日本財団補助金による
1996 年度日中医学協力事業助成報告書

－在留中国人研究者研究助成－

1997 年 3 月 10 日

財団法人 日中医学協会

理事長 中島 章 殿

Ⅰ．研究者氏名 虞 顕（グ エイ）

研究機関 昭和大学医学部第一生理学教室 研究指導者 久光 正 職名 主任教授

所在地〒１４２品川区旗の台１－５－８ 電話 03-3784-8000 内線 8110

Ⅱ．過去の研究歴

１９９０年７月－１９９３年６月 中国輻射防護研究院放射生物効応研究室 助手

１９９３年６月－１９９４年３月 昭和大学医学部第一生理学教室 研修生

１９９４年４月 昭和大学医学部第一生理学教室 特別研究生 現在にいたる

Ⅲ．過去の研究実績

1、Ying Chen, Changju Li, Yuzhong Guo, Baohe Mu, Meilan Cui, Ying Yu, Study on Preventive and Therapeutic Effects of Placenta Factor on Radiation-Induced Damage of Hematopoietic Function (In Chinese), Radition Protection, Vol 14, No.3, 184-189 (1994)

2、山本竜隆, 虞 顕, 青木孝志, 日下史章, 瀬戸 明, 郭 試瑜, 佐藤孝雄, 笠原多嘉子, 久光正：磁気および石英単結晶・励振波動によるマウス・マクロファージ貪食能の増加, 「磁気と生体」研究会誌, Vol.21, No.1, 41-46, 1994

3、虞 顕, 山本竜隆, 瀬戸 明, 郭試瑜, 佐藤孝雄, 笠原多嘉子, 久光正： 磁気刺激により上昇したマウス脾臓NK細胞活性および腹腔マクロファージ貪食能－磁気の治療効果の免疫生理学的解明を求めて, 日本生体磁気学会会誌, Vol 8, No.1, 254-257, May 1995

4、花川 一郎, 山本 竜隆, 郭 試瑜, 虞 顕, 笠原 多嘉子, 久光 正, 桑澤 二郎, 松本 清：交流磁気と血中セロトニン－頭痛症例から－, 「磁気と生体」研究会誌 Vol 22：19-34, 1995

Ⅳ．本年度の研究業績

(1) 学会、研究会等においての口頭発表（学会名・内容）

１、虞 顕、佐藤孝雄、郭 試瑜、笠原多嘉子、久光 正、1996, 4月（福井） 第73回日本生理学会大会、 ラット足三里相当部位鍼刺激による脾臓NK細胞活性上昇作用。

２、虞 顕, 山本竜隆, 瀬戸 明, 郭試瑜, 佐藤孝雄, 笠原多嘉子, 久光正、1996, 6月（東京大学） 第11回日本生体磁気学会大会、 静磁場曝磁によるマウス生体防御能の増強：Biological Response Modifierとの協力作用の観察。

(2) 学会誌等に発表した論文 無 ・ (有) （雑誌名・論文名）

1. Ying Yu, Takako Kasahara, Takao Sato, Shi-Yu Guo, Yan-Qing Liu, Kazuhito Asano and Tadashi Hisamitsu: Enhancement of Splenic Interferon-γ, Interleukin-2 and NK cytotoxicity by S36 Acupoint Acupuncture in F344 Rats, Jpn. J. Physiol. 47, in press (1997)

2. T. Sato, Y. Yu, S.Y. Guo, T. Kasahara and T. Hisamitsu, Acupuncture Stimulation Enhanced the Splenic Natural Killer Cell Cytotoxicity in Rats, Jpn. J. Physiol. Vol.46, 131-136 (1996).

3. 虞 顕, 山本竜隆, 瀬戸 明, 郭試瑜, 佐藤孝雄, 笠原多嘉子, 久光正： 静磁場曝磁によるマウス生体防御能の増強：Biological Response Modifierとの協力作用の観察, 日本生体磁気学会会誌, Vol.9, No.1, 82-83, May 1996

4. 久光正, 笠原多嘉子, 虞 顕, 瀬戸明, 浅野和仁： 交流磁気(50Hz)によるヒト前骨髄性白血病細胞アポトーシス(細胞のプログラム死)の特異的誘導 －In vitro HL-60 細胞DNAの断片化促進－ 日本生体磁気学会会誌, Vol.9, No.1, 86-89, May 1996

Ⅴ．今後の研究計画及び希望

免疫系に神経系の活動が影響をおよぼすことが明らかになっているが、その詳細な機序については多くの疑問点が残されている。今後、特に中枢神経系や自律神経の活動経路や興奮伝達物質についての研究を継続して行いたい。

