

# **Impact of soy fermented product (Zenhua 851) Intake on Human first line of defense**

—Results of Double-blind Study on Healthy Subjects

Using Placebo and Experimental Food

Osaka University of Foreign Studies Health Care Center

Kajiki Osami

Holistic Medicine Science Research Institute

Takahashi Rei

## **Abstract**

This is a study on humans to research the effect of soy fermented product (Zenhua 851) on human immunity. Changes in NK cell activity and other immunity-related blood values are observed after one-time intake and 7-day continuous intake. 15 male subjects of good health conditions are summoned to go through a procedure to screen out the following candidates with possible immunity impairments: (1) those taking anti-inflammatory analgesics, antibiotics, nutrition and complementary foods in the two weeks prior to the experiment; (2) those with flu, diarrhea or a fever resulting from other unknown causes; (3) those using large doses of caffeine (more than five cups of coffee or black tea a day) or smoking heavily (more than 20 cigarettes a day); and (4) those refusing to abstain from smoking on the day of the test. 12 male of them survive the selection procedure, at an average age of  $22.6 \pm 5.1$ . A double-blind crossover method is adopted, using both placebo and experimental product. In the one-time intake experiment, subjects are placed under the same conditions in terms of food, sleep, movement, and environment, from the night before until the end of the experiment, to eliminate the impact on NK cell activity of anything other than the experimental product. The result shows no significant change resulting from the placebo but a significant increase in NK cell activity 5 and 8 hours after taking Zenhua 851. After 7-day continuous intake, a check on NK cell activity, interleukin (IL<sub>2</sub>, IL<sub>2</sub> receptor, IL-12), cd<sub>4</sub>/cd<sub>8</sub> is carried out on the following morning, the result shows no significant change for both placebo and experimental product. Besides, no side-effect is detected during the period of intake. All this shows that Zenhua 851 is capable of enhancing immunity after one-time intake.

# 1. Foreword

Soy fermented product foods became widely available long ago. At the same time, research began on the effect that these foods made with yeast and Natto Kinase have on human body. The animal experiments carried out at China's Fujian Medical University showed that soy bean fermented by adding natural soil bacteria (851 bacteria) is capable of prolonging life. In one of the experiments, Kunming mice were found to be able to swim longer and with better endurance. Immunity tests also indicated an increase in lymphocytes as well as improved cytoimmunity and appetite. The animal experiments therefore have provided clear evidence of enhanced physiological function produced by soy bean fermented with 851 bacteria.

Clinical research to this day has found that intake of soy bean fermented with 851 bacteria can benefit patients suffering from chronic gastritis and viral hepatitis. A double-blind experiment involving 330 volunteers, ages 50-69, showed significant improvements in blood anti-acidification, T cell immunity, estradiol and other sex hormones, lung functions, memory and conversation capabilities.

In addition, in an experiment involving 333 cancer patients receiving chemotherapy and radiotherapy, in which a comparison was made between those taking soy bean fermented with 851 bacteria and others who did not, Zhenhua 851 not only boosted the overall condition of the patients, but also resulted in significantly improved cd<sub>3</sub>, cd<sub>4</sub>, NK cell activity and immune goblin. NK cell activity is a blood test item in clinical research on human immunity. But it varies greatly from one individual to another, and from day to day or moment to moment even inside one individual. For example, it can change a lot after one watches a comedy for an hour or so, or is given a slight stimulus to laugh. With this in mind, it is easy to see why subjects in an experiment have to face environment restrictions in order to eliminate all factors other than experimental foods and to obtain accurate results in measuring NK cell activity.

This research has done just that by establishing an experimental system that has eliminated as much as possible all the other factors to focus on the enhancement of human immunity by soy bean fermented with 851 bacteria. First, we have to make sure that the NK cell activity values of the subjects are randomly scattered. Next, to minimize differences common to the group, the subjects are put under restrictions with regard to food intake, sleep, movement and environment as well as prohibited from taking any food that might affect their NK cell activity other than the experimental ones so as to avoid any cross interference.

