行政院國家科學委員會專題研究計畫 期末報告

分泌型磷脂?A2(sPLA2)在人類微小病毒 B19 感染與誘發自體免疫的角色及機轉研究

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計 畫 主 持 人：徐再靜
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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 102 年 10 月 09 日

中 文 摘 要 ： 感染人類微小病毒 B19 主要會表現的臨床症狀包括傳染性紅斑(erythema infectiosum)、關節痛(arthropathy)、血小板過低(thrombocytopenia)、神經方面疾病(neurologic disorders)、肝炎(hepatitis)、心肌炎(cardiovasculitis)以及自體免疫疾病(autoimmune disorders)。臨床研究中發現急性肝炎病人的肝臟組織切片可偵測到人類微小病毒 B19 DNA 的存在。紅斑性狼瘡(SLE)已知為全身系統性自體免疫疾病，會影響許多器官其中包括肝臟，文獻已指出在紅斑性狼瘡的病人中其肝臟不正常的併發率約為 12%~55% 。然而人類微小病毒 B19 的病毒蛋白對於紅斑性狼瘡(SLE)在肝臟損傷的影響仍不清楚。因此我們將人類微小病毒 B19 的重組蛋白(B19-NS1、B19-VP1u 與 B19-VP2)利用皮下注射的方式注射到 NZB/W F1 狼瘡小鼠體內，藉以瞭解人類微小病毒 B19 重組蛋白(B19-NS1、B19-VP1u 與 B19-VP2)對於紅斑性狼瘡(SLE)在肝臟方面的影響。結果發現相較於 B19 結構蛋白獨立區域 VP1u 或結構蛋白 VP2 蛋白,B19 非結構蛋白 NS1 會造成狼瘡小鼠肝臟發炎損傷的情形產生。B19-NS1 蛋白加重 NZB/W F1 狼瘡小鼠在肝臟方面受損的情形，主要藉由誘導型一氧化氮合成酶 iNOS 蛋白和環氧合酵素 COX2 蛋白的表現量明顯增加與肝臟中顯著淋巴球浸潤的現象，以及經由 TNF-α/ NF-κB (p65)訊號傳遞路徑促進 NZB/W F1 狼瘡小鼠肝臟中基質金屬蛋白酶 MMP9 的表現。

中文關鍵詞： 人類微小病毒 B19、紅斑性狼瘡、結構蛋白獨立區域 VP1u、非結構蛋白 NS1、肝臟損傷

英 文 摘 要 ： Human parvovirus B19 (B19) has been associated with a variety of diseases. However, the influence of B19 viral proteins on hepatic injury in SLE is still obscure. To elucidate the effects of B19 viral proteins on livers in SLE, recombinant B19 NS1, VP1u or VP2 proteins were injected subcutaneously into NZB/W F1 mice, respectively. Significant expressions of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) were detected in NZB/W F1 mice receiving B19 NS1 as compared to those mice receiving PBS. Markedly hepatocyte disarray and lymphocyte infiltration were observed in livers from NZB/WF 1 mice receiving B19 NS1 as compared to those mice receiving PBS. Additionally, significant increases of Tumor Necrosis Factor - α (TNF-α), TNF-α receptor, IκB kinase - α (IKK-α), nuclear

factor of kappa light polypeptide gene enhancer in B-cells inhibitor (IκB) and nuclear factor-kappa B (NF-κB) were detected in livers from NZB/W F1 mice receiving B19 NS1 as compared to those mice receiving PBS. Accordingly, significant increases of matrix metalloproteinase-9 (MMP9) and U-plasminogen activator (uPA) were also detected in livers from NZB/W F1 mice receiving B19 NS1 as compared to those mice receiving PBS. Contrarily, no significant variation on livers from NZB/W F1 mice receiving B19 VP1u or VP2 was observed as compared to those mice receiving PBS. These findings firstly demonstrated the aggravated effects of B19 NS1 but not VP1u or VP2 protein on hepatic injury and provide a clue in understanding the role of B19 NS1 on hepatic injury in SLE.