Ⅵ. 研　究　報　告（日本語、又は英語で書いて下さい。 2,000字程度で記載して下さい。）

Ⅶ. 指導教官の意見

虞 穎君は本学第一生理学教室で神経と免疫に関連した多くの研究プロジェクトに参加し、非常に熱心に研究を行なった。この分野の研究は世界的にもまだ数少なく、今後さらに研究テーマがひろがるものと思われる。虞 穎君が活躍する機会も多いと信じている。本年度の助成に感謝すると共に、今後も可能な援助をお願いしたい。今後の虞 穎君の発展が日中医学の進歩に大いに役立つものと信じている。

昭和大学 医学部 第一生理学教室
主任教授　久光　正

日本財団補助金による
1996 年度日中医学協力事業助成報告書

－在留中国人研究者研究助成－

年　　月　　日

財団法人　日中医学協会

理事長　　中島　　章　殿

Ⅰ．研究者氏名＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

研究機関＿＿＿＿＿＿＿＿＿＿＿＿研究指導者＿＿＿＿＿＿＿＿職名＿＿＿＿＿

所在地〒＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿電話＿＿＿＿＿＿内線＿＿＿＿＿

Ⅱ．過去の研究歴

Ⅲ．過去の研究実績

Ⅳ．本年度の研究業績

(1) 学会、研究会等においての口頭発表（学会名・内容）

(2) 学会誌等に発表した論文　　無　・　有　　（雑誌名・論文名）

Ⅴ．今後の研究計画及び希望

研　究　報　告

本年度、日中医学協力事業助成を受けることができ大変に感謝しております。本助成を有効に使用し、昭和大学医学部第一生理学教室において、次のような多くの研究成果をあげることができました。

A．論文

1、Ying Yu, Takako Kasahara, Takao Sato, Shi-Yu Guo, Yan-Qing Liu, Kazuhito Asano and Tadashi Hisamitsu: Enhancement of Splenic Interferon-γ, Interleukin-2 and NK Cytotoxicity by S36 Acupoint Acupuncture in F344 Rats, Jpn. J. Physiol. Vol 47(2), in press (1997)

別刷ができ上がり次第提出。

2、T. Sato, Y. Yu, S.Y. Guo, T. Kasahara and T. Hisamitsu: Acupuncture Stimulation Enhanced the Splenic Natural Killer Cell Cytotoxicity in Rats, Jpn. J. Physiol. Vol.46(2), 131-136 (1996).

3、虞　穎, 山本竜隆, 瀬戸　明, 郭試瑜, 佐藤孝雄, 笠原多嘉子, 久光正：　静磁場曝磁によるマウス生体防御能の増強：Biological Response Modifierとの協力作用の観察, 日本生体磁気学会会誌, Vol.9, No.1, 82-83, May 1996

4、久光正,　笠原多嘉子; 虞　穎, 瀬戸明, 浅野和仁：交流磁気(50Hz)によるヒト前骨髄性白血病細胞アポトーシス(細胞のプログラム死)の特異的誘導　－in vitro HL-60細胞DNAの断片化促進－　日本生体磁気学会会誌, Vol.9, No.1, 86-89, May 1996

5、成田和広,　虞　穎,　瀬戸　明, 浅野和仁, 朝比奈茂, 佐原正明, 笠原多嘉子, 久光正：超低周波交流磁気の非熱効果による白血病細胞のアポトーシス誘導,「磁気と生体」研究会誌 Vol 23：3-9, 1996

B、学会抄録

1、Yu, Y., Sato, T., Kasahara, T., Guo, S.Y., & Hisamitsu, T: Enhancement of Splenic Natural Killer(NK) Cytotoxicity by Tsusanli (S36) Acupuncture Point Stimulation in Rats. Jpn J Physiol 46 (Suppl) s222, 845, 1996

2、郭　試瑜、佐藤孝雄、虞　穎、笠原多嘉子、久光　正(1996) 水浸拘束ストレスのラット脾臓NK細胞活性抑制作用に対する脾臓交感神経の関与、ストレス科学（第１１回　日本ストレス学会学術総会　抄録集）Vol. 11, No.2, 86, 1996

3、T. Hisamitsu, Ying Yu, Takako Kasahara, Takao Sato, Shi-Yu Guo, Yan-Qing Liu and Kazuhito Asano: Roles of Interleukin-2 and Interferon-γ on the Enhance ment of Splenic Natural Killer (NK) Cytotoxicity by Acupuncture Stimulation in F344 Rats. the 4th World Conference on Acupuncture (W.F.A.S.), Program & Abstracts 332, 1996

