行政院國家科學委員會補助專題研究計畫成果報告

※※※※※※※※※※※※※※※※※※※※※※※※※※※※※

※ PARVOVIRUS B19 在自體免疫疾病之研究 ※

※※※※※※※※※※※※※※※※※※※※※※※※※※※※※

計畫類別：※個別型計畫　　□整合型計畫

計畫編號：NSC89－2314－B－040－009－

執行期間：　88 年 08 月 01 日至 89 年 07 月 31 日

計畫主持人：蔡嘉哲 教授

執行單位：中山醫學院免疫學研究所

中　華　民　國　89　年　09　月　30　日

行政院國家科學委員會專題研究計畫成果報告

PARVOVIRUS B19 在自體免疫疾病之研究

計畫編號:NSC 89-2314-B040-009

執行期限: 88 年 8 月 1 日至 89 年 7 月 31 日

主持人:蔡嘉哲教授　執行機構及單位名稱:中山醫學院免疫學研究所

計畫參與人員: 許靜婷(助理)、徐再靜(講師)、陳佳伶(助理)、

吳怡瑩(研究生)

執行機構及單位名稱: 中山醫學院免疫學研究所

一.中文摘要

利用病毒血清學法、聚合酶連鎖反應、南方墨點法分析被人類微小病毒 B19 感染的血清。血清來源爲 72 位有紅斑性狼瘡病人；23 位爲風顯性關節炎;18 位爲修格連氏症候群；8 位爲雷諾氏症；5 位爲原發性膽汁性肝硬化；4 位爲傳染性紅斑；另外 22 位正常控制組。72 位紅斑性狼瘡中有 7 位及 4 位傳染性紅斑病人有 3 位偵測到有人類微小病毒 B19 DNA，而在其它系統性風濕性疾病無此發現。17 位有 B19 DNA 的病人，血清內只含一種抗 B19 的 IgG 抗體及二種 IgM 抗體，另外 55 位未被 B19 感染的紅斑性狼瘡病人有 27 位測得抗 B19 的 IgG 抗體及 21 位有抗 B19 的 IgM 抗體。所有傳染性紅斑病人皆有抗 B19 的 IgG 及 IgM 抗體。由統計資料可知紅斑性狼瘡病人沒產生抗 B19 抗體含有 B19 DNA 的機率比產生抗 B19 抗體者高（$P<0.05$）。紅斑性狼瘡病人被 B19 感染而沒產生抗 B19 抗體乃因爲疫系統發育不全或服用免疫抑制藥物。臨床上發現紅斑性狼瘡病人有 B19 DNA 存在。低補體症者被感染 B19 的病毒血症的盛行率比未感染者高。

Abstract

Sera from 72 patients with systemic lupus erythematosus (SLE), 23 patients with rheumatoid arthritis (RA), 18 patients with Sjogren's syndrome (SS), eight patients with Raynaud's phenomenon (RP), five patients with primary biliary cirrhosis (PBC), five patients with polymyositis (PM), four patients with erythema infectiosum (EI), and 22 normal controls were examined for parvovirus B19 (B19) infection by serological assays, nested PCR, and Southern blotting. Parvovirus B19 DNA was detected in 17 of 72 patients with SLE, and in three of four patients with EI, but not detected in patients with other systemic

rheumatic diseases. Of the 17 patients with B19 DNA, only one had IgG anti-B19 antibody and two with IgM anti-B19 antibodies, whereas IgG and IgM anti-B19 antibodies were detected in 27 (49.1%) and 21 (38.2%) of 55 SLE patients without B19 DNA, respectively. All sera from the patients with EI contained both IgG and IgM anti-B19 antibodies. B19 DNA was found more common in sera from SLE patients without anti-B19 antibodies than these with anti-B19 antibodies ($p<0.05$). The infection of B19 in patients with SLE may be due to lack of anti-B19 antibodies because of the immunocompromised host or immunosuppressive drugs. The clinical significance of the presence of B19 DNA in patients with SLE was studied. There was a higher prevalence of hypocomplementemia and Raynaud's phenomenon in patients with parvovirus

KEY WORDS: human parvovirus B19, systemic lupus erythematosus, nested PCR, hypocomplementemia,

二.緣由與目的:

