *Sophora subprostrata* CHUN et T. CHEN. Aqueous extracts from these Chinese herbs were made by the ordinary methods, which were described previously (4, 5). The yields of the extracts were 13.4% from *A. capillaris*, 7.4% from *S. doederleinii*, 38.1% from *A. macrocephala* and 17.2% from *S. subprostrata*. The following materials were also employed: benzylpenicillin potassium-treated and lyophilized cells of the *Streptococcus pyogenes* Su strain (A group, type 3) (OK-432, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan), prednisolone (Takeda Chemical Industries, Ltd., Osaka, Japan) and cyclophosphamide (CY, Nakalai Tesque, Inc., Kyoto, Japan). The dose of OK-432 was expressed as units of Klinische Einheit (KE), where one KE contains 0.1 mg of dried whole cells of *S. pyogenes*. They were dissolved or suspended in distilled water for oral administration and in saline for parenteral dosing. For the in vitro assay, the aqueous extracts of Chinese herbs were dissolved in the culture medium described below and sterilized with a 0.45 μ m cellulose acetate membrane.

2. Animals: BALB/c, (BALB/c $\times$ C57BL/6) F₁ (CBF₁) and ddY strains of female mice were purchased from Shizuoka Experimental Animal Center (Hamamatsu, Japan) and maintained in laminar-air-flow cages at 22 $\pm$ 1°C and 60% humidity. They were allowed free access to pellet food and water and used for the experiments when they were 8 to 12 weeks of age.

3. Tumor cells: Meth A tumor cells were obtained from Aichi Cancer Center (Nagoya, Japan) and maintained by weekly passage in the peritoneal cavity of BALB/c mice in our laboratory.

4. Preparation of spleen cell suspension: Spleens were aseptically taken from mice and

gently crushed and separated into single cells by squeezing in Hanks' solution containing 5 U/ml heparin. The cells obtained were passed through a fine stainless steel sieve (200 mesh) and washed twice with Hanks' solution, followed by resuspension in an appropriate medium.

5. Tumor-neutralizing activity of spleen cells: One million Meth A cells were inoculated s.c. into the flanks of BALB/c mice. They were killed 10, 15 and 20 days after the inoculation to provide spleen cells for examining the neutralizing activity against Meth A cells by Winn's assay. Namely, 10⁷ spleen cells from the mice were mixed with 10⁵ Meth A cells. The mixture from one mouse was inoculated s.c. into the flanks of two normal BALB/c mice. Tumor size (the mean of 2 mice) was measured 15 days after the inoculation using vernier calipers in terms of 2 diameters at right angles. Tumor size was expressed as volume ($4/3\pi \times (\text{long diameter}/2) \times (\text{short diameter}/2)^2 \text{ cm}^3$), and the neutralizing activity of spleen cells was represented as the percent tumor size to that of the control mice which had been inoculated with the mixture of normal spleen cells and Meth A cells.

6. Local graft versus host reaction (local GvHR): Ten million spleen cells from CBF₁ and BALB/c mice were inoculated in a volume of 0.1 ml suspension, s.c., into the left and right footpads of CBF₁ mice, respectively. The severity of GvHR was evaluated by the weight difference between the right and left popliteal lymph node of the recipient 8 days after the inoculation.

7. Plaque forming cell (PFC) production in spleen and serum antibody formation: Sheep red blood cells (SRBC) were used as an antigen. SRBC stored in Alsever's solution was washed and resuspended into an appropriate solution [sterilized saline for immunization, Eagle's MEM for PFC assay and phosphate-buffered saline (PBS, 8 g NaCl, 1.43 g Na₂HPO₄·2H₂O and KH₂PO₄ in a liter of distilled water, pH 7.4) for serum antibody titration]. ddY mice were immunized with the i.v. injection of 2 $\times$ 10⁸ SRBC. They were killed 5 and 10 days after the immunization, and spleen weight, number of PFC in the spleen and serum antibody titer were