英文關鍵詞： Human Parvovirus B19 (B19), Systemic lupus erythematosus (SLE), VP1 unique region protein (VP1u), nonstructural protein (NS1), hepatic injury

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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□移地研究心得報告
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目　　錄

中文摘要

感染人類微小病毒 B19 主要會表現的臨床症狀包括傳染性紅斑(erythema infectiosum)、關節痛(arthropathy)、血小板過低(thrombocytopenia)、神經方面疾病(neurologic disorders)、肝炎(hepatitis)、心肌炎(cardiovasculitis)以及自體免疫疾病(autoimmune disorders)。臨床研究中發現急性肝炎病人的肝臟組織切片可偵測到人類微小病毒 B19 DNA 的存在。紅斑性狼瘡(SLE)已知為全身系統性自體免疫疾病，會影響許多器官其中包括肝臟，文獻已指出在紅斑性狼瘡的病人中其肝臟不正常的併發率約為 12%~55% 。然而人類微小病毒 B19 的病毒蛋白對於紅斑性狼瘡(SLE)在肝臟損傷的影響仍不清楚。因此我們將人類微小病毒 B19 的重組蛋白(B19-NS1、B19-VP1u 與 B19-VP2)利用皮下注射的方式注射到 NZB/W F1 狼瘡小鼠體內，藉以瞭解人類微小病毒 B19 重組蛋白(B19-NS1、B19-VP1u 與 B19-VP2)對於紅斑性狼瘡(SLE)在肝臟方面的影響。結果發現相較於 B19 結構蛋白獨立區域 VP1u 或結構蛋白 VP2 蛋白, B19 非結構蛋白 NS1 會造成狼瘡小鼠肝臟發炎損傷的情形產生。B19-NS1 蛋白加重 NZB/W F1 狼瘡小鼠在肝臟方面受損的情形，主要藉由誘導型一氧化氮合成酶 iNOS 蛋白和環氧合酵素 COX2 蛋白的表現量明顯增加與肝臟中顯著淋巴球浸潤的現象，以及經由 TNF-α / NF-κB (p65)訊號傳遞路徑促進 NZB/W F1 狼瘡小鼠肝臟中基質金屬蛋白酶 MMP9 的表現。

關鍵詞: 人類微小病毒 B19、紅斑性狼瘡、結構蛋白獨立區域 VP1u、非結構蛋白 NS1、肝臟損傷

Abstract

Human parvovirus B19 (B19) has been associated with a variety of diseases. However, the influence of B19 viral proteins on hepatic injury in SLE is still obscure. To elucidate the effects of B19 viral proteins on livers in SLE, recombinant B19 NS1, VP1u or VP2 proteins were injected subcutaneously into NZB/W F1 mice, respectively. Significant expressions of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) were detected in NZB/W F1 mice receiving B19 NS1 as compared to those mice receiving PBS. Markedly hepatocyte disarray and lymphocyte infiltration were observed in livers from NZB/WF 1 mice receiving B19 NS1 as compared to those mice receiving PBS. Additionally, significant increases of Tumor Necrosis Factor – α (TNF-α), TNF-α receptor, IκB kinase – α (IKK-α), nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor (IκB) and nuclear factor-kappa B (NF-κB) were detected in livers from NZB/W F1 mice receiving B19 NS1 as compared to those mice receiving PBS. Accordingly, significant increases of matrix metalloproteinase-9 (MMP9) and U-plasminogen activator (uPA) were also detected in livers from NZB/W F1 mice receiving B19 NS1 as compared to those mice receiving PBS. Contrarily, no significant variation on livers from NZB/W F1 mice receiving B19 VP1u or VP2 was observed as compared to those mice receiving PBS. These findings firstly demonstrated the aggravated effects of B19 NS1 but not VP1u or VP2 protein on hepatic injury and provide a clue in understanding the role of B19 NS1 on hepatic injury in SLE.

Key words: Human Parvovirus B19 (B19), Systemic lupus erythematosus (SLE), VP1 unique region protein (VP1u), nonstructural protein (NS1), hepatic injury

Introduction

Systematic lupus erythematosus (SLE) is known as a systemic autoimmune disorder that affects various organs including liver [1]. Various reports have indicated that growing population with liver disease was found in patients with SLE [2-4]. Although the occurrence of liver disease is not routinely screened, the incidence of hepatic abnormality in patients with SLE was reported as varying from 12% to 55% [4].

