行政院國家科學委員會專題研究計畫 成果報告

人類微小病毒 B19 結構蛋白獨立區域 VP1u 在誘發自體免疫反應之功能與分子機制探討(第 3 年)
研究成果報告(完整版)

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計 畫 主 持 人：徐再靜
共 同 主 持 人：曾博修
計畫參與人員：碩士班研究生-兼任助理人員：張舜智
博士班研究生-兼任助理人員：蔡鈞州

報 告 附 件 ：出席國際會議研究心得報告及發表論文

公 開 資 訊 ：本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

中 華 民 國 101 年 10 月 28 日

中 文 摘 要 ： 在我們對 B19 結構蛋白獨立區域(VP1u)的研究結果發現 B19 結構蛋白獨立區域(VP1u)在 B19 感染誘導自體免疫和抗磷脂質抗體的產生扮演著相當重要的角色。研究結果中我們發現(1)具有 anti-B19-VP1u 抗體的抗磷脂質症候群(APS)患者相較於 anti-B19-VP1u 抗體的抗磷脂質症候群(APS)患者，具有四倍高的比例會表現反覆性血管栓塞症狀。(2)此外，我們也發現 anti-B19-VP1u 抗體與 anti-B19 NS1 抗體與成人型史笛兒氏症(AOSD)及紅斑性狼瘡(SLE)發生血球減少及關節炎具有顯著相關。(3)進一步分析抗磷脂質抗體辨識 VP1u 主要抗原決定位，結果發現 1-195 a.a 為磷脂質抗體主要辨識區域並且此區域也具有 sPLA2 活性可促進老鼠巨噬細胞 Raw264.7 活化及 MMP9 的表現。因此我們將繼續分析 B19-VP1u 主要抗原決定位之功能。(4)此外，我們發現注射 B19-VP1u 抗體至 NZB/W F1 狼瘡小鼠會促進血清 IL-1 刍, IL-6 和 TNF-脉細胞激素表現及促進心臟左心室心肌細胞淋巴球浸潤。此外 B19-VP1u 抗體會促進狼瘡小鼠左心室心臟基質金屬蛋白酶 MMP9 活性及 MMP9 蛋白的表現並且透過磷酸化 P38 MAPK 和 NFκB 蛋白訊息傳遞路徑。另外分析狼瘡小鼠左心室心肌梗塞相關蛋白 h-fatty acid binding protein(h-FABP)和 creatine kinase-M/B (CKMB)及心室肥大相關蛋白 atrial natriuretic peptide (ANP)及 brain natriuretic peptide (BNP)，結果發現 B19-VP1u 抗體並不會促進心臟表現心肌梗塞或心室肥大的情形。因此 B19-VP1u IgG 抗體會透過磷酸化 P38 MAPK 和 NFκB 蛋白訊息傳遞路徑造成 NZB/W F1 狼瘡小鼠心臟發炎損傷的情形產生。

中文關鍵詞： 人類微小病毒 B19、結構蛋白獨立區域 VP1u、抗磷脂質抗體、抗磷脂質症候群(APS) 、心肌損傷、紅斑性狼瘡

英 文 摘 要 ： Our previous studies have found that the crucial role of B19-VP1u and its sPLA2 activity in viral infectivity and development of autoimmune diseases (ex: APS and SLE, etc.). However, the precise role and mechanism of B19-VP1u are still unclear. In our studies, we elucidate the function and molecular mechanism of human parvovirus B19-VP1u in induction of autoimmunity. We found that (1) APS patients with anti-B19-VP1u antibody had a 4-fold increased risk for recurrent vascular thrombosis compared with those without anti-B19-VP1u antibody. (2)Higher seroreactivity for anti-B19-VP1u and anti-B19-NS1 IgG were associated with cytopenia in both AOSD and SLE

patients with unique correlation to arthritis in the AOSD. (3) Moreover, we analyzed the antigenic regions on VP1u that confer autoantibody binding in APS patients, several constructs of VP1u truncations were generated. We show a close association of B19 infection with aPL production and suggest B19-VP1u region of 1-195 a.a may be pathogenetic importance in patients with APS and its sPLA2 activity can also active Raw 264.7 mouse macrophages and increase the expression of MMP9 activity. Further studies are necessary to determine. (4)Significant expression of IL-1 刍, IL-6 and TNF-脉 were detected in NZB/W F1 mice receiving rabbit anti-B19-VP1u IgG. Markedly cardiomyocyte disarray and lymphocyte infiltration were observed in left ventricle of hearts from NZB/W F1 mice receiving rabbit anti-B19-VP1u IgG. Additionally, significant increases of MMP9 activity and protein expression were detected in left ventricle of hearts from NZB/W F1 mice receiving B19-VP1u IgG. Accordingly, significant increase of phosphorylated p-38 and NF-kB proteins were observed in left ventricle of hearts from NZB/W F1 mice receiving B19-VP1u IgG. However, no significant variation of cardiac atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), heart-type fatty acid-binding protein (h-FABP) and creatine kinase MB (CK-MB) were detected among all experimental groups. These findings firstly demonstrated the aggravated effects of anti-B19 VP1u IgG on cardiac injury by induction of inflammatory but not myocardial infarction -associated proteins through activation of phosphorylated p-38 and NF-kB signaling.

英文關鍵詞： Human Parvovirus B19 (B19), VP1 unique region protein (VP1u), antiphospholipid antibodies, antiphospholipid syndrome (APS), Cardiac injury, Systemic lupus erythematosus (SLE)

行政院國家科學委員會補助專題研究計畫 □期中進度報告 ■期末報告

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計畫主持人：徐再靜
共同主持人：曾博修
計畫參與人員：蔡鈞州 許懷升 張舜智

本計畫除繳交成果報告外，另含下列出國報告，共 _1 份：
□移地研究心得報告
■出席國際學術會議心得報告
□國際合作研究計畫國外研究報告

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中 華 民 國 101 年 10 月 28 日

目　　錄

中文摘要

在我們對B19結構蛋白獨立區域(VP1u)的研究結果發現B19結構蛋白獨立區域(VP1u)在B19感染誘導自體免疫和抗磷脂質抗體的產生扮演著相當重要的角色。研究結果中我們發現(1)具有anti-B19-VP1u抗體的抗磷脂質症候群(APS)患者相較於anti-B19-VP1u抗體的抗磷脂質症候群(APS)患者,具有四倍高的比例會表現反覆性血管栓塞症狀。(2)此外,我們也發現anti-B19-VP1u抗體與anti-B19 NS1抗體與成人型史笛兒氏症(AOSD)及紅斑性狼瘡(SLE)發生血球減少及關節炎具有顯著相關。(3)進一步分析抗磷脂質抗體辨識VP1u主要抗原決定位，結果發現1-195 a.a為磷脂質抗體主要辨識區域並且此區域也具有 sPLA2 活性可促進老鼠巨噬細胞 Raw264.7 活化及 MMP9 的表現。因此我們將繼續分析 B19-VP1u 主要抗原決定位之功能。(4)此外，我們發現注射 B19-VP1u 抗體至 NZB/W F1 狼瘡小鼠會促進血清 IL-1β, IL-6 和 TNF-α細胞激素表現及促進心臟左心室心肌細胞淋巴球浸潤。此外 B19-VP1u 抗體會促進狼瘡小鼠左心室心臟基質金屬蛋白酶 MMP9 活性及 MMP9 蛋白的表現並且透過磷酸化 P38 MAPK 和 NFκB 蛋白訊息傳遞路徑。另外分析狼瘡小鼠左心室心肌梗塞相關蛋白 h-fatty acid binding protein(h-FABP)和 creatine kinase-M/B (CKMB)及心室肥大相關蛋白 atrial natriuretic peptide (ANP)及 brain natriuretic peptide (BNP)，結果發現 B19-VP1u 抗體並不會促進心臟表現心肌梗塞或心室肥大的情形。因此 B19-VP1u IgG 抗體會透過磷酸化 P38 MAPK 和 NFκB 蛋白訊息傳遞路徑造成 NZB/W F1 狼瘡小鼠心臟發炎損傷的情形產生。