measured. Direct PFC and indirect PFC in the spleen were detected according to Cunningham and Szenberg (6). Rabbit anti-mouse IgG (Fc specific, The Medical and Biological Laboratories, Nagoya, Japan) was used at a final 200-fold dilution for developing indirect PFC. Number of IgG-PFC was calculated as the difference between the numbers of direct and indirect PFCs. The anti-SRBC antibody titer in the serum was measured by enzyme-linked immunosorbent assay (ELISA) as described previously (7). Briefly, SRBC was directly fixed to the bottom of an Aminoplate well (96-well, flat bottom, Sumitomo Bakelite Co., Osaka, Japan) which had been positively charged. Serum samples were diluted with PBS and added to the well. After 2-hr incubation at 37°C, SRBC on the bottom was washed with PBS, added with a 500-fold diluted 2nd antibody of alkaline phosphatase-conjugated affinity purified goat anti-mouse IgM or IgG preparation (Cappel, PA), and incubated 1 hour at 37°C. After washing, the alkaline phosphatase activity in a well was measured by the Kind and King method (8).

8. Mitogen-induced blast transformation of spleen cells: Spleen cells from BALB/c mice were suspended in the culture medium (RPMI-1640 medium containing 10% heat inactivated fetal calf serum (Gibco, NY), 10 unit/ml penicillin G, 100 µg/ml streptomycin and 25 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfate (HEPES)). Five hundred thousand cells in 0.2 ml volume in a well of a flat-bottomed, 96-well culture plate were incubated at 37°C for 96 hours under 5% CO₂ in the presence or absence of 20 µg/ml of phytohemagglutinin-P (PHA-P, Difco, MI) and 50 µg of lipopolysaccharide (LPS, *Escherichia coli* 055: B5, Difco, MI). After the incubation, the amount of DNA per well was measured fluorometrically, and the stimulation index (SI) was calculated as follows: $SI = (\text{DNA amount of stimulated cells}) / (\text{DNA amount of non-stimulated cells})$. The total DNA amount in cultured cells was measured as described previously (4). Briefly, after removing erythrocytes from the cultured cells in a well with 0.75% NH₄Cl solution and washing twice with saline, the residual cells were lysed with 0.2 ml of 0.1%

sodium dodecyl sulfate (SDS) solution. Then, the cell lysate was transferred into a glass tube using 1.8 ml of the SDS solution and mixed with 2 ml of 6 µg/ml ethidium bromide (Nakalai Tesque, Inc., Kyoto, Japan). Fifteen min later, the fluorescence (Ex. 525 nm, Em. 600 nm) of the solution was measured. DNA amount was calculated from the fluorescence standard curve prepared with DNA (Type I, Sigma, MO).

9. Statistics: For all data shown in the figures and tables, the values were expressed as the mean ± S.E.M. Willcoxon's rank sum test (*U*-test) was employed to analyze the significant difference between 2 groups in data for the tumor-neutralizing activity. Student's or Welch's two tailed *t*-test after the *F*-test was used to analyze the significance of difference for the other data. The value of $P < 0.05$ was considered significant.

Results

1. Effect of Chinese herbs on tumor-neutralizing activity of spleen cells in mice bearing Meth A sarcoma: BALB/c mice were given p.o. the aqueous extracts from Chinese herbs in a dose of 250 mg/kg for 20 days from 4 days before the tumor inoculation. Then, their spleen cells were subjected to Winn's assay. As shown in Fig. 1, the spleen cells in the control group exhibited approximately 17% neutralizing activity on days 10 and 15, and then the activity decreased to ~60% on day 20. *A. capillaris* did not affect the activity on any day. *S. doederleinii* significantly prevent the reduction of the activity on day 20. *A. macrocephala* increased the activity significantly on day 10, but did not affect it on days 15 and 20. *S. subprostrata* showed a tendency to increase the activity on days 15 and 20.