Human parvovirus B19 (B19) is known as an important human pathogen, which consists a nonstructural protein (NS1) and two capsid proteins, VP1 and VP2 [5]. Notably, B19 infection has been associated with a wide range of different pathologies and clinical manifestations including erythema infectiosum, arthropathy, thrombocytopenia, neurologic disorders, hepatitis, cardiovasculitis and autoimmune disorders [5-8]. Indeed, various studies have postulated a connection between B19 infection and liver injury. A clinical study reported the existence of B19 DNA in a liver biopsy specimen from a patient with acute hepatitis [9]. Another studies also suggested an important role of B19 infection in acute icteric hepatitis liver injury [10] and acute fulminant hepatitis with bone marrow failure [11]. In addition, B19 infection has been recognized to trigger the acute liver failure in a patient with Wilson disease [12].

Although there is no direct evidence of B19 virus in inducing autoimmune diseases, the association between B19 virus and pathogenesis of autoimmunity has been strongly suggested. Recently, human parvovirus B19 has been associated with SLE [6, 13-16]. However, little is known about the influence of B19 viral proteins on liver injury in SLE. In the present study, various recombinant B19 viral proteins were prepared and injected subcutaneously into NZB/W F1 mice to elucidate the effects of B19 viral proteins on livers in SLE.

Materials and Methods

Animal and treatments

Animal experiments were approved by the Institutional Animal Care and Use Committee at Chung Shan Medical University. Twenty-four female NZB/W F1 mice at week 6 were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and housed under supervision of the Institutional Animal Care and Use Committee at Chung Shan Medical University, Taichung, Taiwan. The animals were kept under a 12-h light-dark cycle and ambient temperature was maintained at 25°C. Animals were free access to water and standard laboratory chow (Lab Diet 5001; PMI Nutrition International Inc., Brentwood, MO, USA). Animal welfare and experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals. All the animals at the age of 8 weeks were randomly divided into 4 equal groups (6 mice each group) and injected subcutaneously with 20ug purified B19-NS1, B19-VP1u, B19-VP2 recombinant proteins or phosphate-buffered saline

(PBS) mixed 1:1 (v/v) with Freund's complete adjuvant (Sigma-Aldrich, UK), respectively. The mice were boosted with the same dose mixed 1:1 (v/v) with Freund's incomplete adjuvant (Sigma-Aldrich, UK) twice a week for 3 times and then sacrificed at the age of 16 weeks by CO_2 asphyxiation. The heart blood and liver tissues were collected and stored at −80 °C until use. The reactivity of anti-sera to dsDNA and B19 viral proteins was examined (Table S2)

Hematoxylin-eosin staining

The liver samples of animals were excised and soaked in formalin and covered with wax. Slides were prepared by deparaffinization and dehydration. They were passed through a series of graded alcohols (100%, 95% and 75%), 15 min 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 min each. At the end, they were soaked with Xylene I and Xylene II. Photomicrographs were obtained using Zeiss Axiophot microscopes.

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 [21]. After blocking with 5% non-fat dry milk in (PBS), antibodies against TNF-α receptor, TNF-α, iNOS, COX-2, MMP-9, uPA, IKK-α, IκB, NF-κB (p65) (Santa Cruz Biotechnology, CA, USA) and β-actin (Upstates, Charlottesville, VA, USA) were diluted in PBS with 2.5% BSA and incubated for 1.5 h 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, CA, USA).

Statistical analysis

All of the statistical analyses were performed using SPSS 10.0 software (SPSS Inc., Chicago, IL). Three independent experiments were repeated. Statistical analyses were performed using the analysis of variance plus posterior multiple comparison test to test the difference. $P < 0.05$ was considered statistically significant. The significant differences were stressed with symbols as shown in figures.

Results

Increased expression of iNOS and COX-2 proteins in NZB/W F1 mice receiving B19-NS1 protein

Human parvovirus B19 has been strongly associated with the pathogenesis of SLE. To investigate the influences of B19 viral proteins on livers in SLE, various inflammatory associated proteins including iNOS and COX-2 were examined. Significantly increased iNOS

protein level was detected in livers from NZB/W F1 mice receiving B19-NS1 as compared to those mice receiving PBS (Fig. 1A). In contrast, no significant variation on iNOS expression in livers from NZB/W F1 receiving B19-VP1u or VP2 was observed as compared to those mice receiving PBS (Fig. 1A). Additionally, significant increase of COX-2 protein was also detected in livers from NZB/W F1 mice receiving B19-NS1 whereas no significant variation on COX-2 expression in livers from NZB/W F1 receiving B19-VP1u or VP2 was observed as compared to those mice receiving PBS (Fig. 1B). Quantified results were shown in the lower panel of figure 1A and 1B.