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Abstract

Our previous studies have found that the crucial role of B19-VP1u and its sPLA2 activity in viral infectivity and development of autoimmune diseases (ex: APS and SLE, etc.). However, the precise role and mechanism of B19-VP1u are still unclear. In our studies, we elucidate the function and molecular mechanism of human parvovirus B19-VP1u in induction of autoimmunity. We found that (1) APS patients with anti-B19-VP1u antibody had a 4-fold increased risk for recurrent vascular thrombosis compared with those without anti-B19-VP1u antibody. (2)Higher seroreactivity for anti-B19-VP1u and anti-B19-NS1 IgG were associated with cytopenia in both AOSD and SLE patients with unique correlation to arthritis in the AOSD. (3) Moreover, we analyzed the antigenic regions on VP1u that confer autoantibody binding in APS patients, several constructs of VP1u truncations were generated. We show a close association of B19 infection with aPL production and suggest B19-VP1u region of 1-195 a.a may be pathogenetic importance in patients with APS and its sPLA2 activity can also active Raw 264.7 mouse macrophages and increase the expression of MMP9 activity. Further studies are necessary to determine. (4)Significant expression of IL-1β, IL-6 and TNF-α were detected in NZB/W F1 mice receiving rabbit anti-B19-VP1u IgG. Markedly cardiomyocyte disarray and lymphocyte infiltration were observed in left ventricle of hearts from NZB/W F1 mice receiving rabbit anti-B19-VP1u IgG. Additionally, significant increases of MMP9 activity and protein

expression were detected in left ventricle of hearts from NZB/W F1 mice receiving B19-VP1u IgG. Accordingly, significant increase of phosphorylated p-38 and NF-kB proteins were observed in left ventricle of hearts from NZB/W F1 mice receiving B19-VP1u IgG. However, no significant variation of cardiac atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), heart-type fatty acid-binding protein (h-FABP) and creatine kinase MB (CK-MB) were detected among all experimental groups. These findings firstly demonstrated the aggravated effects of anti-B19 VP1u IgG on cardiac injury by induction of inflammatory but not myocardial infarction -associated proteins through activation of phosphorylated p-38 and NF-kB signaling.

Key words: Human Parvovirus B19 (B19), VP1 unique region protein (VP1u), antiphospholipid antibodies, antiphospholipid syndrome (APS), Cardiac injury, Systemic lupus erythematosus (SLE)

Introduction

Human parvovirus B19 (B19) is known as an important human pathogen and has been associated with a wide spectrum of clinical manifestations including erythema infectiosum, arthropathy, thrombocytopenia, neurologic disorders, hepatitis, cardiovasculitis and autoimmune disorders [1-4]. B19-VP1-unique region (VP1u) has been reported to have the secreted phospholipases A2 (sPLA2) activity [5-8] and is critical for playing important roles in pathogenesis of B19 infection and development of autoimmunity [2, 9-13].

B19 infection has been recognized as a cause or trigger of autoimmune diseases and is associated with the production of various autoantibodies, including anti-beta2-glycoprotein-I (anti-β2GPI) and anti-cardiolipin antibody (aCL) [14-18]. Many studies have demonstrated that a remarkable similarity exists in the specificity of antiphospholipid antibodies (aPL) between patients with B19 infection and those with systemic lupus erythematosus (SLE) [16-19]. Previous studies have reported that anti-β2GPI and aCL were detected in patients with B19 infection and SLE [16-19]. These finding highlight the remarkable similarity in the aPL between SLE and B19 infection.

Previous investigations have reported that patients with systemic lupus erythematosus (SLE) have strikingly high rates of coronary heart disorders [20-21]. Another study indicated that the cardiac injury in SLE patients mainly focus on pericarditis and myocarditis [22]. Recently, a clinical research indicated that paricarditis and pleuritis are associated with B19 infection in SLE patient [23]. Similar results were also reported in an animal experiment that myocardial pathology and myocardial zymogram were detected in mice after treatment with B19-VP1u recombinant protein [24]. However, the related mechanisms about B19 infection on cardiac injury in SLE are still unclear. In our studies, we elucidate the function and molecular mechanism of human parvovirus B19-VP1u in induction of autoimmunity.

Materials and Methods

Detection of IgM/IgG/DNA against B19-VP in serum from APS patients and Determination of IgG

against B19-VP1u using Immunoblotting

Forty-five patients with APS (36 females and 9 males, mean age 37.0 ± 13.3 years) were enrolled from Taichung Veterans General Hospital. The enrolled subjects with APS were classified into two groups: primary APS and SLE with secondary APS, according to the clinical course by ACR criteria. The Clinical Research Ethics Committee at Taichung Veterans General Hospital approved the study protocol, and informed consent was obtained from each participant. The IgM and IgG against B19-VP were analyzed by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (IBL-America, Minnesota, USA). DNA was extracted using QIA Amp blood kit (QIAagen, Hidden, Germany) as directed by the manufacturer. Nested-PCR for the detection of B19 DNA in serum and Western blotting were performed as described in our previous report [8]. The substrate NBT/BCIP (nitroblue tetrazolium/ 5-bromo-4-chloro-3 indolyl phosphate) was used to detect antigen-antibody complexes.

Determination of IgG against B19-NS-1 using Immunoblotting

The nitrocellulose-transferred proteins were cut into strips and soaked in 5% nonfat dry milk in PBS, for 30 min at room temperature, to saturate irrelevant protein binding sites. Human or rabbit antiserum against B19-NS1 was diluted with 5% nonfat dry milk in PBS, reacted with the nitrocellulose strips and then incubated for 1.5 hr at room temperature. The strips were washed twice with PBS-Tween for 1hr and secondary antibody consisting of alkaline phosphatase conjugated goat anti-human or rabbit IgG antibodies was added. The substrate NBT/BCIP (nitroblue tetrazolium/5-bromo-4-chloro-3 indolyl phosphate) was used to react with alkaline phosphatase for detecting the goat anti-human or rabbit IgG antibodies.

Preparation of recombinant human B19 VP1 unique protein

E. coli (BL21-DE3) clones containing VP1u (wild type)/mutant/truncated cDNA in pET-32a expression vector (Novagene, Cambridge, MA) were grown overnight in one liter L-Broth containing 100ug/ml ampicillin at 37°C with shaking. When the OD 600 reached 0.7-0.9, protein expression was induced by addition of IPTG to a concentration of 1 mM and incubated for another 3 hr. The cells were harvested by centrifugation at 4000 g for 20 min and resuspended in 20 ml sonication buffer (50 mM NaPO4 pH 8/0.25 mM EDTA). Lysozyme was added to a final concentration of 1mg/ml and kept on ice for 30 min. The cells were sonicated (W385, Heat systems-ultrasonic, INC) for a total of 30 min at 5 min intervals, centrifuged 10,000 g for 30 min. The pellet was dissolved with 10 ml buffer B (8 M urea; 0.1M NaH2PO4; 0.02M Tris-HCl; pH 8.0) for 1 hr at room temperature, and centrifuge lysate at 10,000 g for 30 min at room temperature to pellet the cellular debris. The supernatant was loaded onto a Ni-NTA spin column (Qiagen, Chatsworth, CA, USA), and purified specific VP1u/mutant/truncated proteins.

sPLA2 catalytic activity

VP1u proteins were assayed for sPLA2 activity by use of a colorimetric assay (sPLA2

Activity Kit; Cayman Chemical), in accordance with the manufacturer'sinstructions, with dynamic colorimetric measurements (the optical density at 405 nm) determined every minute for 10 min. Results are expressed as micromoles per minute per milliliter.

