行政院國家科學委員會專題研究計畫 成果報告

以 JC virus 當載體輸送細胞激素 IL-6 之干擾 RNA 在治療類風濕性關節炎之角色
研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 95-2314-B-040-027-
執 行 期 間 ：95 年 08 月 01 日至 96 年 07 月 31 日
執 行 單 位 ：中山醫學大學免疫學研究所

計 畫 主 持 人：蔡嘉哲

計畫參與人員：碩士班研究生-兼任助理：郭懿瑩、周孟穎

報 告 附 件 ：國外研究心得報告

處 理 方 式 ：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 96 年 08 月 15 日

行政院國家科學委員會補助專題研究計畫 ＊成果報告 □期中進度報告

（計畫名稱）

計畫類別：＊個別型計畫　　□ 整合型計畫
計畫編號：NSC 95-2314-B-040-027
執行期間：　95 年　08 月 01 日至　96 年 07 月 31 日

計畫主持人：蔡嘉哲
共同主持人：Professor Moncef Zouali
計畫參與人員：

成果報告類型(依經費核定清單規定繳交)：＊精簡報告　□完整報告

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□國際合作研究計畫國外研究報告書一份

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執行單位：

中　華　民　國　96 年　8 月 15　日

FRANCE (Inserm) - TAIWAN (NSC) COOPERATION

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Progress report

—o—o—o—o—o—o—o—

August 2007

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Title of Project

Silencing IL-6 expression by RNA interference (RNAi) using the JC virus-like particles as a transfer vector: Treatment of rheumatoid arthritis in experimental mouse models

French coordinator

Moncef ZOUALI, Director of Research
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Taiwanese coordinator

Gregory J TSAY, Professor, Department of Medicine, and Director,
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Objectives of the collaboration

Rheumatoid arthritis (RA) affects 1% of the adult population. While its etiology remains under investigation, cytokines have been identified as key molecules in RA pathogenesis. Among them, IL-6 has been proposed to contribute to the development of arthritis. Therefore, therapies targeting IL-6 are promising for RA**.** The aim of this proposal is to test the therapeutic effect of silencing genes encoding cytokines known to play a key role in the pathogenesis of RA. RNA interference (RNAi) is a powerful tool to silence gene expression post-transcriptionally and, as such, represents a novel potential therapeutic approach. Indeed, it offers the prospect of high specificity and low immunogenicity. The main obstacle to achieving *in vivo* gene silencing by RNAi technologies is delivery. As a first step towards this goal, we will silence IL-6 expression in mouse models of RA. Since JC virus (JCV) has been proved to be a suitable delivery vector, we will use JCV as a delivery vector to target IL-6 by RNAi. Constructs will be packaged into the virus-like particles (VLPs) of JC virus by osmotic shock. They will be used to silence IL-6 in U937 cells. The VLPs containing IL-6 RNAi will also be injected into arthritic mice to investigate their therapeutic efficacy. Overall, the goals of this project are to find a new therapeutic potential of RNAi for the treatment of RA.

Exchange visits made

Dr. Gregory J Tsay visited Inserm U 606 in Paris and gave a special lecture on 21 November 2006 entitled "*Parvovirus B19 infection and rheumatic autoimmune diseases*".

Dr. Moncef Zouali made a first visit to Chung Shan Medical University (Taichung, Taiwan) from 2 to 9 December 2006 and gave a lecture entitled "*The evolving pathogenesis of SLE*" at the Institute of Immunology on 5 December 2006, and a second, entitled "*Emerging therapeutic strategies in SLE*" at the Department of Medicine (Chung Shan Medical University, Hospital, Taichung) on 6 December 2006.

These two sets of visits allowed several discussions between Dr. Tsay and French scientists in Paris, and between Dr. Zouali and Taiwanese scientists in Taichung. The visits also allowed more detailed and specific discussions of the experiments to be performed within the frame of this collaboration.

From 28 June to 6 July 2007, Dr. Zouali made a second visit to Chung Shan Medical University (Taichung, Taiwan). During this second visit, he gave seminars at Cheng Kung University in Tainan, at Chung Shan Medical University in Taichung, and at Taichung VA Hospital in Taichung.

Experimental progress made

Since there was no data on the use of the JC virus as a delivery vector for cytokines, we had to set up an experimental system. Because IL-10 shRNA was available and because IL-10 is a regulatory cytokine that plays a central role in systemic autoimmune disease, we performed experiments to test JC virus as a delivery vector for IL-10.

First, VLP particles were purified from VP1 of JC virus by sucrose gradient ultracentrifugation. Activity of the purified particles was test by hemagglutination of O type red blood cells. IL-10 shRNA was then packaged into VLP by osmotic shock. Two cell lines (U-937 human histiocytic lymphoma cell line, and RAW-264.7 mouse monocytic cells) were then pseudo-infected with the It was then IL-10 shRNA containing VLP particles. The preparations were then shown to have suppressive effects of IL-10 production by RT-PCR and Real time PCR.

We then tested the *in vivo* activity of IL-10 shRNA on IL-10 production. To this end, BALB/c mice were pre-injected with LPS. After two hours, mice received intravenously IL-10 shRNA packaged into JCV virus-like particles or control preparations. The results showed that the IL-10 release following LPS injection was partially inhibited by IL-10 shRNA at six, 12, 24 and 36 hours. The suppressive effects observed were specific for IL-10, and no such effects were seen on release of TNF-α.

Thus, we have shown that JCV virus-like particles represent a suitable system for gene delivery. We have also demonstrated that IL-10 shRNA can be used to silence efficiently IL-10 gene expression *in vitro* and *in vivo*.

