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IMMUNOMODULATING CHINESE HERBAL MEDICINES

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Traditional Chinese medicine always pays close attention to the strengthening of the patient's general resistence against illness, there are many Chinese herbs used for thousands of years are considered as tonics. Animal experiments and modern clinical trails have shown that quite a number herbs are immunologically active, and most of the tonics are excellent immunomodulating agents, such as polysaccharides or saponins isolated from Astragalus mongholicus, Acanthopanax senticosus *and* Panax notoginseng, *which stimulated macrophages, promoted antibody formation, activated complement and increased T lymphocyte proliferation. Moreover, some of them were proved to be anti-irradiative and protected animals from liver intoxications. On the other hand, some anti-inflammative or anti-pyretic herbs such as* Tripterygium wilfordii, Aconitum *and* Artemisiae *species were proved to have immunosuppressive principles, some of them were now used clinically for the treatment of rheumatoid arthritis, chronic nephritis, systemic lupus erythematosis and various skin disorders. Pharmacological studies revealed that they have depressant effect on most of the humoral-immunity but not on the cell-mediated immunity. Some of them stimulated adrenal cortex functions and prolonged the survival time of transplanted allograft tissues.*

Key words: *Astragalus mongholicus* – *Acanthopanax senticosus* – *Panax notoginseng* – *Tripterygium wilfordii* – *Aconitum* – *Artemisiae* – immunomodulating

The research work on immunomodulating drugs is a new field in pharmacology which was developed only in recent 20 years. It will provide both theoretical significance and therapeutic value in many diseases, including cancer, viral infections, autoimmune diseases, organ transplantation and aging, caused by deficiency or imbalance of the immune functions of patients. The development of a safe and effective immuno-modulator for clinical use has become a major goal of many pharmaceutical investigators. As the traditional Chinese medicine always pays close attentions to the strengthening of the patient's general resistance against illness, many Chinese herbs used for thousands of years are considered as tonics for the improvement of general health. Animal experiments and clinical trails have shown that quite a few herbs are immunologically active, and most of the tonics are excellent immunopotentiating or immunomodulating agents.

In this paper, we reviewed some of our recent publications on the immuno-activities of natural products. Three immunostimulators and three immunosuppressors isolated from traditional Chinese herbs were reported briefly.

The immunological system comprise a complex network of cells including T-cells, B-cells, macrophages and natural killer cells. In addition, many kinds of cytokines, immunoglobulins, interferons and complement molecules play pivotal roles in the up and down regulations of immune functions. Thus mutiple immunoassays both *in vitro* and *in vivo* were used in order to evaluate their final immunoactivities. Lymphocyte proliferation was determined by counting the incorporation rate of ^{3}H-thymidine into splenocyte DNA during synthesis, antibody formation was assayed by counting the plaque-forming cells (PFC) or by mesuring the serum hemolysin unit after mice challenged by sheep red blood cells (SRBC). Other special methods are indicated in the text.

Astragalus mongholicus Bunge is one of the most commonly used Chinese herb which was considered as a bioenergetics, to reinforce the bioenergy of patients suffering from deficiency of vital energy. A polysaccharide APS has been isolated from the root of this herb which is a polymer of glucose and arabinose. This compound is almost non-toxic on mice and can diminish the toxic effects of CCl_4, PHA and

TABLE I

Antagonist effects of APS on immunosuppressors

	Dose mg/kg	Organ wt. (mg/10g) spleen	Thymus	Lymph node	WBC $mm^3 \times 10^{-2}$
Control	–	43 ± 5	29 ± 11	48 ± 13	140 ± 51
APS	200	86 ± 5[b]	24 ± 6	45 ± 7	–
Prednisolene	25	24 ± 3[b]	15 ± 3[b]	21 ± 6[b]	56 ± 22[b]
APS + Pred.	200 + 25	50 ± 5[e]	19 ± 5[b/c]	31 ± 4[a/e]	112 ± 27[e]
CY	100	26 ± 3[b]	15 ± 3[b]	32 ± 7[a]	20 ± 3[b]
APS + CY	200 + 100	35 ± 9[d]	14 ± 3[b]	34 ± 8[a]	18 ± 6[b]

a: $p < 0.01$; *b*: $p < 0.001$ vs Control; *c*: $p < 0.05$; *d*: $p < 0.01$; *e*: $p < 0.001$ vs Prednisolone or CY alone.