Determination of levels of aCL, anti-β2GPI and anti-PhL

We used direct antigen-specific ELISA kits to detect aCL IgG (QUANTA LiteTM ACA IgG III), anti-*β*2GPI IgG (QUANTA LiteTM *β*2GPI IgG) (INOVA Diagnostics Inc., San Diego, CA, USA), and anti-PhL IgG (Louisville APL Diagnostics Inc., GA, USA) according to the manufacture's description. Results were expressed as IgG phospholipid (GPL) units or standard IgG units (SGU) using international reference material. For absorption analysis to assess the effect of binding inhibition, the sera were pre-incubated with 2mM of purified VP1u recombinant proteins for one hour at 37℃ before ELISA kits (aCL, anti-b2GPI and anti-PhL) were performed. Negative values range from 0-20 GPL (aCL), 0-20 SGU (anti-b2GPI antibodies) or 0-15 GPL (anti-PhL). Positive results are greater than 20 GPL (aCL), 20 SGU (anti-b2GPI), and 15 GPL (anti-PhL).

Cell culture

Mouse macrophages RAW 264.7 cells (RAW 264.7) were originally obtained from American type culture collection (ATCC) (Manassas, Va, USA) and were cultured in Dulbecco' s modified Eagle medium (DMEM) supplemented with 10% (v/v) fetal bovine serum at 37℃ and 5% CO2 incubator. RAW 264.7 cells were incubated with various B19-VP1u recombinant proteins for 24 h at 37℃, 5% CO2.

Gel zymography

RAW 264.7 cells were stimulated with peptides and the activities of MMP-2 and MMP-9 in medium were measured by gelatin-zymography assays. Culture supernatant were separated by an 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels containing 0.1% gelatin. Gels were washed for 30min in 2.5% Triton X-100 to remove the SDS and then soaked in the reaction buffer containing 40mM Tris-HCl, pH8.0, 10mM CaCl2 and 0.02% NaN3 for 30 min. Gelatinolytic activity was visualized by staining the gels with 0.5% Coomassie brillant blue R-250, de-stained with methanol-acetic acid water, and relative MMP levels were quantitated by a gel documentation and analysis system (AlphaImager, 2000, Alpha Innotech Corporation).

Animals, passive transfer and heart samples

Thirty female NZB/W F1 mice at age of 8 weeks were purchased from the Laboratory Animal Center of National Taiwan University, Taipei, Taiwan and housed under supervision of the Institutional Animal Care and Use Committee at Chung Shan Medical University, Taichung, Taiwan. Normal rabbit IgG and rabbit anti-B19-VP1u IgG were isolated using Protein A beads (Santa Cruz Biotechnology, CA, USA) described in our previous study [11-12, 28]. The NZB/W F1 mice at the age of 12 weeks were divided into three groups and intravenously

received PBS, normal rabbit IgG (20ug) and rabbit anti-B19-VP1u IgG (20ug) through the tail vein, respectively. The mice were then sacrificed at day 30 (16 weeks) after intravenous injection by CO_2 asphyxiation. The blood samples and heart tissues were collected and stored at −80 °C until use.

Hematoxylin-eosin staining

The left ventricle samples of animals were excised and soaked in formalin and covered with wax [29]. Slides were prepared by deparaffinization and dehydration. They were passed through a series of graded alcohols (100%, 95% and 75%), 15 minutes of each. The slides were then dyed with hematoxylin. After gently rinsing with water, each slide was then soaked with 85% alcohol, 100% alcohol I and II for 15 minutes each. At the end, they were soaked with Xylene I and Xylene II. Photomicrographs were obtained using Zeiss Axiophot microscopes.

Preparation of tissue extract and determination of protein

All procedures were performed at 4℃ in a cold room. The left ventricle samples of cardiac tissue obtained from NZB/W F1 mice were homogenized in 600ul PRO-PREP™ solution (iNtRON Biotech, Korea) by 30 strokes using a Dounce Homogenizer (Knotes Glass, Vineland, NJ). The homogenates were centrifuged at 13,000rpm for 10 minutes at 4℃ and the supernatant was then stored at -80℃ until use. Protein concentration of tissue extracts was determined according to the method described elsewhere [30] using bovine serum albumin as standards.

Immunoblotting

Protein samples were separated in 10% or 12.5% SDS-PAGE and electrophoretically transferred to nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ, USA) described elsewhere [32]. After blocking with 5% non-fat dry milk in (PBS), antibodies were diluted in PBS with 2.5% BSA and incubated for 1.5 hr with gentle agitation at room temperature. The membranes were washed twice with PBS-Tween for 1 h and secondary antibody conjugated with horseradish peroxidase (HRP) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was added. Pierce's Supersignal West Dura HRP Detection Kit (Pierce Biotechnology Inc., Rockford, IL) was used to detect antigen–antibody complexes. Quantified results were performed by densitometry (Appraise, Beckman‐Coulter, Brea, California, USA).

Statistical analyses

Data were analyzed using SPSS 10.0 for windows (Chicago, Illinois, USA). Chi-Square test was used to determine significant differences in categorical variables among groups. The paired t test was used to analyze data for statistical significance. A binary logistic regression analysis was used to evaluate the effect of anti-B19-VP1u IgG on the occurrence of recurrent thrombosis. P value <0.05 was

considered to be statistically significant.

Results

Part-1

Demographic data, clinical characteristics, and the presence of aPL in APS patients

Forty-five patients with APS were classified according to the clinical course of their disease as follows: primary APS (n=10) and SLE with secondary APS (n=35). As illustrated in Table 1, the most commonly associated thrombosis was cerebral vascular accident in both primary APS patients (50%) and SLE patients with secondary APS (25.7%). Significantly higher proportion of usage of immunosuppressive agents including corticosteroids was found in SLE patients with secondary APS than in primary APS patients.

Reactivity of IgG directed B19-VP1u in sera from APS patients

As illustrated in Table 2, all of serum samples from primary APS patients with diagnostic pattern DNA-/IgM-/IgG+ had anti-B19-VP1u activity. The serum sample from one patient with primary APS who had diagnostic pattern DNA+/IgM+/IgG+ showed reactivity to anti-B19-VP1u. Only one primary APS patient with diagnostic pattern DNA-/IgM-/IgG- had a serum sample that showed reactivity to anti-B19-VP1u. All of serum samples from SLE patients with APS who had diagnostic pattern DNA-/IgM-/IgG+ (n=14) showed reactivity to anti-B19-VP1u. One of 4 (25%), three of 4 (75%), and one of 4 (25%) serum samples showed positivity for aCL, anti-β2GPI, and anti-PhL respectively in 4 patients with primary APS who had diagnostic pattern DNA-/IgM-/IgG+.

Clinical characteristics of APS patients with anti-B19-VP1u antibodies

As shown in Table 3, APS patients with anti-B19-VP1u antibodies were more likely than those without anti-B19-VP1u antibodies to have recurrent thrombosis (50.0% vs. 20.0%, $p<0.05$), positivity for aCL (25.0% vs. 0.0%, $p<0.05$) and positivity for anti-PhL (25.0% vs. 0.0%, $p<0.05$). Compared with APS patients without anti-B19-VP1u antibody, those with anti-B19-VP1u antibody had significantly increased risk of recurrent vascular thrombosis, with an odds ratio of 4.000 (95% confidence interval 1.074-14.896). In addition, high sensitivity (100%) and negative predictive value (100%) of anti-B19-VP1u IgG were observed for predicting the reactivity to CL and PhL in APS patients. However, there was no significant connection between the presence of anti-B19-VP1u IgG and clinical features in APS patients. As illustrated in Figure 1, a significant decrease in the levels of aCL, anti-β2GPI, and aPhL antibodies after absorption with B19-VP1u was observed in 20 patients with APS. The inhibition of reactivity to CL, β2GPI and PhL by absorption with purified B19-VP1u was 31.4 to 91.0%, 0.8 to 59.8% and 20.2 to 72.1% respectively. Among APS patients with positivity for aPL, significantly higher degree of inhibition to CL/β2GPI/PhL by B19-VP1u absorption was observed in sera from those with anti-B19-VP1u antibodies than from those without anti-B19-VP1u antibodies (45.6% vs. 21.3%, $p<0.05$, Table 3). Moreover, significantly higher degree of inhibition to β2GPI by B19-VP1u absorption was observed in sera from those with anti-B19-VP1u antibodies when compared to those without anti-B19-VP1u antibodies (38.5% vs. 21.3%, $p<0.05$, Table 3).