Hepatic architecture changes in NZB/W F1 mice receiving B19-NS1 protein

To observe the effects of B19-NS1 protein on hepatic architectures in NZB/W F1 mice, we performed a histopathological analysis on liver tissue stained with hematoxylin and eosin (Fig. 2). More abnormal hepatic architecture and increased interstitial space were observed in livers from NZB/W F1 mice receiving B19-NS1 as compared to those mice receiving PBS, B19-VP1u or VP2, respectively (Fig. 2A). Additionally, markedly lymphocyte infiltration was observed in livers from NZB/W F1 mice receiving B19-NS1 protein as compared to those mice from other groups (Fig. 2B).

Increased expression of TNF-α and TNF-α receptor in NZB/W F1 mice receiving B19-NS1 protein

To clarify the possible signaling involved in the B19-NS1 aggravated liver injury in NZB/W F1 mice, the expressions of TNF-α and TNF-α receptor were examined. Significant increase of TNF-α was detected in livers from NZB/W F1 mice receiving B19-NS1 as compared to those mice receiving PBS (Fig 3A). Consequently, significant increase of TNF-α receptor was also detected in livers from NZB/W F1 mice receiving B19-NS1 as compared to those mice receiving PBS (Fig 3B). In contrast, no significant variation on both TNF-α and TNF-α receptor expression were observed in livers from NZB/W F1 mice receiving B19-VP1u or VP2 as compared to those mice receiving PBS (Fig 3A and 3B). Meanwhile, significantly increased serum TNF-α level was also detected in NZB/W F1 mice receiving B19-NS1 as compared to those mice receiving PBS (Fig 3C). Quantified results were shown in the lower panels of figure 3A and 3B.

Increased expression of IKK-α, IκB and NF-κB (p65) in NZB/W F1 mice receiving B19-NS1 protein

To further investigate the signaling molecules involved in the B19-NS1 enhanced TNF-α expression, the downstream molecules of TNF-α including IKK-α, IκB, NF-κB (p65) were examined. Significant increases of IKK-α and IκB were detected in livers from NZB/W F1 mice receiving B19-NS1 as compared to those mice receiving PBS (Fig 4A and 4B). In contrast, no significant variation on IKK-α and IκB expression were observed in livers from NZB/W F1 mice receiving B19-VP1u or VP2 as compared to those mice receiving

PBS (Fig 4A and 4B). Quantified results were shown in the lower panels of figure 4A and 4B. Moreover, significant increase of NF-κB (p65) was also detected in livers from NZB/W F1 mice receiving B19-NS1 as compared to those mice receiving PBS whereas no significant variation on NF-κB (p65) level was observed in NZB/W F1 mice receiving B19-VP1 u or VP2 as compared to those mice receiving PBS (Fig 5). Quantified results were shown in the lower panel of figure 5.

Increased MMP-9 and uPA expression in NZB/W F1 mice receiving B19-NS1 protein

MMP-9, a consequent molecules in TNF-α signaling, is known as an indicator playing important roles in hepatic disorders. To further examine the effect of B19-NS1 protein on MMP-9 expression, Immunoblots were preformed to examine the expression of MMP-9. Significant increase of MMP-9 was detected in livers from NZB/W F1 mice receiving B19-NS1 as compared to those mice receiving PBS (Fig 6A). Additionally, the expression of uPA protein, an upstream activator of MMP-9, was also examined. As shown in figure 6B, significant increase of uPA protein was observed in liver from NZB/W F1 mice receiving B19-NS1 as compared to those mice receiving PBS (Fig 6B). In contrast, no significant variation on both MMP9 and uPA expression were observed in livers from NZB/W F1 mice receiving B19-VP1u or VP2 as compared to those mice receiving PBS (Fig 6A and 6B). Quantified results were shown in the lower panels of figure 6A and 6B.