This experiment aims to evaluate as accurately as possible the impact of soy fermented products (Zhenhua 851) on human NK cell activity and to verify its effect in immunity enhancement.

## 2. Experimental subjects

Only male candidates were selected during the screening process to eliminate the impact of female menstrual cycle.

Out of 15 healthy males with normal white blood cell count, 12 candidates emerged without prior history of special diseases or alcoholism after eliminating those with immunity impairments (see Table 1). Total abstention from alcohol was verified two days before the experiment started. The average age of these 12 subjects is about  $22.6\pm 5.1$ . They were fully briefed on the purpose of the experiment.

Table 1 Candidates Eliminated

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- (1) those taking anti-inflammatory analgesics, antibiotics, nutrition and health care products in the two weeks prior to the experiment
  - (2) those with a fever resulting from flu, diarrhea or other unknown causes in the two weeks prior to the experiment
  - (3) those smoking heavily (more than 20 cigarettes a day) or refusing to abstain from smoking during the experiment; and
  - (4) those using large doses of caffeine (more than five cups of coffee and black tea)
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## 3. Experimental method

### (1) Experiment food

The foods used in the experiment, both soy fermented product (Zhenhua 851) and a placebo made of rice flour, were supplied in the form of pills by Ledun Pharmaceutical Company. The daily dose of nine pills (3,237mg) contains 2,400mg of Zhenhua 851, the nutrition composition of which comprises amino acid, vitamin and minerals as shown in Table 2. The same additive was used for both the placebo and Zhenhua 851, two of which can not be distinguished in appearance as confirmed by Ledun Pharmaceutical Company.

Table 2 Zhenhua 851 Composition

Composition	Content per 100g	Analytical Method
Water	2.9g/100g	Loss on drying
Protein	53.8g/100g	Kjeldahl method
Fat	2.7g/100g	Soxhletextractor
Ash	8.9g/100g	
Sugars	27.1g/100g	
Dietary fiber	4.5g/100g	Oxygen weight
General bacteria	3.5×10 <sup>2</sup> /g	Standard plate culture method
Coliform	Negative	BGIB method

Table 3 Amino Acid, Vitamin and Mineral Content per 1g of Zhenhua 851

Amino acid	Alanine	25.1mg	Methionine	5.2mg
	Arginine	15.2mg	Phe	14.8mg
	Aspartate	86.5mg	Praline	10.4mg
	Cystine	2.9mg	Valine	17.4mg
	Glutamic	28.7mg	Threonine	16.0mg
	Glycocoll	20.8mg	Tryptophan	4.9mg
	Histidine	5.1mg	Tyrosine	11.1mg
	Isoleucine	14.8mg	Lysine	21.1mg
	Leucine	31.0mg	Ornithine	3.4mg
	Serine	14.1mg		
Vitamin	Vitamin A	354.11U	Vitamin E	4.61U
	Vitamin B2	0.7mg	Vitamin K	8.7µg
	Vitamin C	27.3mg		
Trace elements	Lead (II)	0.19mg	Iron	0.83mg
	Potassium	46.7mg	Calcium	23.2mg
	Selenium	58.3µg	Magnesium	11.1mg
	Sodium	3.5mg		

## (2) Subjects distribution

For this double-blind crossover experiment using both placebo and experimental food, 12 subjects are randomly divided into two groups by Dr. Takahashi Rei who is not directly involved in the experiment. The subjects, six in each group, are coded a, b, c, d, e, and f in Group A and g, h, j, k, l, and m in Group B. Before the experiment, their age, height, weight, and blood cell composition were checked to verify that no significant distinctions existed.