Recognition of truncated type VP1u proteins by anti-His antibody, rabbit anti-VP1u antibody, anti-phospholipid antibody and anti-β2GPI antibody

Figure 2A illustrates the schematic diagram of three truncated VP1u with N-terminal deletions. The four PCR products of truncated VP1u (Figure 2B) and rVP1u proteins were purified using the affinity chromatography on immobilized nickel column and demonstrated with SDS-PAGE (Figure 2C). Rbbit anti-VP1u antibodies (Figure 2D) were used to identify the purified recombinant proteins. Human anti-PhL antibodies (Figure 2E), and anti-β2GPI antibodies (Figure 2F) were used to identify the purified recombinant proteins. These data indicated that truncated type VP1u proteins confer dominant binding reactivity in sera from patients with APS patients.

Table 1
Characteristics of 45 patients with antiphospholipid syndrome (APS).[a]

Characteristics	Primary APS ($n=10$)	SLE with APS ($n=35$)
Age at study entry (y)	35.0 ± 9.3	37.6 ± 14.3
Duration of disease (y)	3.2 ± 4.2	6.6 ± 5.3
Females	8 (80.0%)	27 (77.1%)
Ever smoker	1 (10.0%)	3 (8.6%)
Number of subjects with ≧2 thrombosis	4 (40.0%)	11 (31.4%)
Thrombosis by subtype		
Cerebral vascular accident	5 (50.0%)	9 (25.7%)
Livedo reticularis	4 (40.0%)	8 (22.9%)
Deep vein thrombosis	2 (20.0%)	4 (11.4%)
Peripheral arterial thrombosis	1 (10.0%)	4 (11.4%)
Miscarriage late (≧1 in 2nd or 3rd trimester)	1 (10.0%)	2 (5.7%)
Myocardial infarction	0 (0.0%)	3 (8.6%)
Other thrombosis	3 (30.0%)	17 (48.6%)
Used medications		
Corticosteroids	4 (40.0%)	33 (94.2%)*
Hydroxychlorquine	10 (100.0%)	34 (97.1%)
Other immunosuppressive agents	2 (20.0%)	30 (85.7%)*

SLE: systemic lupus erythematosus.
[a] Data were presented as mean ± SD or number (percentage).
* $p<0.05$, vs. patients with primary APS, determined by the paired t test.

Table 3
Characteristics of 45 patients with APS according to the presence of anti-B19-VPlu antibodies.[a]

Characteristics	Anti-B19-VPlu (−) ($n=25$)	Anti-B19-VP1u (+) ($n=20$)
Age at study entry (y)	37.5 ± 14.4	36.5 ± 12.1
Duration of disease (y)	59.9 ± 59.0	83.7 ± 65.8
Females	18 (72.0%)	147 (85.0%)
Number of subjects with		
≧2 thrombosis	5 (20.0%)	10 (50.0%)*
Positivity for aCL	0 (0.0%)	5 (25.0%)*
Positivity for anti-β_2GPI	9 (36.0%)	10 (50.0%)
Positivity for aPhL	0 (0.0%)	5 (25.0%)*
Absorption inhibition degree, %		
Inhibition to CL/β_2GPI/PhL[b]	21.3 ± 11.3	45.6 ± 22.3**
Inhibition to β_2GPI[b]	21.3 ± 11.3	38.5 ± 17.0*

B19-VP1u: B19-VP1 unique region protein; aCL: anti-cardiolipin antibodies; anti-β2GPI: anti-beta2-glycoprotein-I antibodies; aPhL: antiphospholipid antibodies.
[a] Data were presented as mean ± SD or number (percentage).
[b] Among patients with aCL, anti-β2GPI, or anti-PhL antibodies.
* $p<0.05$, vs. patients with patients with anti-B19-VPlu (−), determined by the paired t test.
** $p<0.01$, vs. patients with patients with anti-B19-VPlu (−), determined by the paired t test.

Table 2
The diagnostic patterns of B19 infection, serology of anti-B19-VP1u, and antiphospholipid antibodies in 45 patients with antiphospholipid syndrome (APS).[a]

Diagnostic patterns of parvovirus B19 infection	Anti-B19-VP1u ($n=20$)	Positivity of aCL	Positivity of anti-β_2GPI	Positivity of anti-PhL
Primary APS ($n=10$)				
$DNA^-/IgM^-/IgG^-$ ($n=5$)	1	1 (25%)	1 (25%)	1 (25%)
$DNA^-/IgM^-/IgG^+$ ($n=4$)	4	1 (25%)	3 (75%)	1 (25%)
$DNA^+/IgM^+/IgG^+$ ($n=1$)	1	1 (100%)	1 (100%)	0 (0%)
SLE with APS ($n=35$)				
$DNA^-/IgM^-/IgG^-$ ($n=21$)	0	0 (0%)	8 (38%)	0 (0%)
$DNA^-/IgM^-/IgG^+$ ($n=14$)	14	2 (14.3%)	6 (43%)	3 (21.4%)
$DNA^+/IgM^+/IgG^+$ ($n=0$)	0	0	0	0

SLE: systemic lupus erythematosus; B19-VP1u: B19-VP1 unique region protein; aCL: anti-cardiolipin antibodies; anti-β2GPI: anti-beta2-glycoprotein-I antibodies; anti-PhL: antiphospholipid antibodies.
[a] Data were presented as number (percentage) among the diagnostic patterns of B19 infection.

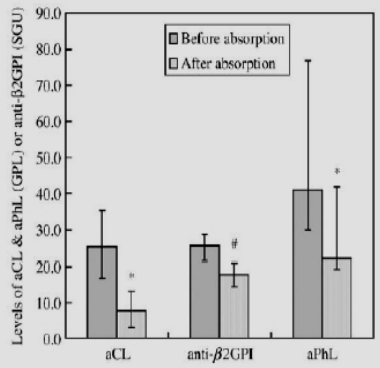


Fig. 1. Changes in the levels of anti-cardiolipin antibodies (aCL), anti-beta2-glycoprotein-I (anti-β2GPI), and antiphospholipid antibodies (aPhL) in 20 patients with antiphospholipid syndrome. Data are expressed as median levels (the 25th to the 75th percentile). *$p<0.05$, #$p<0.001$, vs. before absorption, determined by the Wilcoxon signed rank test.

Part-2

Results of B19 DNA, anti-B19 IgM and IgG detection in sera

Since parvovirus B19 cannot be grown routinely *in vitro*, B19 infection is generally diagnosed via direct detection of B19 DNA (virological) and by anti-B19 IgM and IgG (serological). Based on the advances in diagnosis of B19 infection [1, 33], none of AOSD patients or controls had detectable circulating B19 DNA or detectable anti-B19-VP1/2 IgM. In contrast, 43 (50.0%) of AOSD patients, 27 (46.6%) of SLE patients and 30 (46.9%) of controls had detectable anti-B19-VP1/2 IgG, indicating past B19 infection. As illustrated in Table 2, significantly higher positive rates for anti-B19-VP1u and anti-B19-NS1 antibodies were observed in AOSD patients (39.5% and 46.5% respectively) and SLE patients (34.5% and 36.2% respectively) than in healthy controls (14.1% and 15.6% respectively).

The association of anti-B19-VP1/2 IgG, anti-B19-VP1u IgG and anti-B19-NS1 IgG with clinical characteristics in AOSD patients

When compared to AOSD patients and SLE patients without evidence of past B19 infection, those with anti-B19-VP1/2 IgG were more likely to have leucopenia and thrombocytopenia (Table 3). AOSD patients and SLE patients with anti-B19-VP1u or anti-B19-NS1 antibodies were also more likely to have leucopenia and thrombocytopenia when compared to those without anti-B19-VP1u or anti-B19-NS1 antibodies. AOSD patients with anti-B19-VP1u or anti-B19-NS1 antibodies were more likely to have arthritis when compared to those without these antibodies (Table 3). However, no significant differences in age at study entry, duration of disease, proportion of female, or other clinical characteristics were observed between patients with serological positivity and those with serological negativity for B19 infection.