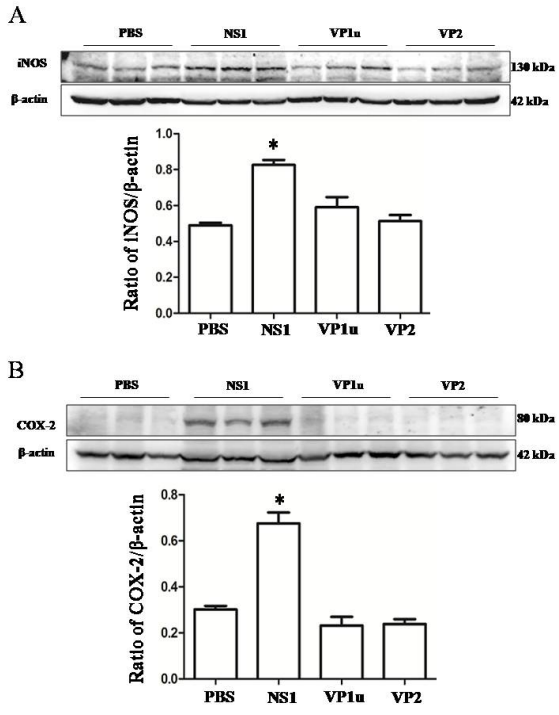


Figure 1

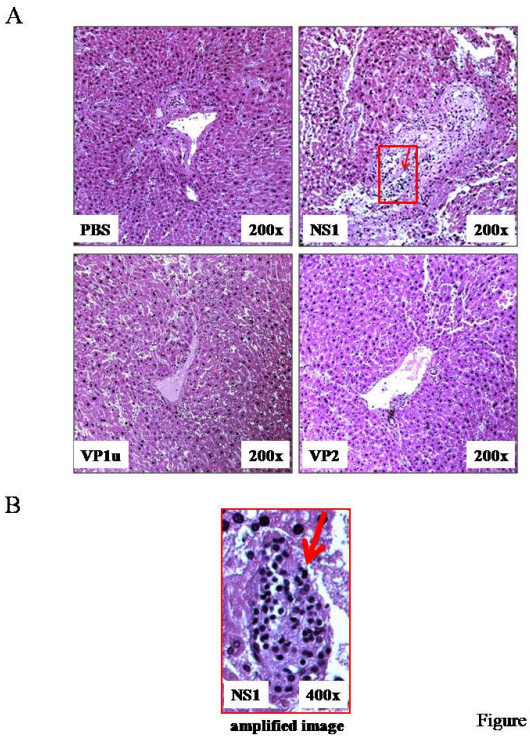
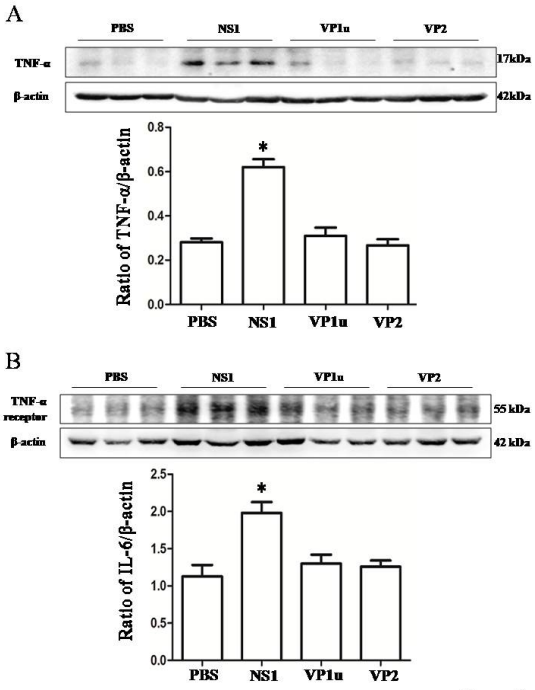