Table 4 The conditions of subjects before the experiment

		Test Date	Starting day of 1st stage		Starting day of 2nd stage	
		Subject Group	Group A (6)	Group B (6)	Group A (6)	Group B (6)
		Subject	a. b. c. d. e. f.	g. h. j. k. l. m.	a. b. c. d. e. f.	g. h. j. k. l. m.
		Test food	Placebo	Zhenhua851	Zhenhua851	Placebo
Age (years)			22.2±3.3	23.0±6.9	22.2±3.3	23.0±6.9
NK cell activity		%	50.3±9.1	46.7±7.6	36.3±8.7*	40.0±15.0
WBC count		×1000/μl	58.5±16.0	56.2±10.5	58.7±9.9	65.2±14.2
<b>WBC differential count</b>	Stab form neutrophil	%	3.7±1.0	4.0±1.4	3.5±1.6	4.7±0.8
	Segmented neutrophil	%	47.7±7.6	53.7±5.1	44.8±10.5	49.3±2.1
	Eosinophil	%	6.5±3.8	5.2±1.8	7.0±4.3	4.5±2.1
	Basophil	%	0.2±0.4	0.3±0.5	0.5±0.6	0.5±0.5
	Lymphocyte	%	36.7±6.2	30.5±5.7	39.0±11.0	35.5±3.5
	Monocyte	%	5.3±1.5	6.3±1.9	5.2±2.0	5.5±1.4
Red blood cell count		×10000/μl	469±26	520±37	477±25	514±26
Thrombocyte		×10000/μl	21.4±7.0	23.2±4.0	23.5±7.6	23.9±4.4
Body weight		Kg	67.3±11.5	65.9±12.5	67.4±11.3	66.0±12.4
Stature		cm	168.9±9.6	170.1±10.1	168.9±9.6	170.1±10.1
BP	SBP	mmHg	122.5±12.5	123.5±13.8	123.8±15.4	127.4±15.9
	DBP	mmHg	75.4±8.9	69.8±9.1	75.8±8.4	72.4±7.6
Pulse		Beats/min	66.8±6.2	64.8±8.1	65.4±6.2	67.2±7.4

Wilcoxon test : \* p<0.05 (Significant difference for conditions of subjects between starting day of two stage)

### (3) Experimental procedure

Table 5 shows how the experiment is carried out.

The experiment is divided into two stages with an intermission of two weeks in between. The experimental foods that the two groups take are crossed over the two periods.

Table 5 Experiment Timetable

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1<sup>st</sup> stage:

Day before experiment

- 19:00 Assembly and orientation
- 20:00 Supper (sushi) and watching TV
- 23:00 Go to bed

1<sup>st</sup> day

- 6:30 Get up and wash face
- 8:00 1<sup>st</sup> blood drawing (before intake)  
Take orally 9 pieces of test food after blood drawing and then watch TV
- 10:00 2<sup>nd</sup> blood drawing (2 hours after intake)
- 11:00 Lunch (noodle and sushi)
- 13:00 3<sup>rd</sup> blood drawing (5 hours after intake)  
Watch TV and listen to music
- 16:00 4<sup>th</sup> blood drawing (8 hours after intake)

2<sup>nd</sup> – 6<sup>th</sup> days

Morning Take orally 9 pieces of experimental food on an empty stomach

7<sup>th</sup> day (last day)

Morning Take orally 9 pieces of experimental food on an empty stomach

- 19:00 Watch TV together
- 20:00 Supper (sushi)
- 23:00 Go to bed

Day after experiment

- 6:30 Get up and wash face
- 8:00 5<sup>th</sup> blood drawing (on an empty stomach without taking any experimental food)

2<sup>nd</sup> stage (3 weeks after the 1<sup>st</sup> stage)

Same as 1<sup>st</sup> stage (experimental food taken)

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#### (4) Blood test items

Per the experiment timetable, four blood drawings were performed before and after intake of experimental foods on the starting day of each stage. The same was done on the day following the end of the 7-day period. The specific blood test items are listed below.

Group A: blood drawings from the start of each stage till 8:00am on the morning after the end of the experiment.

Blood test items: WBC, RBC, differential count, HGB, HCT, OT, GPT,  $\gamma$ -GTP, NK cell activity, interleukin-2(IL-2) and receptor IL-12

Group B: 2<sup>nd</sup> to 4<sup>th</sup> blood drawings of each stage.