Comparison between AOSD or SLE patients and controls

Significantly higher frequencies of leucopenia, anemia or thrombocytopenia in AOSD patients with serologic evidence of past B19 infection were observed when compared with anti-B19-VP1/2-positive controls (18.6% vs. 3.3%, $p<0.05$; 46.5% vs. 10.0%, $p<0.001$; 32.6% vs. 0.0%, $p<0.001$; respectively). Similarly, significantly higher frequencies of leucopenia, anemia or thrombocytopenia in SLE patients with serologic evidence of past B19 infection were observed when compared with anti-B19-VP1/2-positive controls (51.9% vs. 3.3%, $p<0.001$; 59.3% vs. 10.0%, $p<0.001$; 44.4% vs. 0.0%, $p<0.001$; respectively).

Table 1
Clinical characteristics of patients with adult-onset Still's disease (AOSD), patients with systemic lupus erythematosus (SLE), and healthy controls (HC)#.

Characteristics	AOSD (n = 86)	SLE (n = 58)	HC (n = 64)
Age at study entry (y)	37.1 ± 13.4	35.0 ± 12.0	38.4 ± 9.9
Proportion of females	68 (79.1%)	52 (89.7%)	52 (81.3%)
Fever (≧38 °C)	86 (100%)	46 (79.3%)	NA
Rash	66 (76.7%)	40 (69.0%)	NA
Sore throat	59 (68.6%)	7 (12.1%)**	NA
Arthritis	55 (64.0%)	28 (48.3%)	NA
Lymphadenopathy	28 (32.6%)	9 (15.5%)*	NA
Hepatosplenomegaly	8 (9.3%)	6 (10.3%)	NA
Liver dysfunction (ALT > 44 U/l)	27 (31.4%)	2 (3.4%)**	0 (0.0%)**
Hyperferritinemia (levels≧300 μg/l)	78 (90.7%)	NA	NA
Leucocytosis (WBC > 10.0×10^9/l)	49 (57.0%)	2 (3.4%)**	0 (0.0%)**
Anemia (hemoglobin < 11.3 g/dl)	33 (38.4%)	29 (50.0%)	4 (6.3%)**
Thrombocytosis (platelet > 400×10^9/l)	22 (25.6%)	1 (1.7%)**	0 (0.0%)**
Leucopenia (WBC < 4.0×10^9/l)	8 (9.3%)	19 (32.8%)**	1 (1.6%)
Thrombocytopenia (platelet < 100×10^9/l)	14 (16.3%)	15 (25.9%)	0 (0.0%)**
Positivity for ANA (titer > 1:160)	2 (2.3%)	54 (93.1%)**	NA
Positivity for aCL	5 (5.8%)	22 (38.0%)**	NA
Positivity for anti-β_2GPI	4 (4.7%)	18 (31.0%)**	NA

#Data are presented as mean ± SD or number (percentage); NA: not applicable.
ALT: alanine transaminase; ANA: antinuclear antibodies; aCL: anti-cardiolipin antibodies; anti-β2GPI: anti-beta2-glycoprotein-I antibodies. *p<0.05, **p<0.001, vs. AOSD patients.

Table 2
Results of serological tests for parvovirus B19 in patients with adult-onset Still's disease (AOSD), patients with systemic lupus erythematosus (SLE), and healthy controls (HC).

		AOSD (n = 86)		SLE (n = 58)		HC (n = 64)		OR (95% CI)		
		N	(%)	n	(%)	n	(%)	AOSD vs. SLE	AOSD vs. HC	SLE vs. HC
IgG anti-B19-VP1/2	+	43	50.0	27	46.6	30	46.9	1.15	1.13	0.99
	-	43	50.0	31	53.4	34	53.1	(0.59–2.24)	(0.59–2.17)	(0.48–2.01)
IgM anti-B19-VP1/2	+	0	0.0	0	0.0	0	0.0	–	–	–
	-	86	100	58	100	64	100			
IgG anti-B19-VP1u	+	34	39.5	20	34.5	9	14.1	1.24	4.00	3.22
	-	52	60.5	38	65.5	55	85.9	(0.62–2.48)	(1.75–9.13)**	(1.32–7.82)*
IgG anti-B19-NS1	+	40	46.5	21	36.2	10	15.6	1.53	4.70	3.07
	-	46	53.5	37	63.8	54	84.4	(0.77–3.03)	(2.12–10.42)***	(1.30–7.25)*

IgG anti-B19-VP1/2: IgG antibodies against B19-VP1/2; IgM anti-B19-VP1/2: IgM antibodies against B19-VP1/2; VP1u: VP1-unique region protein; NS1: nonstructural protein-1; OR: Odds ratio; 95% CI: 95% confidence interval. *p<0.05, **p<0.005, ***p<0.001, was determined by Chi-Square test with Yate's correction for continuity.

Table 3
The association of clinical characteristics with serological positivity for parvovirus B19 in patients with adult-onset Still's disease (AOSD) and patients with systemic lupus erythematosus (SLE).

	Anti-B19-VP1/2 IgG		Anti-B19-VP1u IgG		Anti-B19-NS1 IgG	
AOSD (n = 86)	(+, n = 43)	(−, n = 43)	(+, n = 34)	(−, n = 52)	(+, n = 40)	(−, n = 46)
SLE (n = 58)	(+, n = 27)	(−, n = 31)	(+, n = 20)	(−, n = 38)	(+, n = 21)	(−, n = 37)
Leucopenia (WBC < 4.0×10^9/l)						
AOSD (n = 8)	8 (18.6%)**	0 (0.0%)	8 (23.5%)***	0 (0.0%)	7 (17.5%)*	1 (2.2%)
SLE (n = 19)	14 (51.9%)**	5 (16.1%)	11 (55.0%)*	8 (21.1%)	12 (57.1%)**	7 (18.9%)
Anemia (hemoglobin < 11.3 g/dl)						
AOSD (n = 33)	20 (46.5%)	13 (30.2%)	17 (50.0%)	16 (30.8%)	19 (47.5%)	14 (30.4%)
SLE (n = 29)	16 (59.3%)	13 (41.9%)	10 (50.0%)	19 (50.0%)	12 (57.1%)	17 (45.9%)
Thrombocytopenia (platelet count < 100×10^9/l)						
AOSD (n = 14)	14 (32.6%)***	0 (0.0%)	13(38.2%)***	1 (1.9%)	13 (32.5%)***	1 (2.2%)
SLE (n = 15)	12 (44.4%)**	3 (9.7%)	10(50.0%)**	5 (13.2%)	9 (42.9%)*	6 (16.2%)
Skin rash						
AOSD (n = 66)	32 (74.4%)	34 (79.15%)	24 (70.6%)	42 (80.8%)	29 (72.5%)	37 (80.4%)
SLE (n = 40)	19 (70.4%)	21 (67.7%)	13 (65.0%)	27 (71.1%)	15 (71.4%)	25 (67.6%)
Arthritis						
AOSD (n = 55)	32 (74.4%)	23 (79.4%)	27 (79.4%)*	28 (53.8%)	31 (77.5%)*	24 (52.2%)
SLE (n = 28)	17 (63.0%)	11 (35.5%)	13 (65.0%)	15 (39.5%)	14 (66.7%)	14 (37.8%)
Sore throat						
AOSD (n = 59)	25 (58.1%)	34 (79.1%)	19 (55.9%)	40 (76.9%)	23 (57.5%)	36 (78.3%)
SLE (n = 7)	5 (18.5%)	2 (6.5%)	4 (20.0%)	3 (7.9%)	4 (19.0%)	3 (8.1%)
Lymphadenopathy						
AOSD (n = 28)	19 (44.2%)*	9 (20.9%)	15 (44.1%)	13 (25.0%)	16 (40.0%)	12 (26.1%)
SLE (n = 9)	5 (18.5%)	4 (12.9%)	5 (25.0%)	4 (10.5%)	5 (23.8%)	4 (10.8%)
Hepatosplenomegaly						
AOSD (n = 8)	4 (9.3%)	4 (9.3%)	2 (5.9%)	6 (11.5%)	3 (7.5%)	5 (10.9%)
SLE (n = 6)	5 (18.5%)	1 (3.2%)	4 (20.0%)	2 (5.3%)	4 (19.0%)	2 (5.4%)
Liver dysfunction						
AOSD (n = 27)	15 (34.9%)	12 (27.9%)	11 (32.4%)	16 (30.8%)	14 (35.0%)	13 (28.3%)
ALT levels (U/l)						
AOSD, mean ± SD	51.2 ± 47.5	46.9 ± 53.3	51.4 ± 52.1	47.5 ± 49.5	50.1 ± 49.3	48.1 ± 51.6
Hyperferritinemia (serum levels≧300 μg/l)						
AOSD (n = 78)	41 (95.3%)	37 (86.0%)	33 (97.1%)	45 (86.5%)	39 (97.5%)	39 (84.8%)
Ferritin levels (μg/l)						
AOSD, mean ± SD	2919 ± 5201	2064 ± 4689	3393 ± 5757	1902 ± 4284	2875 ± 5230	2158 ± 4708