Figure 2



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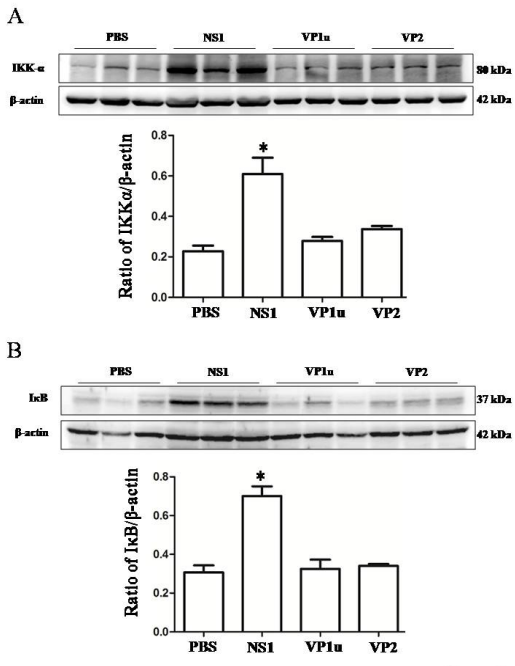

Figure 4

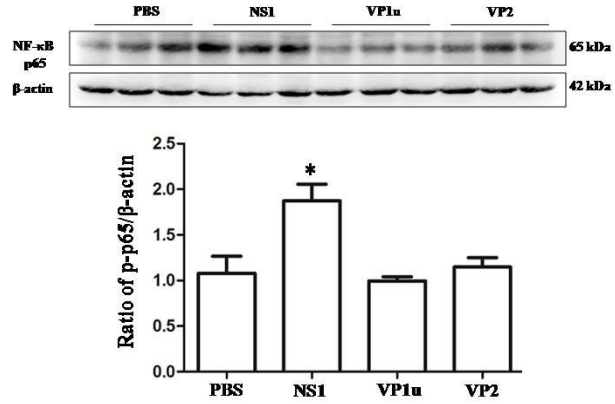

Figure 5

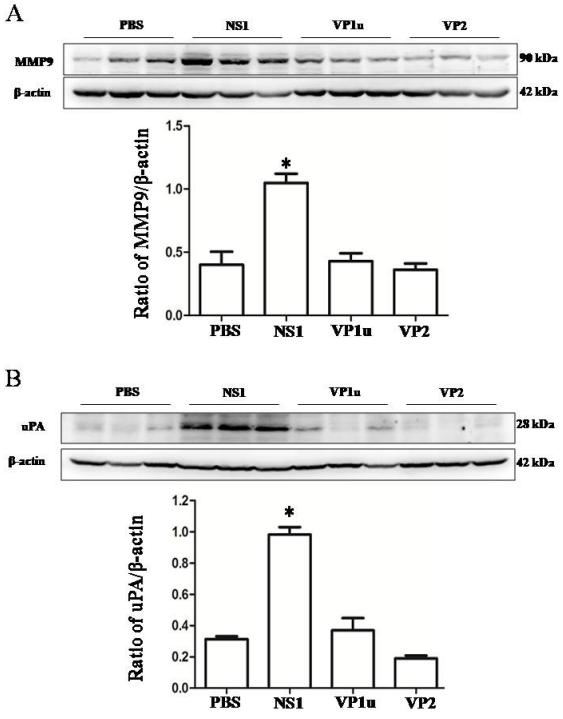

Figure 6

Discussion

Although B19 infection has been implicated in pathogenesis of liver diseases and development of SLE, the effects of B19 and its viral proteins including NS1, VP1u and VP2 on hepatic injury in SLE is still obscure [6, 9-16]. In the present study, we revealed the

aggravated effects of B19 NS1 protein on hepatic injury in NZB/W F1 mice by significantly enhancing the expressions of iNOS and COX-2 proteins and lymphocyte infiltration. Additionally, significant increase of MMP-9 through TNF-α / NF-κB (p65) signaling was also detected.