TABLE II

Effect of APS on phagocytosis

	Time (hr)	Phagocytic % Control	APS	Phagocytic index Control	APS
Expt. 1	1/2	0.8	17.5	0.013	0.35
	3	15	23.2	0.21	0.34
	6	8	19.5	0.15	0.43
Expt. 2	1/2	0.6	10	0.03	0.52
	3	10.2	22	0.18	0.76
	6	8.4	18.8	0.48	1.05

prednisolone. Intraperitoneal injection of this polysaccharide enlarges the spleen and increases the antibody forming cells as well as promotes the synthesis of RNA, DNA and protein in the spleen. It also promotes significantly mice macrophage phagocytosis but increases only slightly the rate of lymphocyte proliferation. (Shanghai Institute of Material Medica, 1979) (Tables I, II).

Acanthopanax senticosus Harms or *Eleutherococcus senticosus* Maxim *(Araliaceae)* is a small tree distributed widely in northeast China. The root of this plant is used as tonics as well as sedative agent. We found the polysaccharide PES possess significant immunopotentiating activities. It is composed with glucose, galactose and arabinose (33:2:1) m.w. about 7000 with $1 \rightarrow 3$ linked α-D-glucopyranosyl residues. It is effective in potentiating the antibody response against sheep red blood cells and in stimulating the phagocytosis of the macrophages in mice (Xu et al., 1983). PES can lessen the intoxication of some hepatotoxins, prolonged the survival time of X-ray irradiated mice, increase WBC counts and inhibits the growth of transplanted S180 tumor. Recently it was found that PES has an interferon promoting effect, the interferon titer was 5-10 times higher in S801 and S7811 leukemic cell cultures by adding PES after primed with NDV-F as an inducer (Fig. 1).

In the same species, another *Acanthopanax gracilistylus* W. W. Smith studied in our laboratory showed also to be an "adaptogen" for their ability to increase the resistance or adaptation ability of men and animals to different unfavourable factors of physical, chemical and biological origins. A triterpine compound which increases antibody formation and T lymphocyte proliferation has been found.