2. Effect of Chinese herbs on local GvHR: The local GvHR was induced in BALB/c mice by inoculating spleen cells of CBF₁ mice as described under Methods. The recipient mice were given p.o. the aqueous extracts of Chinese herbs in doses of 250 and 500 mg/kg for 12 days from 4 days before the inoculation. Control mice were administered water as a vehicle. None of the Chinese herbs affected the GvHR except that

Fig. 1. Effect of Chinese herbs on Meth A tumor-neutralizing activity in spleen cells of female BALB/c mice. Mice, 8 weeks old, were transplanted with 10^6 cells of Meth A tumor, s.c., into their flanks. The aqueous extracts (250 mg/kg) were given p.o. for 20 days from 4 days before the tumor transplantation. Five mice in each group were killed 10, 15 and 20 days after the transplantation to provide spleen cells for Winn's assay (Meth A cells : spleen cells=1:100). Each point indicates the mean $\pm$ S.E.M. of 5 animals. *: Statistically significant difference from the control at $P < 0.05$.

500 mg/kg of *S. subprostrata* suppressed the response (Fig. 2).

3. Effect of Chinese herbs on PFC production in spleen and serum antibody formation: The aqueous extracts from Chinese herbs were given p.o. for 9 days from 4 days before the SRBC immunization. CY was given p.o., and OK-432 was given i.p. for 5 days from the immunization. The Chinese herbs hardly affected the spleen weight at any dose on both days 5 and 10, but CY showed a tendency to decrease the weight on day 5, and OK-432 significantly increased the weight on day 10 (Table 1). Tables 2 and 3 show the effect of herbs on the number of PFCs per spleen and serum antibody titer, respectively. *A. capillaris* showed only a

tendency to increase IgM- and IgG-PFC on day 10 in a dose of 250 mg/kg as well as IgM and IgG antibodies in serum. These effects of *A. capillaris* were not remarkable as a whole. *S. doederleinii* in a dose of 250 mg/kg showed a tendency to increase IgG-PFC on both days 5 and 10 as well as IgG antibody in the serum. Five hundred mg/kg of this herb increased IgM- and IgG-PFC markedly on day 10 as well as IgG antibody on day 5 and IgM- and IgG-antibodies on day 10. *A. macrocephala* in doses of 250 and 500 mg/kg increased all parameters of PFC, except for no increase of IgG-PFC on day 5 at the dose of 250 mg/kg, while IgM and IgG antibodies increased on day 10. *S. subprostrata* at both doses increased IgG-

Fig. 2. Effect of *A. capillaris* (A), *S. doederleinii* (B), *A. macrocephala* (C) and *S. subprostrata* (D) on local GvHR in female CBF₁ mice. The million spleen cells from CBF₁ and BALB/c mice were inoculated s.c. into the left and right feet of 8 week-old CBF₁ mice, respectively. Recipient mice were p.o. given the aqueous extracts for 12 days from 4 days before the inoculation. GvHR was evaluated by the weight difference between the right and left popliteal lymph nodes 8 days after the inoculation. Each column represents the mean $\pm$ S.E.M. of 7 to 8 animals. *: Statistically significant difference from the control at $P < 0.05$.

Table 1. Effect of Chinese herbs on spleen weight of mice

	Dose (mg/kg)	Spleen weight (mg)	
		Day 5	Day 10
Control		135 $\pm$ 7.8	117 $\pm$ 5.7
<i>A. capillaris</i>	250	135 $\pm$ 11.0	120 $\pm$ 6.9
	500	141 $\pm$ 10.0	109 $\pm$ 4.1
<i>S. doederleinii</i>	250	130 $\pm$ 9.4	113 $\pm$ 9.8
	500	140 $\pm$ 7.2	124 $\pm$ 5.9
<i>A. macrocephala</i>	250	140 $\pm$ 10.2	113 $\pm$ 3.6
	500	135 $\pm$ 12.7	119 $\pm$ 9.0
<i>S. subprostrata</i>	250	141 $\pm$ 5.7	123 $\pm$ 9.7
	500	129 $\pm$ 7.4	107 $\pm$ 5.3
CY	10	125 $\pm$ 8.6	105 $\pm$ 9.5
OK-432	1 KE/animal	150 $\pm$ 7.2	172 $\pm$ 12.3**