研究テーマ: **Enhancement of Splenic Interferon-γ, Interleukin-2 and NK Cytotoxicity by S36 Acupoint Acupuncture in F344 Rats**

研究内容：

Introduction

Acupuncture is well known as one of the Chinese traditional medical treatments and it is used for health maintenance throughout the world. In recent years, it has been reported that acupuncture stimulation applied to a specific location of the human body, such as the Tsusanli acupuncture point (S36 acupoint), favorably modulates the immune function of HBsAg carrier and decreases the HBsAg positive rates. Combined acupuncture and moxibustion on supplementary acupoints inhibits the development of cancer cells and results in a prolonged survival of cancer patients.

There is an established concept that NK cells are important as the first line of host defense and as one of the final effector cells against certain tumors, viruses and infections. Several cytokines have been shown to affect NK cell proliferation or cytolytic activity. Of these, IL-2 and IFN-γ have been the most extensively studied. They have been proved to augment the cytolytic activity of NK cells to attach to tumor cells and viruses and kill these organisms. These reports may suggest that acupuncture enhances NK cell activities and results in favorable modification of the clinical conditions in the patients described above.

Our previous report and unpublished data revealed that successive acupuncture treatment applied to S36 acupoint (but not to the abdominal muscle) for three days enhanced splenic NK cytotoxicity, which peaked ($p<0.01$) on the first day after final treatment and gradually declined thereafter in Wistar rats. However, the mechanism of acupuncture on NK cell activities is unknown. The present study was designed to examine the possible mechanism of acupuncture on NK cell activities in an inbred strain of F344 rats.

Materials and Methods

Experimental design:

Electro-acupuncture stimulation was carried out between 3:00 P.M. and 5:00 P.M. for three days. Non-acupunctured control rats were restrained in acrylic rectangular boxes with no special treatment. On the first day after the final treatment, rats were anesthetized by intraperitoneal injection with 50 mg/kg pentobarbital sodium. The spleens were removed aseptically, divided into two portions and weighed. One portion was used to measure splenic NK cytotoxicity and the remainder was dissolved, weighed, immersed in 500 μl cold RPMI 1640 medium, and homogenized by glass tissue homogenizer in an ice cold water bath. The homogenates were then centrifuged at 8000×G for 1h at 4℃ and the supernatants were collected, sterilized by passing through a 0.22 μm filter and stored at -80°C until used for cytokine assay

Acupuncture on S36 acupoint and abdominal muscle in Inbred F344 rats

Electro-acupuncture stimulation was applied to S36 acupoint and abdominal muscle.

Briefly, two sterilized acupuncture needles were inserted perpendicularly about 5 mm into the anterior tibial muscle at the S36 point and the external oblique abdominal muscle. Electrical stimulation pulse with voltage ranging from 1 to 5V, duration of 1 msec and frequency of 1 Hz delivered from an acupuncture stimulator, was applied by two outlets through the two needles. The intensity of electrical stimulation was determined to be the minimum voltage to cause moderate muscle contraction. Electro-acupuncture stimulation of S36 acupoint was applied to the left S36 acupoint for 1h, followed by one 1h stimulation to the right in the same rat. Electro-acupuncture stimulation of abdominal muscle was applied bilaterally for 2h. The stimulation protocol was repeated each day for three consecutive days to rats under restriction in acrylic rectangular boxes.

NK cytotoxicity assay

Splenic NK cytotoxicity was measured in a standard 4-h ^{51}Cr release assay.

Cytokine assays

IL-2 assay: IL-2 in extracts was analyzed according to the ability to support the growth of IL-2 dependent T-cell line (CTLL-2 cell line).

IFN-γ assay: IFN-γ in extracts were measured by the Mouse IFN-γ ELISA Test Kit (Genzyme).

Results

The effect of acupuncture applied to the S36 acupoint on splenic NK cytotoxicity

The present experiments were designed to examine splenic NK cytotoxicity affected by acupuncture applied to the S36 acupoint in F344 rats. NK cells were prepared from rats on the first day after final treatment and cultured with target cells at E:T ratios of 100:1, 50:1, 25:1 and 12.5:1. The experiments were repeated five times with similar results. The data expressed in Fig.1 represents the mean ± S.E.M. of combined LU data from all rats. The splenic NK cytotoxicity of the S36 acupoint acupunctured group (17.7±0.5LU/10^7 effector cells) was significantly higher ($p<0.001$) than that of the abdominal muscle acupunctured group (13.4±0.9LU/10^7 effector cells) and the non-acupunctured control group (13.7±0.6LU/10^7 effector cells). In contrast, there was no difference between abdominal muscle acupunctured group and non-acupunctured control group.