Blood test items: NK cell activity

NK cell activity is measured by SY-Cr separation value which indicates the damage to object cells K-562 after NK cells are cultivated there.

Table 6 Determination procedure of NK cell activity

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[1] Preparation of effector cells

- ① Peripheral blood separate effector cells with Specific weight - centrifugal separation (Conray-Ficoll:d=1.077)
- ② Wash cells with PBS 2 times
- ③ Add 10% FBS-RPMI-1640 medium
- ④ The number of cells to adjust:  $1 \times 10^6$  个/ml

[2] Preparation of target cells ( $^{51}\text{Cr}$  labeled): K562

- ① Collecting of K562 cells in logarithmic growth phase With centrifugal separation, Adding sodium chromate ( $\text{Na}^{51}\text{CrO}_4$ ) of  $50 \sim 100\mu\text{Ci}$ .
- ②  $37^\circ\text{C}$ . cultivate 1 hour.
- ③ Wash target cells with PBS 2 times.
- ④ Add 10% FBS-RPMI-1640 medium .
- ⑤ The number of cells to adjust:  $1 \times 10^6$  个/ml .

[3] Culture

- ① The effector cells, target cells were added to cell culture plate respectively .
- ② Hole of Maximum release add 1N-HCl, hole of natural release addd 10% FBS-RPMI- 1640 medium, hole of test release add effector cells (E/T=20) .
- ③ Centrifugation culture plate at 800r/min, 5 time.
- ④ Culture in 5%  $\text{CO}_2$  culture box 3.5 hours.

[4] Determination

- ① Take supernatant from each hole.
- ② Determine by  $\gamma$ -ray counter.
- ③ Calculation of NK cell activity.

$$\text{NK cell activity}(\%) = \frac{\text{experimental release (cpm)} - \text{spontaneous release (cpm)}}{\text{maximal release (cpm)} - \text{spontaneous release (cpm)}} \times 100\%$$

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### **(5) Statistical method**

Non-parametric method is used throughout the experiment, error of two groups error below 5%.

## **4. Experimental result**

On the days starting the 1<sup>st</sup> and 2<sup>nd</sup> stages, the subjects showed no significant differences in their health conditions and physical examinations. White cell count in Group B increased somewhat but in no significant way during the 2<sup>nd</sup> stage. Differential count did not show any significant change either. But NK cell activity in Group A was significantly lower on the starting day of the 2<sup>nd</sup> stage than during the 1<sup>st</sup> stage while that in Group B did not show any significant drop during the 2<sup>nd</sup> stage.

### **(1) NK cell activity**

Table 6 shows the change in NK cell activity that all the subjects went through after intake of test foods. Fig. 1 shows the average NK cell activity values of the 12 subjects before intake. Those taking the placebo showed no significant change 2 hours, 5 hours, and 8 hours after intake. On the other hand, those taking Zhenhua 851 experienced a significant increase by approximately 30% 5 hours and 8 hours after intake. This shows the superiority of Zhenhua 851 over the placebo in enhancing NK cell activity.

The results measured on the morning following the 7-day continuous intake showed no significant change in NK cell activity with regard to both Zhenhua 851 and the placebo. This shows that long-time intake of Zhenhua 851 does not have any accumulative effect despite its short-term enhancement of NK cell activity.