IgG anti-B19-VP1/2: IgG antibodies against B19-VP1/2; IgM anti-B19-VP1/2: IgM antibodies against B19-VP1/2; VP1u: VP1-unique region protein; NS1: nonstructural protein-1; WBC: white cell count; ALT: alanine aminotransferase. *p<0.05, **p<0.01, ***p<0.001, vs. serologic negative group.

Part-3

Recombinant B19-VP1u proteins reveal sPLA2 activity and MMP9 activity [25]

The full length and N-terminal deletion of recombinant B19-VP1u protein, and B19-VP1uD175A protein were purified (Fig 1A and Fig 2A) and analyzed for sPLA2 activity (Fig 1B and 2B). As shown in Figure 1B, significant decreases of sPLA2 activity were observed in the 21-227 a.a and 91-227 a.a as compared to the other group. To examine whether B19-VP1u and its sPLA2 enzymatic activity could influence the activities of the ECM degrading proteases such as MMP9 or MMP2, the effect of B19-VP1u on the secretion of MMPs was investigated using a zymography assay. As shown in Figure 1C and 2C, significant decreases of MMP9 activity was observed in the experimental group treated with B19-VP1u 21-227 a.a or 196-227 a.a as compared to the other group. Quantified results are shown in the right panel of Fig 1C and 2C. No sPLA2 activity was detected in B19-VP1uD175A as previously shown. Furthermore, we show a close association of B19 infection with aPL production and suggest B19-VP1u region of 1-195 a.a may be pathogenetic importance in patients with APS (Fig 2D).

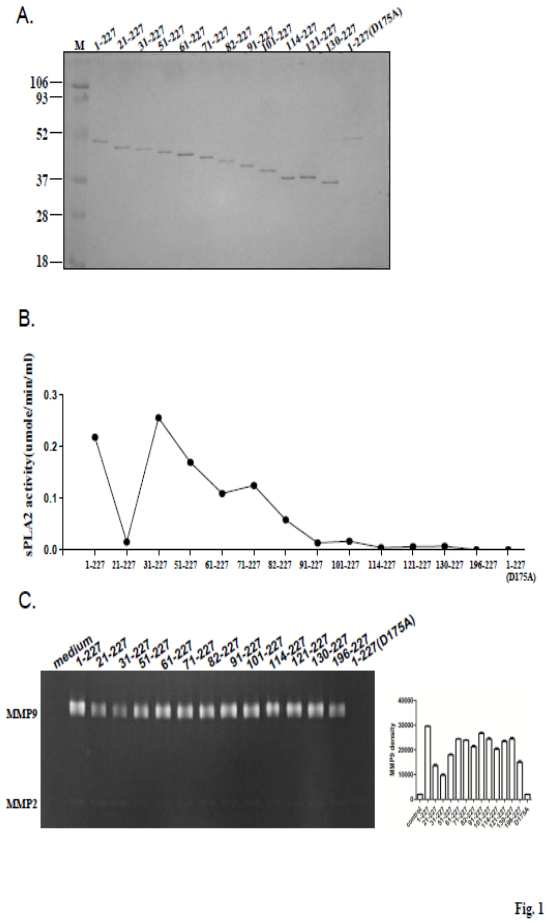


Fig. 1

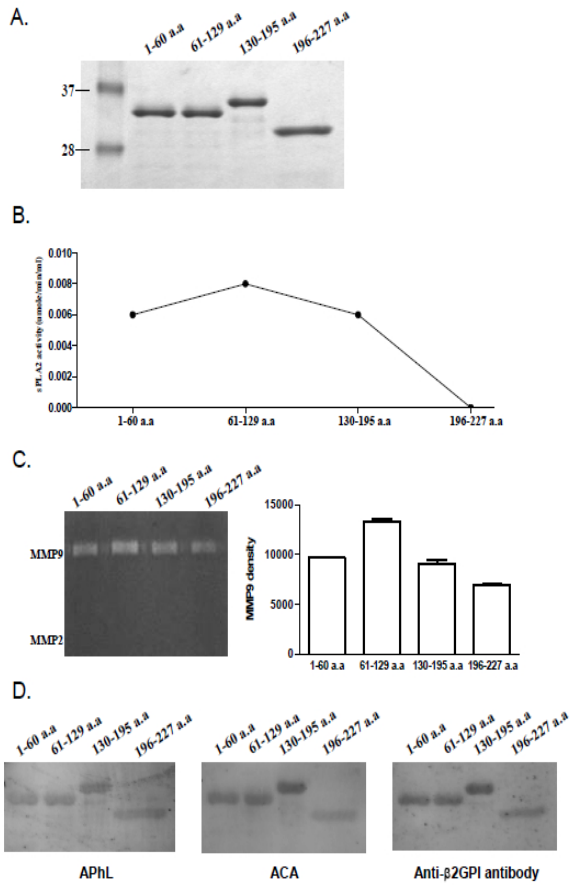


Fig. 2

Part-4

Increased expression of IL-1β, IL-6 and TNF-α in NZB/W F1 mice receiving rabbit anti-B19-VP1u IgG [25]

Passively transfer of IgG against B19-VP1u has been demonstrated playing crucial role in

development of autoimmunity. To investigate the influence of rabbit anti-B19-VP1u IgG on hearts of NZB/W F1 mice, various cardiac inflammatory associated proteins such as IL-1β, IL-6 and TNF-α were examined. Figure 1A revealed the loading control and levels of serum IL-1β, IL-6 and TNF-α protein in NZB/W F1 mice receiving PBS, normal rabbit IgG and rabbit anti-B19-VP1u IgG. The protein levels of IL-1β, IL-6 and TNF-α in serum from NZB/W F1 mice receiving rabbit anti-B19-VP1u IgG were significant higher than in those receiving PBS or normal rabbit IgG (Fig 1B, 1C and 1D).

Cardiac architecture changes in NZB/W F1 mice receiving rabbit anti-B19-VP1u IgG [25]

To observe the effects of anti-B19-VP1u IgG on cardiac architectures in NZB/W F1 mice, we performed a histopathological analysis of left ventricular tissue of hearts stained with hematoxylin and eosin (Fig. 2). Abnormal myocardial architecture and increased interstitial space were observed in left ventricle tissue of hearts from NZB/W F1 mice receiving anti-B19-VP1u IgG (Fig. 2C) compared to those mice receiving PBS (Fig. 2A) or normal rabbit IgG (Fig. 2B). Additionally, lymphocyte infiltration was observed in left ventricle tissue of hearts from NZB/W F1 mice receiving rabbit anti-B19-VP1u IgG (Fig. 2D).