Elevated inducible nitric oxide synthase (iNOS) has been reported in patients with SLE [22]. According to a recent clinical research of 72 SLE patients, significant higher oxidative stress including iNOS level has been associated with the SLE Disease Activity Index (SLEDAI) and recognized to the pathogenesis of SLE [23]. Similar result was also observed in lupus-prone mice [24]. Accordingly, cyclooxygenase type 2 (COX-2) is also known to play pivotal roles in development of inflammatory diseases and associated with the pathogenesis of SLE [25-26]. A previous study has demonstrated that some COX-2 inhibitors are able to suppress the production of pathogenic autoantibodies to DNA by causing autoimmune T-cell apoptosis [27]. Similar result was also demonstrated in a lupus-prone murine model [28]. These studies did stress the roles of iNOS and COX-2 in development of SLE. In the current study, significant increases of iNOS and COX-2 expression were detected in livers from NZB/W F1 mice receiving B19-NS1 but not VP1u or VP2 as compared to those mice receiving PBS. By the way, markedly lymphocyte infiltration was observed in NZB/W F1 mice receiving B19-NS1. These findings suggested the effect of B19-NS1 but not VP1u or VP2 on aggravating the hepatic injury in SLE by enhancing the expression of iNOS and COX-2.

Matrix metalloproteinase-9 (MMP-9) has been postulated with the pathogenesis of autoimmune diseases including SLE [29]. Various studies have reported that elevated MMP-9 activity plays crucial role in development of SLE in both human and lupus-prone mice [30-31]. Indeed, the cleavage of myelin basic protein or type II gelatins by MMP-9 will generate remnant epitopes and contribute to develop autoimmunity [32]. Additionally, TNF-α is known to be involved in the signal pathway of MMP-9 induction, which degrades extracellular matrix in the inflammatory responses [33]. Accordingly, IKK and IKB, the downstream molecules for TNF-α induced MMP-9 expression, are known to be activated prior to the transcription of MMP-9 promoter through NF-κB sites [34-35]. Consistently, our experimental results revealed that B19-NS1 aggravatedly induces the expression of MMP-9 in livers of NZB/W F1 mice via TNF-α signaling, which also elicits the activation of IKK-α, IκB and NF-κB.

B19 NS1 is known as a cytotoxic protein with multi-function. Besides the ATPase and DNA helicase activity [36], B19 NS1 protein has been described as a transactivator of the viral p6- as well as a variety of cellular promoters. These include the promoter region controlling the expression of TNF-α and IL-6 genes [37-38]. Elevated levels of TNF-α have been shown to be present in patients during the acute and convalescent phases of B19

infection [39]. The prolonged or continuous presence of these proinflammatory cytokines during acute-convalescent and persistent B19 infection, respectively, may result in the induction of long lasting clinical symptoms and autoimmune reactions [6]. Although causality is often difficult to conclude, these studies did imply that B19 NS1 is possibly involved in the induction of autoimmunity and can't be negated [6, 39]. Briefly, NZB/W F1 is a well known spontaneously lupus mice model [40], which has been used for investigating SLE for several decades. Therefore, these findings firstly demonstrated the aggravated effects of B19 NS1 but not VP1u or VP2 protein on hepatic injury and provide a clue in understanding the role of B19 NS1 on hepatic injury in SLE.

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42. 蔡鈞州 (中山醫學大學微生物免疫研究所博士論文, 指導教授:徐再靜) 人類微小病毒 B19 蛋白對狼瘡小鼠肝臟影響之研究

計畫成果自評部份

研究內容與計畫相符。

本計畫研究成果已發表如下:

1. Tsai CC, Chiu CC, Tzang BS, **Hsu TC*.** Human Parvovirus B19 nonstructural (NS)-1 protein induces liver injury of NZB/W F1 mice through MAPK and IKK mediated NF-kB signaling pathway. Oct. 3-6, 2012. 15th Biennial Meeting European Society for Immunodeficiency, Florence, Italy. (*Corresponding author) (國際會議 海報論文)
2. Tsai CC, Chiu CC, Hsu JD, Hsu HS, Tzang BS, **Hsu TC***. Human parvovirus B19 NS1 protein aggravates liver injury in NZB/W F1 mice. PLoS One. 2013; 8(3): e59724. (*Corresponding author)

出席國際學術會議心得報告

日期：101 年 9 月 20 日

計畫編號	101-2314-B-040-008
計畫名稱	分泌型磷脂酶 A2(sPLA2)在人類微小病毒 B19 感染與誘發自體免疫的角色及機轉研究
出國人員姓名 服務機關及職稱	徐再靜教授 中山醫學大學微生物免疫研究所
會議時間地點	2012.09.05~2012.09.8 The Scottish Exhibition and Convention Centre, Glasgow, UK
會議名稱	第三屆歐洲免疫學會議 **3rd European Congress of Immunology (ECI2012)**
發表論文題目	Beneficial effects of Ocimum gratissimum aqueous extract on rats with CCl4-induced acute liver injury