Human parvovirus B19 (B19) was discovered in England in 1975 and has been associated with a variety of clinical manifestations including rash, thrombocytopenia, leukopenia, fetal wastage, hypocomplementemia, autoimmune hemolytic anemia, arthritis and vasculitis. It is the causative agent of erythema infectiosum (EI). B19 is a small, nonenveloped virus containing a single-stranded DNA of 5,600 nucleotides and composed of two capsid proteins, VP1 (781 amino acids [aa]) and VP2 (554 aa), and a nonstructural protein, NS1. B19 infection is found worldwide in persons of any age. Most of the population becomes infected at some points, with up to 15% developing infection between 1 and 5 years of age, 15%~60% from age 5 to 19 years, and 30% to 60% in adult. The detection of anti-VP1 and anti-VP2 antibodies is the basis for the diagnosis of acute or past B19 virus infections. The dominant humoral immune response during early convalescence is to VP2 and during late convalescence to the VP1. Anti-VP1 and anti-VP2 antibodies play a major role in limiting B19 infection in man. The association of B19 infection and autoimmune diseases has been suggested, although the exact relationship between this viral infection and these disorders is not understood. Recently it was suggested that B19 may exacerbate or even induce SLE. There are striking analogies between the clinical features and hematologic findings of SLE and those of B19 infection. It was the aim of this study to investigate the role and the clinical significance of B19 infection in patients with SLE.

三.結果:

Anti-parvovirus B19 IgG and IgM were only detected in one (5.9%) and two (11.8%) of 17 serum samples from SLE patients with B19 DNA, respectively, whereas IgG and IgM anti-parvovirus B19 were detected in 27 (49.1%) and 21 (38.2%) of 55 serum samples from SLE patients without B19 DNA, respectively. Parvovirus B19 DNA was found more common in SLE patients without anti-B19 IgG and IgM than these with anti-B19 IgG and IgM antibodies ($p<0.05$). The relationship between the

presence of B19 DNA and clinical manifestations of SLE was studied. As shown in Table 1, the prevalence of hypocomplementemia and Raynaud's phenomenon was higher in patients with B19 viremia. No correlation could be found between the presence of B19 DNA and other clinical manifesations of SLE.

四.討論.

The relationship of parvovirus B19 infection and SLE is an issue of interest. There are striking similarities between B19 infection and SLE. It is difficult to differentiate B19 infection from SLE presentation clinically. The occurrence of parvovirus B19 infection in adults has been documented in some patients with systemic autoimmune diseases. Parvovirus B19 may accompany with a transient subclinical state of autoimmunity and may mimic or exacerbate SLE. It may be implicated in the development of SLE as well as other chronic arthropathies. In this study, the presence of IgG and IgM anti-B19 antibody in sera from SLE patients with B19 DNA were much lower than these without B19 DNA ($p<0.05$). Kurtzman et al demonstrated that the production of the antibody to capsid protein played a major role in limiting parvovirus infection in man. It has also been reported that in the immunocompetent host, production of B19-specific antibodies results in clearing of the viraemia within a few days, whereas in immunocompromised patients extended viral persistence. The appearance of B19 specific antibodies might alter the course of viral infection leading to the establishment of persistently active infection. The persistent infection of parvovirus B19 in our patients with SLE may be due to lack of antibodies against parvovirus B19 because of the immunocompromised host or the use of immunosuppressive agents.

Since B19 DNA was only detected in patients with SLE and EI, their clinical significance was further studied. Hypo-complementemia and Raynaud's phenomenon were significantly found to be more common in patients with B19 viraemia than these without B19 DNA. Parvovirus B19 infection may have exacerbated the clinical course of SLE. However, there was no apparent association between the presence of B19 DNA and with other clinical manifestations such as skin rash, arthritis, and proteinuria in patients with SLE. Recently, cytomegalovirus infection has been found to be a highly significant risk factor for Raynaud's phenomenon. Viral infection may inprint the course of SLE leading to specific clinical subsets. These preliminary findings require more confirmations to elucidate the significance of the presence of B19 DNA in SLE.

五.計畫結果自評:

研究內容與原計畫相符程度爲 100%, 達成預期目標約 80%。目前之研究成果已被接受刊登於 Rheumatology (2000)雜誌, 因此我們之方向及目標是正確的，同時也給予我們信心繼續完成計畫並作更深入之研究。

六.參考文獻

1. Cossart YE, Field AM, Cant B, Widdows D.Parvovirus like particles in human sera. Lancet 1975; 1:72-3.

2. Naides SJ, Scharosch LL, Foto F, Howard EJ. Rheumatologic manifestations of human parvovirus B19

infection in adult: initial two-year clinical experience. Arthritis Rheum 1990; 33:1297-1309.

3. Takahashi Y, Murai C, Munakata ST, Ishii T, Ishii K, Saiyoh T, Sawai T, *et al.* Human parvovirus B19 as a causative agent for rheumatoid arthritis. Proc. Natl. Acad. Sci. USA. 1998; 95:8227-32.

4.Nesher G, Osborn TG, Moore TL. Parvovirus infection mimicking systemic lupus erythematosus. Semin Arthritis Rheum 1995; 24:297-303.