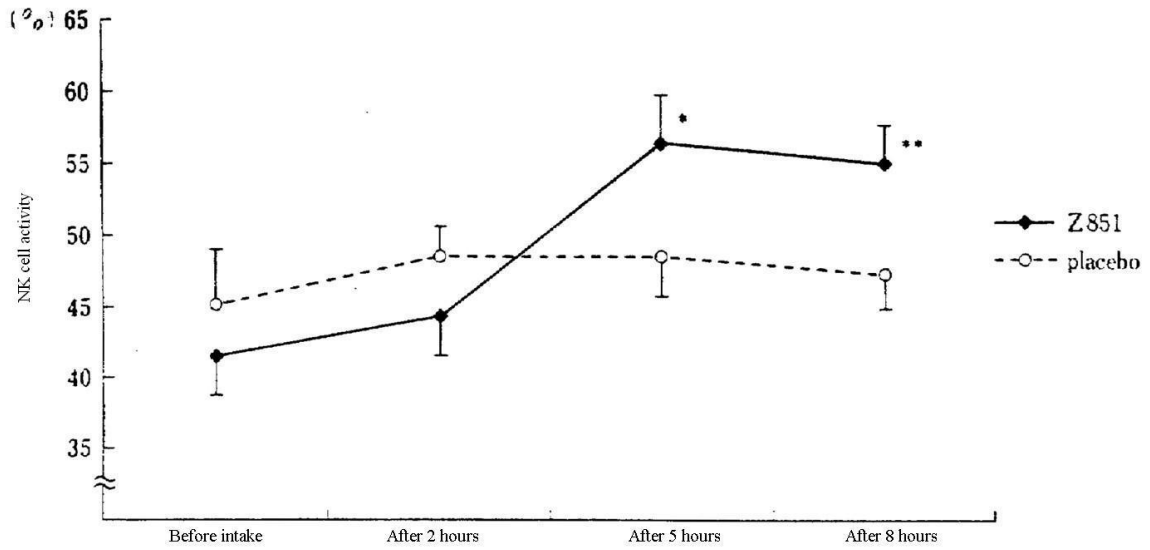
Table 7 After intake the variations of NK cells activity

	Subject	NK cell activity					NK cell activity increased rate (Value than before the experiment)			
		Before intake	After 2 hour	After 5 hour	After 8 hour	After 7 day	After 2 hour	After 5 hour	After 8 hour	After 7 day
Zhenhua 851	a	43	61	70	69	64	1.42	1.63	1.60	1.49
	b	39	44	65	48	40	1.13	1.67	1.23	1.03
	c	29	45	56	56	47	1.55	1.93	1.93	1.62
	d	30	42	63	47	37	1.40	2.10	1.57	1.23
	e	49	60	65	61	62	1.22	1.33	1.24	1.27
	f	28	35	69	65	38	1.25	2.46	2.32	1.36
	g	51	35	31	37	34	0.69	0.61	0.73	0.67
	h	43	34	59	64	57	0.79	1.37	1.49	1.33
	j	53	33	47	54	36	0.62	0.89	1.02	0.68
	k	55	57	56	50	45	1.04	1.02	0.91	0.82
	l	35	40	45	52	19	1.14	1.29	1.49	0.54
	m	43	47	49	56	26	1.09	1.14	1.30	0.60
		ave.±S.D.	41.5±9.5	44.4±10.1	56.3±11.5*	54.9±9.0**	42.1±13.7	1.07	1.36 <sup>#</sup>	1.32 <sup>#</sup>
Placebo	a	49	58	41	53	56	1.18	0.84	1.08	1.14
	b	36	47	41	48	25	1.31	1.14	1.33	0.69
	c	45	41	40	47	27	0.91	0.89	1.04	0.60
	d	54	45	39	32	37	0.83	0.72	0.59	0.69
	e	62	54	53	42	49	0.87	0.85	0.68	0.79
	f	56	42	56	58	39	0.75	1.00	1.04	0.70
	g	34	41	54	53	39	1.21	1.59	1.56	1.15
	h	60	60	66	54	73	1.00	1.10	0.90	1.22
	j	21	47	51	48	55	2.24	2.43	2.29	2.62
	k	56	56	58	51	60	1.00	1.04	0.91	1.07
	l	34	52	50	50	17	1.53	1.47	1.47	0.50
	m	35	39	33	30	26	1.11	0.94	0.86	0.74
		ave.±S.D.	45.2±13.0	48.5±7.3	48.5±9.7	47.2±8.6	41.9±16.9	1.07	1.07	1.04

Wilcoxon test: \*p<0.05, \*\*p<0.01 (Significant differences before and after intake)