Increased MMP-9 activity and protein expression in NZB/W F1 mice receiving rabbit anti-B19-VP1u IgG [25]

Elevated MMP-9 activity has linked to a variety of cardiac disorder. To further examine the effect of anti-B19-VP1u IgG on pathogenesis of heart in NZB/W F1 mice, MMPs activity and protein expression were detected. Significant increase of MMP9 activity was observed in left ventricular tissue of hearts from NZB/W F1 mice receiving rabbit anti-B19-VP1u IgG compared to those mice receiving PBS or normal rabbit IgG, respectively (Fig. 3A). The ratio of MMP-9/MMP-2 was shown in lower panel of Fig 3A. The protein level of MMP-9 and MMP-2 were also examined by Western blotting. Significant increase of MMP-9/α-tubulin ratio was detected in left ventricle tissue of hearts from NZB/W F1 mice receiving anti-B19-VP1u IgG compared to those receiving PBS or normal rabbit IgG, respectively (Fig 3B). However, no significant variation was observed in MMP-2/α-tubulin ratio among all experimental groups (Fig. 3B). Quantified results of MMP-9/α-tubulin and MMP-2/α-tubulin ratio were shown in the lower panel of Fig 3B.

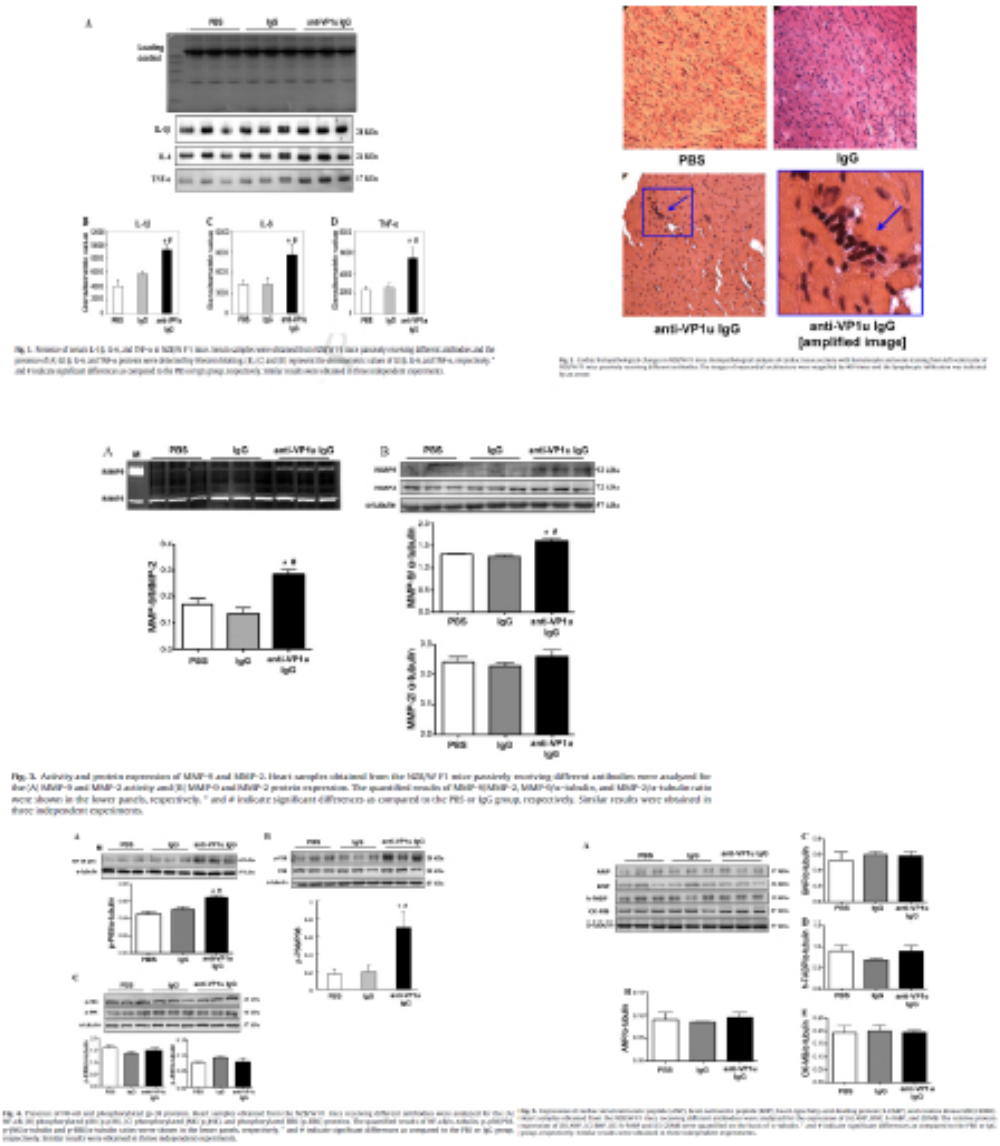
Increased expression of NF-kB and phosphorylated p-38 proteins in NZB/W F1 receiving rabbit anti-B19-VP1u IgG [25]

To clarify the possible signaling involved in activation of MMP9 in hearts of NZB/W F1 mice receiving rabbit anti-B19-VP1u IgG, various signaling molecules including NF-kB (p65), Erk1/2-p, p38-p, and JNK-p were examined. Notably, significant increase of NF-kB (p65) protein was detected in left ventricle tissue of hearts from NZB/W F1 mice receiving anti-B19-VP1u IgG compared to those receiving PBS or normal rabbit IgG, respectively (Fig. 4A). Similar results were shown in the phosphorylation of p38 protein in left ventricle tissue

of hearts from NZB/W F1 mice receiving anti-B19-VP1u IgG compared to those receiving PBS or normal rabbit IgG, respectively (Fig. 4B). However, no significant variation in phosphorylation of ERK and JNK proteins were observed in all experimental groups (Fig. 4C). Quantified results were shown in left panels of figure 4A, 4B and 4C.

Expression of myocardial infarction (MI) markers in NZB/W F1 mice receiving rabbit anti-B19-VP1u IgG [25]

To investigate the effects of anti-B19-VP1u IgG on induction of myocardial infarction (MI) associated proteins, ANP, BNP, h-FABP and CK-MB were detected by Western blotting (Fig. 5A). Notably, no significant variation of ANP, BNP, h-FABP and CK-MB proteins was observed in left ventricle tissue of hears from NZB/W F1 mice receiving rabbit anti-B19 VP1u IgG compared to those mice receiving PBS or normal rabbit IgG, respectively. Quantified results of ANP/α-tubulin, BNP/α-tubulin, h-FABP/α-tubulin and CKMB/α-tubulin were show in Fig 5B, 5C and 5D.



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計畫成果自評部份

研究內容與計畫相符，為探討人類微小病毒 B19 結構蛋白獨立區域 VP1u 在誘發自體免疫反應之功能與分子機制。研究結果讓我們更進一步了解到人類微小病毒 B19 結構蛋白獨立區域及抗 B19-VP1u 抗體與抗磷脂質抗體及抗磷脂質症候群的密切關係,以及抗 B19-VP1u 抗體與紅斑性狼瘡疾病心肌發炎的密切關係。這些線索可提供對於人類微小病毒 B19-VP1u 蛋白在自體免疫疾病致病機轉中扮演重要的角色。本計畫部份研究成果發表如下

(1) Chen DY, Tzang BS, Chen YM, Lan JL, Tsai CC, **Hsu TC***. The association of anti-parvovirus B19-VP1 unique region antibodies with antiphospholipid antibodies in patients with antiphospholipid syndrome. **Clin Chim Acta** 2010; 411:1084-1089. (*Corresponding author) (SCI) (NSC97–2314- B-040–009 and NSC98–2314-B-040–008-MY3)

(2) Tzang BS, Lin TM, Tsai CC, Hsu JD, Yang LC, **Hsu TC***. Increased cardiac injury in