5. Shade RO, Blundell MC, Cotmore SF, Tattersall P, Astell CR. Nucleotide sequence and genome organization of human parvovirus B19 isolated from the serum of a child during aplastic crisis. J Virol 1986; 58:921-36.

6. Thurn J. Human parvovirus B19-historical and clinical review. Rev Inf Dis 1988;10:1005-11.

7. Yoshimoto K, Rosenfeld S, Frickhofen N, Kennedy D, Kajigaya S, and Young NS. A second neutralizing epitope of B19 parvovirus implicates the spike region in the immune response. J Virol 1991; 65:7056-7060.

8. Lin KH, You SL, Chen CJ, Wang CF, Yang CS, Yamazaki S. Seroepidemiology of human parvovirus B19 in Taiwan. J Med Virol. 1999 Feb;57(2):169-73.

9. Morre TL, Bandlamudi R, Alam SM, Nesher G. Parvovirus infection mimicking systemic lupus erythematosus in a pediatric population. Semin Arthritis Rheum 1999; 28:314-18.

10. Trapani S, Ermini M, Falcini F. Human parvovirus B19 infection: its relationship with systemic lupus erythematosus. Semin Arthritis Rheum 1999; 28:319-25.

11. Cope AP, Jones A, Brozovic M, Shafi MS, Maini RN. Possible induction of systemic lupus erythematosus by human parvovirus. Ann Rheum Dis 1992; 51:803-4.

12. Kalish RA, Knopf AN, William Gary G, Canoso JJ. Lupus-like presentation of human parvovirus B19 infection. J Rheum 1992; 19:169-71.

13. Cope AP, Jones A, Brozovic M, Shafi MS, Maini RN. Possible induction of systemic lupus erythematosus by human parvovirus. Ann Rheum Dis 1992; 51:803-4.

14. Chassagne PL. Mejjad O. Gourmelen O. Moore N. Le Loet X. Deshayes P. Exacerbation of systemic lupus erythematosus during human parvovirus B19 infection. Br J Rheum 1993; 32:158-59.

15. Fawaz-Estrup F. Human parvovirus infection:rheumatic manifesations, angioedema

C1 esterasis inhibitor defeciency, ANA positively and possible onsets of systemic lupus erythematosus. J Rheumatol 1996;23:1180-5.

16. Tolfvenstam T, Ruden U, and Broliden K. Evaluation of serological assays for identification of parvovirus B19 immunoglobuin M. Clin Diagn Lab Immunol 1996;3:147-50.

17. Rosenfeld SJ, Yoshimoto K, Kajigaya S, Anderson S, Young NS, Field A, Warrener P, *et al.* Unique region of the minor capsid protein of human parvovirus B19 is exposed on the virion surface. J Clin Invest 1992; 89:2023-29.

18. Bansal GP, Hatfield JA, Dunn FE, Kramer

AA, Brady F, Riggin CH, Collett MS, *et al.* Candidate recombinant vaccine for human B19 parvovirus. J Infect Dis 1993; 167:1034-44.

19.Kawase M, Momoeda M, Young NS, Kajigaya S. Most of the VP1 unique region of B19 parvovirus is on the capsid surface. Virol 1995; 211:359-66.

20. Kurtzman GJ, Cohen BJ, Field AM, Oseas R, Blaese M, Young NS. Immune response to B19 parvovirus and an antibody defect in persistent viral infection. J Clin Invest 1989; 84:1114-23.

21. Stratta P, Canavese C, Ciccone G, Stanti S, Quaglia M, Ghisetti V, Marchiaro G, *et al.* Correlation between cytomegalovirus infection and Raynaud's phenomenon in lupus nephritis.

Nephron 1999;82:145-54.

Table I. Clinical manifesations of systemic lupus erythematosus patients with and those without B19 DNA by nested PCR.

Clinical feature	with B19 DNA (n=17)	without B19 DNA (n=55)	P value
Fever	3 (17.6)	4 (7.3)	
Rash	11 (64.7)	32 (58.2)	
Arthritis	12 (70.6)	38 (69.1)	
Serositis	3 (17.6)	3 (5.5)	
CNS	1 (5.9)	1 (1.8)	
RP	4 (23.5)	3 (5.5)	
High ESR	3 (17.6)	15 (27.3)	
Cytopenia	8 (47.1)	23 (41.8)	
ANA	13 (76.5)	37 (67.3)	
Anti-dsDNA	6 (35.3)	14 (25.5)	
ENA	8 (47.1)	19 (34.5)	
Low C3/C4	8 (47.1)	9 (16.4)	P< 0.01
High AST/ALT	0 (0)	3 (5.5)	
Proteinuria	6 (35.3)	18 (32.7)	
aCL	2 (11.8)	4 (7.3)	