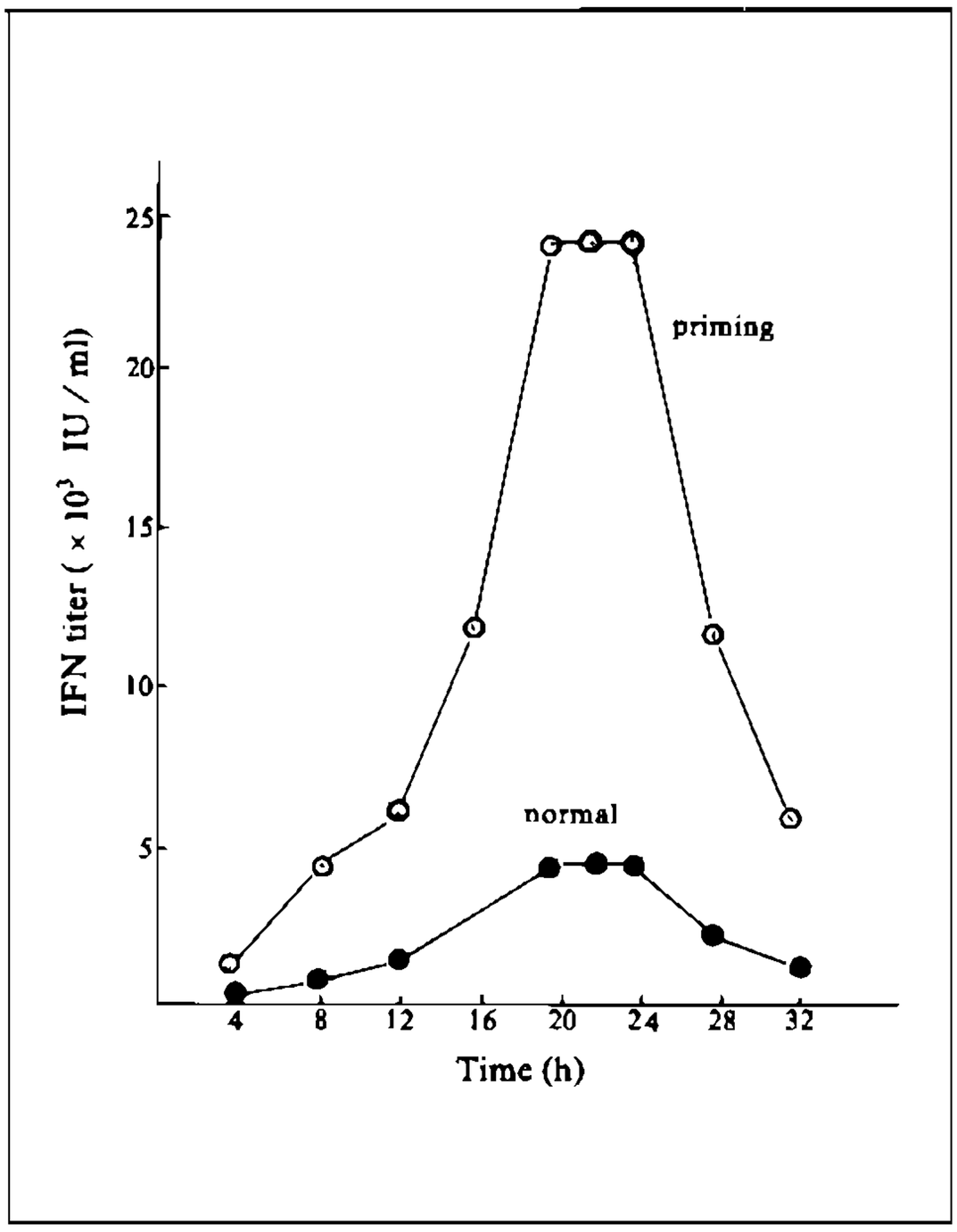

Fig. 1 : kinetic effect of interferon inducing of PES on S801 leukemia cells.

Pannax notoginseng (Burk) F. H. Chen is a famous Chinese traditional herb grown in southwest China which has multiple activities such as analgetic, hematostatic and tonic. It contains several known Ginseng saponins such as ginsenoside Rb_1 Rd, Re and Rg, now some new saponins were also isolated and identified. We found that the total saponin PNS caused a marked increase in serum complement and hemolysin levels, especially in immuno-deficient animal models, such as in EAE (experimental allergic encephalomyelitis), a T-lymphocyte-mediated autoimmune disease in rats and guinea pigs, PNS antagonized the suppression of DTH and protected the EAE rats from paralysis and death. The polysaccharide of *Panax notoginseng* (PNB) stimulated T lymphocyte proliferation both *in vivo* and *in vitro*, antagonized the action of T cell suppressor cyclosporin A. It also promoted antibody formation and interleukin production. It is low toxic and may be developed into a therapeutic drug for some immuno-deficient diseases (Tables III, IV).

Tripterigium wilfordii Hook f. belonging to the family of *Celastraceae* used for the treatment of fever, chillness, edema and carbuncle. In the last 20 years, it has been used increasingly to treat rheumatoid arthrits, chronic nephritis and various skin disorders including psoriasis, systemic lupus erythematosis, and allergic angitis with promising results. Clinical practice proved that some patients received the crude extract of the plant decreased the testicular volume and caused azoospermia. Many compounds have been isolated from this plant. Triptolide and tripdiolide showed significant antileukemic activity against mice L-1210 and P388 leukemias *in vivo* at 0.1 mg/kg and markedly prolonged the survival time of mice with L615 Leukemia. 0.2 mg/kg triptolide ip had a depressant effect on the humoral-mediated immunity as assayed by the hemolysin test, but not on the cell-mediated immunity as monitored by the graft versus host reaction and the tumor-concomittent immunity (Zhang, 1981). Tripterine 0.1 μg/ml inhibited *in vitro* the proliferation of mice splenic cells induced by mitogens PHA, Con A. PWM and LPS. The immunosuppressive effect of triptonide was revealed by an elevation of plasma cortisol and a lowering of ascorbic acid and cholesterol content in adrenal gland. The stimulation effects on adrenal cortex may be also a factor to inhibit the inflammatory process. Recently, tripterygium glycosides preparation (GTW) was used clinically which showed satisfactory effects on RA and SLE with less side effect (Zheng, et al., 1983). Our works are in progress (Table V).