Current and future projects

Since recent evidence indicates that IL-10 levels are increased in SLE patients (Ref.). The results we have obtained with IL-10 shRNA prompt us to apply the gene delivery system we have developed to manipulation of experimental lupus. To that end, we will use two experimental lupus models: (NZBxNZW) F_1 and MRL-*lpr* mice. The effects will be tested on autoantobody production, proteinuria, and glomerulonephritis.

In parallel, we will use the JC virus gene therapy system to deliver an IL-6 RNAi construct to mice with experimental arthritis [collagen-induced arthritis. (CIA) and K/BxN (KRN/C57Bl/6J x NOD/Ltj) mice]. The preparation will be administered systemically or intra-articularly. Assessment of the effects of administration of RNAi into these mice by clinical, radiographic, histologic and immunohistochemical means will be performed. Assessment of the effects of RNAi injection on expression of inflammatory mediators and cytokines related to bone destruction, on osteoclast maturation, and on expression of the RANK/RANKL/OPG system in the joints.

Publications

- Tsay, G., and Zouali, M. (2006). Human parvovirus B19, signaling and systemic autoimmunity. **Biochem Pharmacol**. *72,* 1453-1459.

- Suppression of IL-10 expression by RNAi using JC virus as a gene transfer vector, manuscript in preparation.

Cooperative activities requested for the period of 1 August 2007 – 31 July 2008.

French scientists:
Two exchange visits, 10 days each, to Taiwan.

Taiwanese scientists:
One exchange visit for two scientists for one week to France.

國際合作計畫赴國外研究心得報告

計畫編號	NSC 95-2314-B-040-027
計畫名稱	以JC virus 當載體輸送細胞激素IL-6 之干擾RNA 在治療類風濕性關節炎之角色
出國人員姓名 服務機關及職稱	蔡嘉哲/中山醫學大學/教授兼所長
出國時間及地點	2006/11/20-2006/11/22 法國巴黎
合作研究機構	法國國家衛生研究院
合作計畫名稱	以 JC virus 當載體輸送細胞激素 IL-6 之干擾 RNA 在治療類風濕性關節炎之角色
合作計畫主持人	Professor Moncef Zouali
出國事由	學術交流

一、內容及成果：

Parvovirus B19 infection and rheumatic autoimmune diseases

It has been known for some time that viruses are implicated in rheumatic disorders such as SLE, RA, Sjogren's syndrome, and systemic sclerosis. The etiology of rheumatic diseases is unknown, but both genetic and environmental factors are involved in the pathogenesis of these disorders. It is presumed that environmental factors especially viruses trigger development in the genetically predisposed. We sometimes see patients with arthralgia or arthritis following viral infection. I'll talk B19 and HCV in more detail latter.

Arthritis can follow infection with several viral agents included: B19 as their name, only 23 nm, is one of the most frequent causes of virus-induced arthritis. Arthritis with HBV infection occurs in adolescents and young adults, begins acutely with migratory arthralgias (25%) involving the PIP, and MCP, also found in patients with PAN and vasculitis. Most of these patients resolve in 3 weeks. In1989, HCV was established and HCV has been found to be strikingly associated with autoimmune diseases and serological markers of autoimmunity. Rubella arthritis is not seen in children, but in one sixth in adult, involve small joints. Rubella vaccination is associated with a high frequency (40%) of arthritis on knees, particularly in young female. These occur 2 or 3 weeks after vaccination.

Hypothesis for the viral infections in autoantibody mediated blood autoimmune disease. Two events: first, a production of autoantibodies by a first infections agent and by second infection involved secretion of proinflammatory cytokines, followed by interferon-γ and enhance the phagocytic activity of macrophages and finally, increased phagocytosis of blood cells.

Molecular mimicry has been proposed for the pathogenesis of AID. Based on the similarities between viral proteins and host elements in patients with SLE. Apoptosis plays an important role

in autoimmune diseases. Evidence is accumulating that modification of autoantigens during apoptosis. The immunological events following infection fall into a beneficial and a detrimental stage. Human parvovirus B19 as a causative agent for rheumatoid arthritis. Parvovirus infection mimicking SLE, Lupus-like presentation of human parvovirus B19 infection in patients with SLE
B19 is a small single-stranded DNA virus that contains 5600 nucleotides. The genes of the left side of the genome encode the NS1 and those on the right side encode VP1 and Vp2. VP1 interacts with cellular receptor; VP2 involved in antigenic recognition by neutralizing Antibody. aCL was positive in three of the four patients included three with anti-β2 glycoprotein I. ANCA was detected in all patients included three p-ANCA and one c-ANCA. Two patients with both antibodies had polyarthritis for more than 6 months.

Our patients with B19 infection had the production of ANCA and aCL. These indicate parvovirus B19 infection may be linked to the induction of an autoimmune response. We used nested PCR to test for the presence of B19 DNA in the blood of patients with AID. Positive samples showed the expected molecular size of the 322 bp PCR product. Parvovirus B19 DNA was detected in 17 of 72 patients with SLE and in three of four patients with erythema infectiosum (EI), but not in patients with other AID. There was a higher prevalence of hypocomplementaemia and Raynaud's phenomenon in patients with B19 viraemia.

此行在 95/11/21INSERM 的演講[Parvovirus B19 infection and rheumatic autoimmune diseases]及學術研討活動，與法國國家衛生研究院的各位學者們及研究領域的許多研究人員有相當多的互動，與大家分享在自體免疫疾病中，病毒及感染上的領域，反應與討論相當熱烈，也激發了更多的研究靈感及方向。

二、建議事項

今後應多多參予歐洲的學術研討會，加強台灣與歐洲之研究合作，歐洲之研究水準極高，是值得台灣在學術研究領域上的學習目標，此行的學術交流也能為台灣在醫學研究上多開啟一扇窗。

三、攜回資料

2006.11.21 INSERM

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