The effect of acupuncture applied to the S36 acupoint on IL-2 and IFN-γ levels of splenic aqueous extracts

Since T cell cytokines IL-2 and IFN-γ are generally believed to be responsible for enhancement of NK cytotoxicity, These experiments were carried out to examine whether or not acupuncture on S36 acupoint enhances T cell cytokine productions and result in an increase in NK cytotoxicity. To do this, extracts were prepared from acupunctured and control F344 rats on the first day after the final treatment and endogenous cytokine activities were examined . As shown in Table 1, the IL-2 level in the extracts of S36 acupoint acupunctured rats (31.5±2.3 U/g) was significantly higher than that of abdominal muscle acupunctured rats (18.5±3.3 U/g, $p<0.001$) and non-acupunctured control rats (21.3±1.1 U/g, $p<0.001$). The level of IFN-γ in S36 acupoint acupunctured rats (617±39 pg/g) also showed a significantly higher level than that of abdominal

muscle acupunctured rats (460±37 pg/g, $p<0.01$) and non-acupunctured control rats (419±19 pg/g, $p<0.001$). However, there was no difference between abdominal muscle acupunctured and non-acupunctured control rats.

To determine whether the increase in splenic NK cytotoxicity after acupuncture treatment of S36 acupoint was related to the increase in the levels of IL-2 and IFN-γ, the splenic NK cytotoxicity was plotted against the IL-2 and IFN-γ production for individual rat. As shown in Fig. 2, the shift from splenic NK cytotoxicity correlated not only with the shift of the IL-2 level (simple linear regression, $r=0.62$, $p<0.01$), but also with the increase in IFN-γ production (simple linear regression, $r=0.49$ $p<0.01$). In addition, as shown in Fig.3, the increase in IFN-γ production was accompanied by an increase in the IL-2 level (simple linear regression, $r=0.53$, $p<0.01$).

Discussion

Traditional Chinese acupuncture, especially on S36 acupoint, is used as one type of supplementary therapy for the treatment of acute and chronic diseases such as viral infectious disease and cancer in China. Several mechanisms have been proposed to explain the therapeutic effect of acupuncture. These include 1) regulation of the endocrine control system in the body (hormone regulation); 2) regulation of nervous control system (neurotransmitter regulation) and 3) increase in function of the immune system. However, the mechanism(s) by which acupuncture favorably modify the clinical conditions of these diseases is not well known. The present results clearly showed that electro-acupuncture stimulation on bilateral S36 acupoint once a day (1h) for three days significantly increased splenic NK cytotoxicity as compared to that of abdominal muscle stimulated and non-stimulated control rats (Fig.1). It is also showed that acupuncture of the S36 acupoint enhanced the levels of IL-2 and IFN-γ in the spleen (Table1). There are significantly positive correlations between the levels of cytokines and their splenic NK cytotoxicity (Fig.2,3).

IL-2 is primarily released by activated helper T cells and acts as the central role in the regulation of NK cell activities. IFN-γ is released not only by helper T cells but also by IL-2 activated NK cells and serves as one of the important factors in the up-regulation of NK activities. Together with these reports and the present results, it is a possible that enhancement of NK cytotoxicity by acupuncture on S36 acupoint is owing to, in part, the increase in both IL-2 and IFN-γ production.

The conclusions from this study can be restated as follows: 1) acupuncture on S36 acupoint increases splenic NK cytotoxicity. 2) At least, as one of the mechanism(s), IL-2 and IFN-γ play important roles in the regulation of immune functions especially splenic NK cytotoxicity in S36 acupoint acupunctured animals.

研究成果の発表

得られた研究成果は第74回日本生理学会大会(3月25-27日、浜松)にて発表予定である。また、5月発行のJapanese Journal of Physiology (Vol.47, No.2)に掲載予定である。