Alkaloids isolated from different species of *Aconitum* such as 3-acetyl-aconitine(3-Ac), bulleyaconitine (Bul) and yunaconitine (YAc) were highly effective on analgesic, anti-inflammation and immunosuppression. Our work showed that YAc 50 μg/kg ip daily markedly prolonged the survival time of transplanted allograft heart tissue in ICR mice and was comparable to prednisolone. YAc ip 20 μg/kg inhibit spleen PFC counts but increased serum complement level as well as phagocytic activity of RES in mice. Besides, YAc inhibited the increased vascular permeability, the leukocyte migratory response and other inflammatory process such as swelling and granuloma formation. YAc neither reduced the weights of thymus and adrenal gland in rats, nor prolonged the survival time of adrenalectomized rats indicates that the action of YAc does not depend on stimulation of the pituitary-adrenal axis (Li et al., 1987) (Table VI).

TABLE III

Effect of PNB on spleen lymphocyte transformation responses to several mitogens *in vitro* ($\bar{x} \pm SD$), cpm x 10^{-2}

Conc. μg/ml	Control	Con A 5 μg/ml	PHA 10 μg/ml	PWM 10 μg/ml	LPS 10 μg/ml
0	2 ± 1	198 ± 22	99 ± 6	92 ± 10	138 ± 21
100	2 ± 1	171 ± 10	107 ± 14	88 ± 6	120 ± 20
200	3 ± 1	221 ± 21	208 ± 25^b	90 ± 14	133 ± 22
400	5 ± 1^b	224 ± 43	257 ± 36^b	108 ± 21	150 ± 12
800	11 ± 1^c	371 ± 51^b	232 ± 29^b	137 ± 16^a	130 ± 17

a: $p < 0.05$; *b*: $p < 0.01$; *c*: $p < 0.001$ vs blank.

TABLE IV

Effect of ip PNB on spleen lymphocyte blastogenesis in ICR mice *(in vivo)* ($\bar{x} \pm SD$)

	Dose mg/kg x d	No. of mice	^{3}H-TdR incorporation cpm/3 x 10^5 cells
Control	–	13	4246 ± 725
PNB	2.5 x 3	13	6886 ± 1280^a
	5.0 x 3	13	5450 ± 826^a

a: Compared with control $p < 0.01$.

TABLE V

Immunosuppressive effects of *Tripterygium wilfordii* glycosides

	Dose mg/kg	Hemolysin HC50	PFC /10^6 cell	Lymphocyte proliferation cpm x 10^{-2}	
				in vivo	*in vitro*
Control	–	231 ± 54	245 ± 73	458 ± 60	508 ± 36
GTW	20	165 ± 74^a	138 ± 33^a	387 ± 55	174 ± 61^b

a: $p < 0.05$; *b*: $p < 0.001$ vs Control.

TABLE VI

Effect of YAc on the survival time of transplanted mouse heart

	Daily ip dose	Starting on	Mice	Heart tissue survival days
Control	–	–	13	8.7 ± 1.2
Prednisolone	25 mg/kg	d 1	12	11.8 ± 2.5^b
YAc	50 μg/kg	d 7	10	10.3 ± 2.4^a
Control	–	–	17	11.8 ± 2.0
Prednisolone	25 mg/kg	d 7	16	12.3 ± 2.1
YAc	50 μg/kg	d 7	18	12.4 ± 2.2

Heart transplantation were operated on d 0. Survival heart tissues were examined by ECG. ($\bar{x} \pm SD$; *a*: $p < 0.05$; *b*: $p < 0.01$, compared with control).

TABLE VII

Effect of im artemether 2 mg/kg daily for 3 d on circulating immune complexes (CIC, mg protein/ml serum) and total complement (C, complement hemolysis U/ml serum)

	Mice	Days after infection 0	7	9
CIC	Malarial	0.53 ± 0.12(8)	0.82 ± 0.12(7)[a]	1.14 ± 0.35(6)[a]
	Treated		0.68 ± 0.18(7)[b]	0.60 ± 0.20(6)[c]
C	Malarial	30 ± 2(5)	22 ± 5(3)[a]	8 ± 3(3)[a]
	Treated		49 ± 21(3)[b]	40 ± 14(3)[c]

Number of Balb/c mice in the parentheses. ($\bar{x}$ ± SD); *a*: $p < 0.01$ compared with normal mice; *b*: $p < 0.05$; *c*: $p < 0.01$ compared with malarial mice.

TABLE VIII

Effect of im artemether 2 mg/kg daily for 3 d on mouse RBC rosette formation. $\bar{x}$ ± SD.

	Mice	C3b receptor rosette (%)	CIC rosette (%)
Normal	7	22 ± 15	10 ± 9
Malarial	5	0.9 ± 0.7[a]	0.9 ± 0.9[c]
Treated	5	38 ± 15[a/b]	26 ± 9[a/b]

a, *b*: were same to Table VII.