Mann-whitney test : <sup>#</sup>p<0.05 (Significant difference between the measured food)

Fig.1 After intake the variations of NK cells activity



Friedman test :  $p < 0.01$  Wilcoxon test : \* $p < 0.05$ , \*\* $p < 0.01$

**(2) Check value of immune cells such as interleukin and other immunity-related blood test values**

WBC, lymphocytes,  $cd_4/cd_8$ , IL-2, receptor IL-2 and IL-12 values before and after intake are shown in Table 8. After intake of Zhenhua 851, white cell count went up significantly and IL-2 receptor values also showed an unexpected increase. The other items did not change in any significant way. L-2 and IL-12 were mostly undetected, making it difficult to make any comparisons.

**(3) GOT, GPT and  $\gamma$ -GTP**

Table 9 lists the GOT, GPT and  $\gamma$ -GTP values before and after taking placebo and Zhenhua 851. The values show no significant change. In addition, individual tests have also produced the same result.

Table 8 After intake changes in immune function in cells

		WBC		Lymphocyte		cd4/cd8	
		Before intake	After 7 days	Before intake	After 7 days	Before intake	After 7 days
Zhenhua 851	a	6700	8400	37.9	33.6	2.5	1.8
	b	5000	4800	47.5	39.9	1.8	2.1
	c	4600	5500	48.7	29.8	1.1	1.0
	d	7100	7600	42.3	37.1	1.4	1.6
	e	6300	6700	45.6	46.1	2.0	1.6
	f	5500	4500	19.6	26.2	1.9	2.0
	g	5500	8200	33.5	30.3	0.8	0.8
	h	5700	7100	23.3	53.5	0.9	0.6
	j	5600	6900	30.4	42.0	1.9	1.3
	k	5400	10100	26.5	24.0	1.0	0.9
	l	4100	4100	36.7	35.6	0.7	0.8
	m	7400	7100	32.2	38.0	1.2	1.5
		ave.±S.D.	5742±983	6750±1771*	35.4±9.5	36.3±8.4	1.4±0.6
Placebo	a	7400	7600	35.9	35.8	2.7	2.0
	b	5400	6300	41.5	45.0	2.1	1.6
	c	4200	5800	39.1	41.3	1.4	1.0
	d	8100	8300	32.6	36.0	1.7	1.4
	e	5700	5300	41.9	43.7	1.9	1.7
	f	4300	4800	26.1	22.0	1.7	2.0
	g	5800	5300	36.9	31.2	0.8	0.9
	h	6000	4800	35.0	51.6	0.8	0.9
	j	6600	5900	39.8	31.3	1.8	1.7
	k	7800	7000	32.9	28.4	0.9	1.0
	l	4500	5700	35.3	44.0	0.7	0.7
	m	8400	8300	36.3	40.2	1.3	1.3
		ave.±S.D.	6183±1483	6258±1259	36.1±4.4	37.5±8.3	1.5±0.6

Wilcoxon test: \*p<0.05 (Significant differences before and after intake)

The variations of Interleukins

IL-2		IL-2 receptor		IL-12	
Before intake	After 7 days	Before intake	After 7 days	Before intake	After 7 days
S.F.	5.2	224	231	10.6	7.6
S.F.	S.F.	267	267	21.3	13.3
S.F.	17.7	156	152	6.9	S.F.
S.F.	S.F.	162	166	S.F.	S.F.
S.F.	9.6	250	271	S.F.	11.6
S.F.	S.F.	324	213	S.F.	12.9
S.F.	S.F.	267	296	S.F.	S.F.
S.F.	14.8	322	375	S.F.	S.F.
5.5	S.F.	245	269	S.F.	S.F.
6.5	S.F.	279	347	8.3	S.F.
9.1	S.F.	259	376	S.F.	S.F.
S.F.	S.F.	199	207	S.F.	S.F.
		246.2±53.8	264.2±75.0		
10.5	S.F.	240	237	14.3	S.F.
21.2	S.F.	284	255	33.3	10.9
9.0	S.F.	356	144	13.9	10.0
S.F.	S.F.	215	174	S.F.	S.F.
S.F.	S.F.	298	302	S.F.	S.F.
8.5	S.F.	232	397	S.F.	S.F.
S.F.	S.F.	276	213	S.F.	10.4
S.F.	S.F.	376	291	5.6	10.2
5.4	S.F.	428	269	19.2	11.4
13.6	S.F.	272	218	33.0	28.5
14.3	S.F.	197	185	23.0	S.F.
11.9	S.F.	216	163	16.6	S.F.
		282.5±71.6	237.3±71.2*		