ddY mice were immunized i.v. with 2×10^8 SRBC. The aqueous extracts were given p.o. for 9 days from 4 days before the SRBC immunization. CY was given p.o. and OK-432 was given i.p. for 5 days from the immunization. Each group included 8 mice except for the group of 7 mice given 500 mg/kg of *A. macrocephala* on day 10 and the group of 7 mice given 500 mg/kg of *S. subprostrata* on day 10. Measurements were done 5 and 10 days after the immunization. **: Statistically significant difference from the control at $P < 0.01$.

PFC on day 5 and increased IgM- and IgG-PFC on day 10. In this case, 250 mg/kg of the herb increased IgM antibody and 500 mg/kg increased IgG antibody on day 10. CY suppressed IgM- and IgG-PFCs on day 5 as well as IgM and IgG antibodies, but not

on day 10. OK-432 increased IgG-PFC on day 5 and IgM- and IgG-PFC on day 10 as well as IgG antibody on day 10.

4. Effect of Chinese herbs on mitogen-induced blast transformation of spleen cells: Spleen cells of BALB/c mice were incubated

Table 2. Effect of Chinese herbs on PFC formation in the spleen of mice

	Dose (mg/kg)	Day 5			Day 10		
		IgM-PFC	IgG-PFC	IgM-PFC	IgG-PFC	IgM-PFC	IgG-PFC
Control		395±25	430±138	41±8	233±55		
<i>A. capillaris</i>	250	449±61 (1.1)	533±158 (1.2)	63±7 (1.5)***	352±58 (1.5)		
	500	531±61 (1.3)	333±169 (0.8)	44±5 (1.1)	275±45 (1.2)		
<i>S. doederleinii</i>	250	371±36 (0.9)	681±126 (1.6)	49±6 (1.2)	443±97 (1.9)***		
	500	415±42 (1.1)	322±54 (0.7)	77±14 (1.9)*	455±109 (2.0)***		
<i>A. macrocephala</i>	250	532±17 (1.3)**	415±71 (1.0)	80±8 (2.0)**	302±65 (1.3)		
	500	504±59 (1.3)	560±122 (1.3)	66±12 (1.6)	392±73 (1.7)		
<i>S. subprostrata</i>	250	443±40 (1.1)	600±128 (1.4)	75±10 (1.8)*	378±62 (1.6)		
	500	443±53 (1.1)	552±105 (1.3)	66±7 (1.6)*	403±66 (1.7)***		
CY	10	130±18 (0.3)**	167±28 (0.4)***	51±7 (1.2)	305±64 (1.3)		
OK-432	1 KE/animal	428±31 (1.1)	757±118 (1.8)***	100±10 (2.4)**	612±129 (2.6)*		

See legends for Table 1. Data represent the number of PFCs ($\times 10^3$) per spleen, with the ratio to the control in parentheses. *, **: Statistically significant difference from the control at $P<0.05$ and $P<0.01$, respectively. *** indicates $P<0.1$.