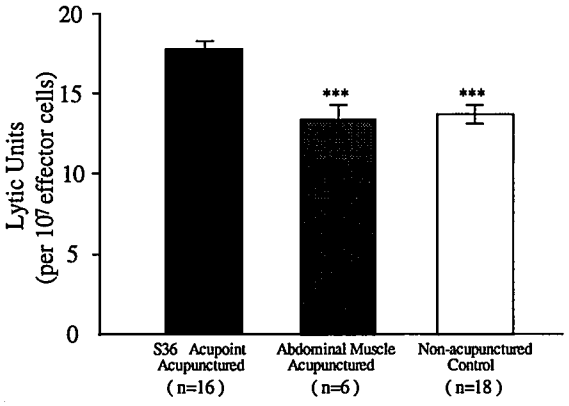

Figure. 1. Effect of acupuncture on splenic NK cytotoxicity in F344 rats Results are presented as the mean ± S.E.M. of LU, where 1LU presents the number of effector cells per 10^7 mediating 50% target cells lysis. S36 acupoint acupunctured group displayed a significantly higher NK cytotoxicity compared to the abdominal muscle acupunctured group and non-acupunctured control group. One-way ANOVA followed by Fisher's PLSD test revealed that $F(2, 37)=16.02$, $p<0.0001$. ***, $p< 0.001$ for S36 acupoint acupunctured group vs. abdominal muscle acupunctured group and non-acupunctured control group.

Table 1. Effect of Acupuncture on the IL-2 and IFN-γ Levels of Splenic Aqueous Extracts in F344 Rats

Group	IL-2 (U/g)	IFN-γ(pg/g)
S36 acupoint Acupunctured	31.5 ± 2.3 (n=13)	618 ± 39 (n=13)
Abdominal Muscle Acupunctured	18.5 ± 3.3*** (n=6)	460± 37** (n=6)
Non-acupunctured Control	21.3 ± 1.1*** (n=14)	419 ± 19*** (n=14)

Extracts of spleen were taken from acupunctured and control rats. The levels of IL-2 and IFN-γ in these extracts were detected. Data presented here are mean ± S.E. M. of three of four different experiments which gave reproductive results. One-way ANOVA followed by Fisher's PLSD test revealed significant effect of acupuncture of S36 acupoint on IL-2 level ($F_{(2, 30)}=10.6$, $p<0.001$) as well as IFN-γlevel ($F_{(2,30)}=12.2$, $p<0.001$).

** $p<0.01$ for S36 acupoint acupunctured group vs. abdominal muscle acupunctured group. *** $p<0.001$ for S36 acupoint acupunctured group vs. abdominal muscle acupunctured group and non-acupunctured control group.

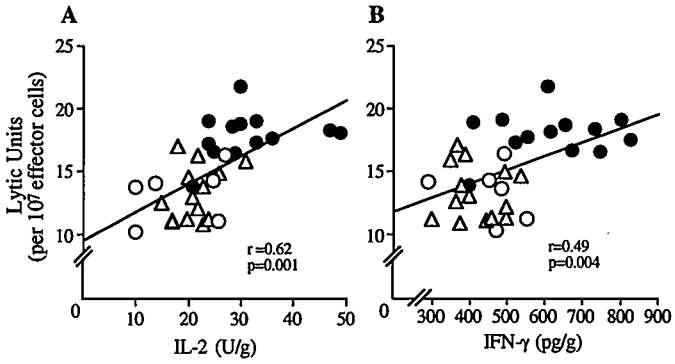

Figure. 2. Correlation of the splenic NK cytotoxicity with two cytokine levels in individual S36 acupoint acupunctured (●; n=13), abdominal muscle acupunctured (○; n=6) and non-acupunctured control (△; n=14) rats A: the splenic NK cytotoxicity plotted against the IL-2 level (r=0.62, p=0.001 by simple linear regression analysis). B: the splenic NK cytotoxicity plotted against the IFN-γ production (r=0.49, p=0.004 by linear regression analysis).

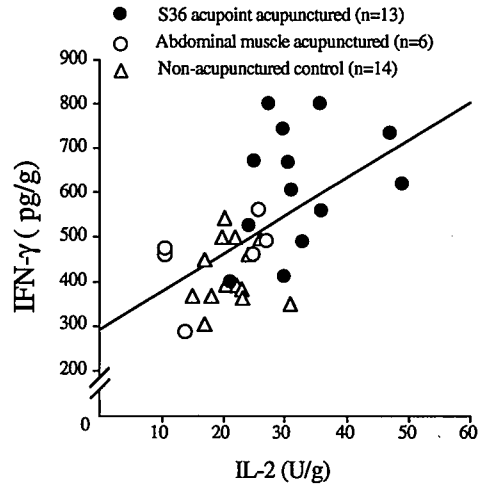

Figure. 3. Correlation of the production of IFN-γ and IL-2 level in the extracts of individual acupunctured and control rats. Results presented are the production of IFN-γ plotted against IL-2 level (r=0.53, p=0.002 by simply linear regression analysis).