The herb *Artemisiae annua* Linn has been used as an anti-malarial drug for more than thousand years. Recently, artemisinine (Qinghaosu) has been isolated and shown to possess potent inhibitory effects on malarial infections both in animals and patients. It is higher in efficacy than chloroquine, with a quicker effect and lower toxicity, especially in treating those patients with cerebral malaria. Immunological studies shown that artemisinine inhibited mice spleen cell proliferation and hemolysin formation with no cytotoxicity. Since the intensity of the immune response and the associated complement activation may be important factors in the pathogenesis of cerebral and fulminating malaria, we studied a semi-synthesized derivative artemether, on mice red blood cell immunity. The results showed that artemether in 2 mg/kg lowered serum circulating immune complexes, maintained normal RBC membrane C3b receptors and serum complement levels, protected the infected mice from death (Li & Liang, 1986). It is suggested that artemether exerts some modulating effects on RBC immunity other than its schizonts killing activity (Tables VII, VIII, Fig. 2).

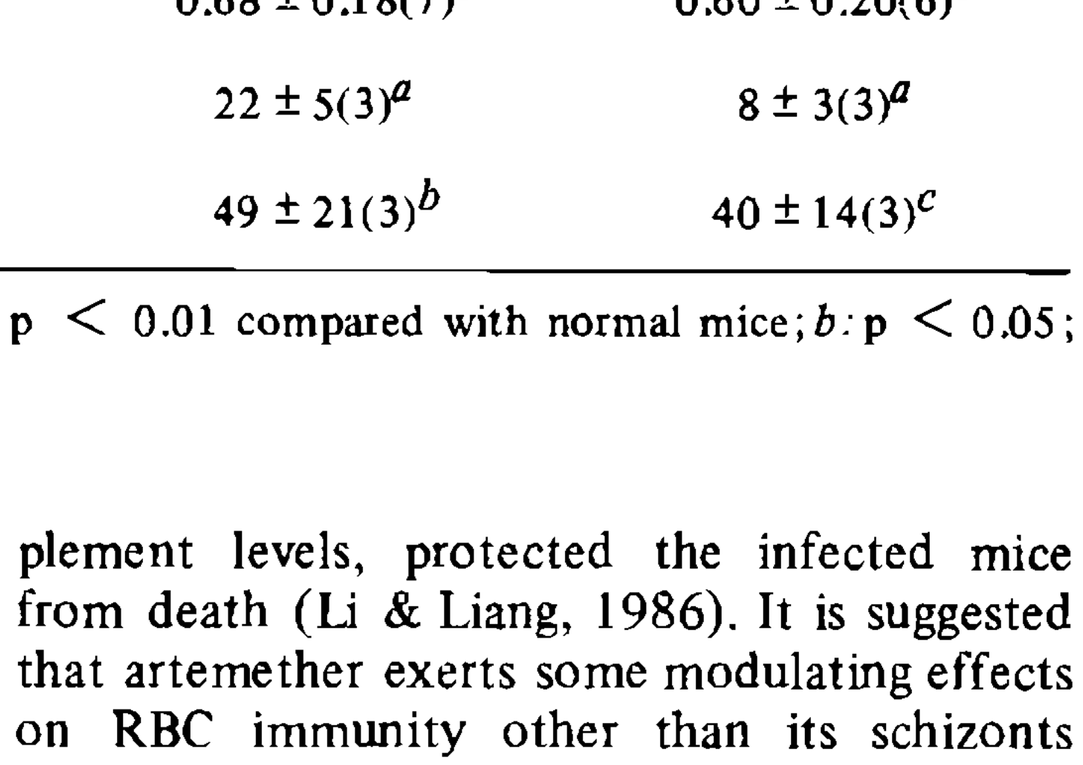

Fig. 2: serum total complement level increased after artemether emulsion 32 mg/kg iv or oil solution 8 mg/kg im on 3 monkeys inoculated with *P. cyonomolgi*.

CONCLUSION

Traditional Chinese medicine, as well as the folk medicine in Brazil, are great treasures of the people. It offers significant potential for new drug discovery and development. Our Institute is willing to cooperate with scientists from other countries to join the research. We

believe that new drugs that cure cancer, AIDS and make human longevity related to immunomodulators will be developed in the next century and will have great contributions to the health of mankind.

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