S.F. = Sensitivity following

Table 9 Liver function test values after intake

	GOT		GPT		γ-GTP	
	Before intake	After 7 days	Before intake	After 7 days	Before intake	After 7 days
Zhenhua 851	19.1±5.7	21.5±4.9	19.9±22.9	21.6±20.0	13.8±5.9	14.2±6.0
Placebo	20.5±6.8	21.7±6.1	20.8±23.3	21.0±24.0	13.6±6.6	14.6±6.0

Wilcoxon test: not significant

## 5. Discussion

This double-blind experiment that we have conducted using placebo indicates that Zhenhua 851 is capable of enhancing NK cell activity, which peaks 5 hours after intake and is still sustained 8 hours after intake. When tested 24 hours after the 7-day continuous intake experiment, NK cell activity shows almost no change compared with pre-intake. This tells us that NK cell activity rapid growth between 2 and 5 hours after intake, and then disappears after 24 hours. It also indicates that continued intake will not have an accumulative effect and that there is no adverse effect 24 hours after intake. Zhenhua 851 is capable of enhancing human immunity and its long-time use is safe to human body.

NK cell is one of the most important immune cells in human cellular immunity. As a large granular lymphocyte (LGL), its cytoplasm contains rich particles. NK cells exist inside human body and their killing power is not subject to the restrictions of major histocompatibility complex (MHC). This is how a hypothesis explains the enhancement of NK cell activity after Zhenhua 851 intake: macrophages are activated and cytokines like IL-2 or IL-12 are involved in the activation of lymphocytes which induce interleukins, resulting in enhanced NK cell activity. In particular, IL-12 is the first to be called a “NK cell stimulator” because of its prominent role in activating NK cells. Research carried out to this day has found that those infected with AIDS virus have significantly lower periphery lymphocyte IL-12 and NK cell activity. After given IL-12, their NK cell activity can be restored to a normal level.

The experiment also looked into IL-2 receptor and IL-12 values before and after 7-day continuous intake. The result shows only a slight upward tendency in IL-2 receptor without any significant change. IL-2 and IL-12 are mostly undetected. No significant change is observed. This tells us that the enhancement of NK cell activity hours after intake is undoubtedly related to increasing IL values. There is a need for further research in future on the relationship between cytokines and NK cell activity as well as on the impact of long-time intake on the activation of cytokines.

To this day, there has hardly been any relatively complete report on scientific experiments measuring food immunity conducted on humans. The fact that Zhenhua 851 is capable of enhancing human immunity points to an epoch-making invention. Up to now, apart from oral intake, one can engage in medium-intensity short-duration exercise to increase NK cell activity within hours. Experiments show that NK cell activity grows significantly after a few minutes of exercise of medium-plus intensity. Moreover, they also show that about three hours of strong laughter stimulation will have the same effect. These activities, however, only apply to healthy people and can not benefit

many of those facing strict restrictions on exercise or unable to enjoy a happy life because of chronic diseases or cancer. For these people, all it takes is to take Zhenhua 851 orally at a regular hour everyday. This very nutritious health care product enhances immunity for both healthy people and those suffering from chronic diseases.

Zhenhua 851 is selling very well in China and U.S. . Blood tests have shown no side-effect resulting from taking Zhenhua 851. Its efficacy and safety have also been proved. Because it benefits both people of good health and those suffering from chronic diseases, it won't be long before Zhenhua 851 achieves very wide popularity.