Table 3. Effect of Chinese herbs on antibody formation in the serum of mice

	Dose (mg/kg)	Day 5			Day 10		
		IgM (1/250 ^a)	IgG (1/250 ^a)	IgM (1/100 ^a)	IgG (1/5000 ^a)	IgM (1/100 ^a)	IgG (1/5000 ^a)
Control		0.42±0.03	0.53±0.05	0.51±0.05	0.60±0.08		
<i>A. capillaris</i>	250	0.42±0.05 (1.0)	0.53±0.08 (1.0)	0.59±0.03 (1.2)	0.70±0.04 (1.2)		
	500	0.44±0.04 (1.0)	0.54±0.08 (1.0)	0.60±0.05 (1.2)	0.62±0.06 (1.0)		
<i>S. doederleinii</i>	250	0.44±0.02 (1.0)	0.65±0.05 (1.2)	0.48±0.03 (0.9)	0.78±0.07 (1.3)		
	500	0.46±0.03 (1.1)	0.67±0.05 (1.3)***	0.59±0.03 (1.2)	0.81±0.07 (1.4)***		
<i>A. macrocephala</i>	250	0.49±0.02 (1.2)	0.54±0.03 (1.0)	0.67±0.04 (1.3)*	0.71±0.06 (1.2)		
	500	0.45±0.03 (1.1)	0.56±0.06 (1.1)	0.64±0.06 (1.3)	0.76±0.08 (1.3)		
<i>S. subprostrata</i>	250	0.47±0.02 (1.1)	0.59±0.05 (1.1)	0.65±0.06 (1.3)***	0.62±0.07 (1.0)		
	500	0.37±0.03 (0.9)	0.62±0.06 (1.2)	0.53±0.04 (1.0)	0.86±0.11 (1.4)***		
CY	10	0.20±0.02 (0.5)**	0.25±0.04 (0.5)**	0.53±0.03 (1.0)	0.51±0.05 (0.9)		
OK-432	1 KE/animal	0.39±0.03 (0.9)	0.60±0.05 (1.1)	0.43±0.03 (1.0)	0.86±0.05 (1.4)*		

See legends of Table 1. ^a: Final dilution of serum samples for titration. *, **: Statistically significant difference from the control at $P<0.05$ and $P<0.01$, respectively. *** indicates $P<0.1$.

Fig. 3. Effect of *A. capillaris*, *S. doederleinii*, *A. macrocephala* and *S. subprostrata* on PHA-P or LPS induced blast transformation of spleen cells from BALB/c mice. Spleen cells (5×10^6 /well) were incubated with the aqueous extracts in the presence (○, □) or absence (●, ■) of 2×10^{-5} g/ml of PHA-P or 5×10^{-5} g/ml of LPS at 37°C for 4 days, respectively. Blastogenesis of lymphocytes was estimated by measuring the amount of DNA with a fluorometric method using ethidium bromide. Each datum indicates the mean $\pm$ S.E.M. of triplicate sets. C: Control. *, **: Statistically significant difference from the control at $P < 0.05$ and $P < 0.01$, respectively.

with 10^{-7} – 10^{-4} g/ml of the aqueous extracts from Chinese herbs in the presence or absence of $20 \mu\text{g}$ of PHA-P or $50 \mu\text{g}$ of LPS at 37°C for 96 hr. SIs of the control were between 1.94 and 2.14 in PHA-P stimulation and between 2.70 and 3.62 in LPS stimulation (Fig. 3). *A. capillaris* showed a tendency to increase SI induced by PHA-P at lower concentrations (10^{-7} – 10^{-6} g/ml) and reduced the index at higher concentrations (10^{-5} – 10^{-4} g/ml), and it caused a concentration-dependent reduction of SI induced by LPS. *S. doederleinii* at the highest concentration (10^{-4} g/ml) showed a tendency to reduce SI by PHA-P and reduced SI by LPS. *A. macrocephala* increased both SIs with PHA-P and, particularly, with LPS. *S. subprostrata* reduced both SIs by PHA-P and LPS at the highest concentration (10^{-4} g/ml). None of these Chinese herbs affected the DNA amount of cells incubated without

mitogens.

Discussion

There have been many reports (1–3, 9–13) about the immunological response against Meth A tumor in the syngeneic host, BALB/c mice. North and Bursucker (9) reported that the cells having the $\text{Ly-1}^+\text{2}^+$ phenotype of cytotoxic T cells in mice play an important role in the concomitant immunity, showing tumor resistance. Others (10, 11, 14) described the importance of the cells having the $\text{Ly-1}^+\text{2}^-$ phenotype of helper/delayed hypersensitivity T cells in mice. Previously, we (5) showed that Meth A tumor specific delayed type hypersensitivity induced in BALB/c mice after the primary tumor inoculation was detectable until 15 days, but decayed drastically by day 20 in inverse proportion to the tumor growth. Similarly, the neutralizing activity in the