會議經過

經過30多小時的班機航程,終於抵達位於英國格拉斯哥之蘇格蘭展覽會議中心(The Scottish Exhibition and Convention Centre, Glasgow, UK),當地天氣已進入秋天,溫度約8~13度。在國際會議暨展示中心辦理報到後,取得相關會議資料便前往開幕會場。整個會議會場有四個展館,場地相當舒適及廣大,海報場地就容納近2000張看板。會議最主要是集合歐洲17國從事免疫學研究學者進行交流,根據大會統計本次參與的國家高達30個,而台灣報名參加的人數有30人。會議議程9月5日-9月8日,分為82個免疫主軸做廣泛深入的研究分析探討，包括熱門主題: (1) TLR (2) Inflammasomes (3) Lymphocyte Signalling Mechanisms (4) Monocyte, Macrophage and DC Functions (5) Lymphocyte Development (6) Rheumatoid Arthritis and Lupus (7) Asthma/Allergy (8) Advances in Biologics 1 - Antibodies, Related Molecules and ivlg (9) Advances in Biologics 2 - New Developments (10) Autophagy (11) Tumour Immunology (12) Hepatitis and Liver Immunology。

與會心得

由於會議時間相當緊湊，因此大家都把握時間在場內外進行學術討論。本會議約有將近五千名學者參與。這次主題中在2011年諾貝爾獎Dendritic cell重要性開幕,是會場討論重點,也更顯現對先天免疫及後天免疫研究之重要性。因此歐洲免疫會議之行的收穫相當豐富,也對相關免疫研究也有進階的認知。另外,展場中也有一些新的研究試劑廠商參展,透過訪談也瞭解歐洲免疫研究相關產品市場。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與進行學術交流。

Poster session: Hepatitis & Liver Immunology

P0974

Beneficial effects of Ocimum gratissimum aqueous extract on rats with CCl_4-induced acute liver injury

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Purpose/Objective: Ocimum gratissimum (OG) is known as a food spice and traditional herb, which has been recommended for the treatment of various diseases. Herein we investigated the effects of OG leaf aqueous extract (OGAE) on reducing hepatic injuries in rats after CCl4 challenging.

Materials and methods: To investigate the hepatoprotective effect of OG aqueous extract (OGAE), male Wistar rats challenged by carbon tetrachloride (CCl4) were used as the animal model of chronic hepatic injury. Catalase assay (CAT) and DPPH assay was assessed in CCl4-administrated rats. Expression and phosphorylation of protein was determined and quantitated by immunoblotting using specific antibodies and densitometric analysis.

Results: Significantly increased serum catalase and DPPH levels were detected in CCl4-administrated rats that were treated with OGAE or sylimarin as compared to those rats that were treated with saline or CCl4. In contrast, significantly decreased stress proteins including HSP70 and iNOS were observed in livers of CCl4-administrated rats that were treated with OGAE or sylimarin as compared to those rats that were treated with saline or CCl4. Moreover, significant decreases of MMP-9/MMP-2 ratio, uPA, phosphorylated ERK (p-ERK) and NF-κB (p-P65) were detected in livers of CCl4-administrated rats that were treated with OGAE or sylimarin as compared to those rats that were treated with saline or CCl4.

Conclusions: These findings imply that OGAE can efficiently inhibit CCl4-induced liver injuries in rats and may therefore be a potential food or herb for preventing liver injuries.

無研發成果推廣資料

101 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：101-2314-B-040-008-
計畫名稱：分泌型磷脂?A2(sPLA2)在人類微小病毒 B19 感染與誘發自體免疫的角色及機轉研究	

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	0	100%	人次	
		博士生	1	0	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	1	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	1	0	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。)	無

	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)

研究結果首度發現相較於人類微小病毒 B19 結構蛋白獨立區域 VP1u[具有分泌型磷脂胺 A2(sPLA2)]及結構蛋白 VP2, B19 非結構蛋白 NS1 會加重狼瘡小鼠肝臟發炎損傷的情形產生。這些線索有助於瞭解 B19 病毒蛋白對於紅斑性狼瘡肝臟損傷扮演的重要角色。