NZB/W F1 mice received antibody against human parvovirus B19 VP1 unique region protein. **Mol Immuno** 2011; 48: 1518-1524. (*Corresponding author) (SCI) (NSC97–2314-B-040–009 and NSC98–2314- B-040–008-MY3)

(3) Chen DY, Chen YM, Lan JL, Tzang BS, Lin CC, **Hsu TC***. Significant Association of Past Parvovirus B19 Infection with Cytopenia in Both Adult-Onset Still's Disease and Systemic Lupus Erythematosus Patients. **Clin Chim Acta** 2012; 413:855-860. (*corresponding author) (SCI) (NSC98–2314- B-075A–003-MY3 and NSC98–2314-B-040–008-MY3)

出席國際學術會議心得報告

計畫編號	98-2314-B-040-008-MY3
計畫名稱	人類微小病毒 B19 結構蛋白獨立區域 VP1u 在誘發自體免疫反應之功能與分子機制探討(第三年)
出國人員姓名 服務機關及職稱	徐再靜教授/所長 中山醫學大學微生物免疫研究所
會議時間地點	2011.11.17~2011.11.19 新加坡(Singapore)- Suntec 國際會議暨展示中心
會議名稱	第五屆亞洲自體免疫學大會 The 5th Autoimmunity Congress Asia (ACA 2011)
發表論文題目	INCREASED CARDIAC INJURY IN NZB/W F1 MICE RECEIVED ANTIBODY AGAINST HUMAN PARVOVIRUS B19 VP1 UNIQUE REGION PROTEIN

會議經過

2011年11月16日下午五時搭乘華航班機由台北出發,由於班機起飛時間被延誤1小時,因此抵達新加坡及出關已是深夜十時。因為11月是新加坡的雨季,因此17日下午到達會場時原本豔陽天突然來個3小時的雷陣雨,讓我領略到熱帶氣候的天氣變化差異。在Suntec國際會議暨展示中心辦理報到後取得相關會議資料便及參與會議。會場入口及空間安排並不如預期而這個會議最主要是集合亞洲從事自體免疫學研究學者進行交流。會議議程11月17日-11月19日分為幾個主軸做廣泛及深入的研究分析，包括八大主題: (1) Autoantibodies: implications in laboratory and in clinical practice (2) Autoimmunity 2012 (3) Diagnostics (4) Therapy of autoimmune diseases (5) The mosaic of autoimmunity (6) B Cells in Autoimmunity (7) Novel pathways in autoimmune diseases (8) Autoimmunity: Pathogenetic mechanisms (9) IVIG – An old, yet novel therapy for autoimmune diseases (10) Infection and autoimmunity (11) Vitamin D, innate immunity and autoimmune diseases (12) Autoantibodies and genetics of autoimmune diseases。會議時間相當緊湊,因此大家把握時間在場內外進行交流討論。本會議約有將近兩百名學者參與。

與會心得

會場內看板標示了所有參加會議的 14 個國家之國旗與出席會議人員的名字，非常醒目。而中華民國國旗標誌當然也與我的名字一同出現在這個會議看板上，台灣這次有 19 人參加。我所發表的壁報論文在壁報間展示，被安排在第一批展出時間並且分類於 Animal Model，看板號碼為 4 號。自體抗體和自體免疫疾病及臨床檢驗試劑是目前在重要的熱門研究主題，因此會議開始便以此主題作廣泛深入的探討。此外自體免疫疾病治療也獲得熱烈討論。另外在生技產品上也有新的研發，像自體抗體 Panel 分析也可以相當快速檢驗，也於此次會議中首度發表並介紹使用及應用。因此此次新加坡會議之行的收穫相當豐富。而這次能順利的出國進行學術交流和參與免疫盛會，吸取新知，實在很不容易。所幸有國科會大力的支持經費與鼓勵我們新一代的研究學者出國進行學術訪問。在此特別致上最衷心的感謝。也期待日後繼續能有機會參與國外的重要學術會議。

Topic: 1 ANIMAL MODELS

Title: INCREASED CARDIAC INJURY IN NZB/W F1 MICE RECEIVED ANTIBODY AGAINST HUMAN PARVOVIRUS B19 VP1 UNIQUE REGION PROTEIN

Author(s): **T.-C. Hsu**[1], B.-S. Tzang[2,3], T.-M. Lin[1], C.-C. Tsai[1]

Institute(s): [1]*Institute of Microbiology and Immunology, Chung Shan Medical University,* [2]*Department of Biochemistry, School of Medicine, Chung Shan Medical University,* [3]*Institute of Biochemistry and Biotechnology, Chung Shan Medical University, Taichung, Taiwan R.O.C.*

Text: **Introduction:** Human parvovirus B19 (B19) infection has been postulated to both myocardial injury and development of systemic lupus erythematosus (SLE). **Objectives:** However, the influence of anti-B19-VP1u antibodies on cardiac disorders in SLE is still obscure. Aims and **Methods:** To elucidate the effects of anti-B19-VP1u IgG in SLE, passive transfer of PBS, normal rabbit IgG or rabbit anti-B19-VP1u IgG was injected intravenously into NZB/W F1 mice, respectively. **Results:** Significant expression of IL-1β, IL-6 and TNF-α were detected in NZB/W F1 mice receiving rabbit anti-B19-VP1u IgG. Markedly cardiomyocyte disarray and lymphocyte infiltration were observed in left ventricle of hearts from NZB/W F1 mice receiving rabbit anti-B19-VP1u IgG. Additionally, significant increases of matrix metalloproteinase-9 (MMP9) activity and protein expression were detected in left ventricle of hearts from NZB/W F1 mice receiving B19-VP1u IgG. Accordingly, significant increase of phosphorylated p-38 and NF-kB proteins were observed in left ventricle of hearts from NZB/W F1 mice receiving B19-VP1u IgG. However, no significant variation of cardiac atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), heart-type fatty acid-binding protein (h-FABP) and creatine kinase MB (CK-MB) were detected among all experimental groups. **Conclusions:** These findings firstly demonstrated the aggravated effects of anti-B19 VP1u IgG on cardiac injury by induction of inflammatory but not myocardial infarction -associated proteins through activation of phosphorylated p-38 and NF-kB signaling.

Author Keywords: Human Parvovirus B19 (B19), VP1 unique region protein (VP1u), Cardiac injury, Systemic lupus erythematosus (SLE)

國科會補助計畫衍生研發成果推廣資料表

日期:2012/10/24

國科會補助計畫	計畫名稱: 人類微小病毒B19結構蛋白獨立區域VP1u在誘發自體免疫反應之功能與分子機制探討
	計畫主持人: 徐再靜
	計畫編號: 98-2314-B-040-008-MY3　　學門領域: 血液科腫瘤科風濕免疫及感染

無研發成果推廣資料

98 年度專題研究計畫研究成果彙整表

計畫主持人：徐再靜	計畫編號：98-2314-B-040-008-MY3
計畫名稱：人類微小病毒 B19 結構蛋白獨立區域 VP1u 在誘發自體免疫反應之功能與分子機制探討	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	2	2	100%	人次	
		博士生	1	1	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	3	3	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	3	3	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	2010 年榮獲中華民國免疫學會『莊淑綺女士紀念傑出醫學研究獎』 榮獲 99 年度國科會補助大專校院獎勵特殊優秀人才 榮獲 100 年度國科會補助大專校院獎勵特殊優秀人才 榮獲 101 年度國科會補助大專校院獎勵特殊優秀人才 2012 年榮獲中華民國免疫學會財團法人沈水德翁文教基金會優秀論文獎

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：■已發表 □未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

研究結果讓我們更進一步了解到人類微小病毒 B19 結構蛋白獨立區域及抗 B19-VP1u 抗體與抗磷脂質抗體及抗磷脂質症候群的密切關係,以及抗B19-VP1u抗體與紅斑性狼瘡疾病心肌發炎的密切關係。這些線索可提供對於人類微小病毒 B19-VP1u 蛋白在自體免疫疾病致病機轉中扮演重要的角色,並且對 B19 疫苗研發有所助益。