control was detected on days 10 and 15 after tumor transplantation, then decreased on day 20 to a level lower than that of normal spleen (Fig. 1). The mechanisms for the decay of the immunity seen on day 20 after the Meth A-inoculation have not been clear, although North (12) and DiGiacomo and North (13) reported the induction of suppressor T cells which down-regulated effector T cells in concomitant tumor immunity. The administration of *S. doederleinii*, *A. macrocephala* and *S. subprostrata* enhanced the tumor neutralizing activity in the spleen on day 10 or 15, and it prevented the decrease of the activity on day 20. *A. capillaris* did not affect the activity. It was, therefore, reconfirmed that these herbs except for *A. capillaris* could augment the immune response to resist the tumor growth. However, the precise mechanisms of the enhancing activity of these Chinese herbs on the tumor immune response are still unclear. Moreover, there is still much debate about whether delayed type hypersensitivity effector T cells (10, 11, 14, 15) or cytotoxic T cells (9, 12, 13, 15), or perhaps even both of them play a more important role in the host defense mechanisms against tumors. There are possibilities that all these T cell subsets as well as activated macrophages (3, 10) contribute to the tumor-neutralizing activity found in this study.

We also examined the effect of the 4 kinds of Chinese herbs on immune responses other than tumor immune response to clarify their immunopharmacological profiles.

It has been established that the local GvHR is mediated primarily by the Lyt-1⁺2⁺ phenotype of cytotoxic T cells recognizing the major histocompatibility antigens. Markwick et al. (16) reported that not only graft lymphocytes but also recipient lymphocytes participate in the local GvHR. None of the Chinese herbs enhanced the GvHR, suggesting that these herbs do not affect this kind of cytotoxic T cell.

These Chinese herbs did not affect the spleen weight, differing from what was observed in the case of OK-432. Such a result indicates that these herbs have less side effects on immunological organs. *A. capillaris* hardly affected the humoral antibody formation to SRBC. On the contrary, the

other 3 herbs including *S. doederleinii*, *A. macrocephala* and *S. subprostrata* enhanced the humoral antibody formation, especially IgM and IgG antibodies on day 10.

Mitogen-induced lymphocyte proliferation in vitro is thought to be a model for the lymphocyte proliferation occurring in the immunological responses in vivo. It is well-known that PHA-P stimulates T cells and LPS stimulates B cells of murine lymphocytes to cause them to transform and proliferate. *A. capillaris* suppressed the lymphocyte proliferation with PHA-P and LPS even at the relatively low concentration of 10⁻⁵ g/ml. Previously, we reported that this herb inhibited the tumor proliferation in vitro, and also this herb suppressed the subcutaneous growth of Meth A tumors in BALB/c-nu/nu mice. From the previous findings and the present result that *A. capillaris* showed no effect on the tumor-neutralizing activity, it is thought that *A. capillaris* exhibits the antitumor activity through a direct cytotoxic action against the tumor. A suppressive effect of *A. capillaris* on the mitogen-induced lymphocyte proliferation might be a reflection of this cytotoxic action. Here, it should be noticed that *A. capillaris* did not show any immunosuppressive action to GvHR and humoral antibody formation as was generally seen by carcinostatic agents such as 5-fluorouracil and CY. Also, *A. capillaris* did not affect the resting cells in vitro. *S. doederleinii* and *S. subprostrata* suppressed the responses with PHA-P and LPS only at the highest dose. The enhancing activity of *A. macrocephala* on the LPS-induced lymphocyte proliferation may be related to its stimulating activity on humoral antibody formation as well as its immunological antitumor activity. Further investigations on the latter 3 herbs will be required to clarify the relationship between their humoral immunity-stimulating activity and their enhancing activity on concomitant tumor immunity, including the tumor-neutralizing activity of spleen cells and tumor-induced delayed type hypersensitivity.

Acknowledgment: We thank Tsumura, Inc. for the generous gifts of the Chinese herbs employed in this study.

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