行政院國家科學委員會補助專題研究計畫成果報告

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計畫主持人：蔡嘉哲 教授

本成果報告包括以下應繳交之附件：

- □赴國外出差或研習心得報告一份
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- □出席國際學術會議心得報告及發表之論文各一份
- □國際合作研究計畫國外研究報告書一份

執行單位：中山醫學大學免疫學研究所

中 華 民 國 90 年 10 月 15 日

行政院國家科學委員會專題研究計畫成果報告

Parvovirus B19 感染引起的體液性免疫反應之研究

The study of humoral immunity in Parvovirus B19 infection

計畫編號:NSC89-2314-B040-022

執行期限: 89 年 8 月 1 日 至 90 年 7 月 31 日

主持人: 蔡嘉哲 中山醫學院免疫學研究所

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一. 中文摘要:

人類微小病毒 B19 在全身性紅斑性狼瘡和肝炎病人中發現。為了解這些病人感染情形及感染 B19 基因序列差異,因此進行本研究,結果發現 VP1 unique region 之變化可分為九型,命名為 TW-1 至 TW-9,其中全身性紅斑性狼瘡病人以第五及第一型為主,而 B 型肝炎病人以第三型為主, C 型肝炎病人以第九型為主。進一步利用 Phylogenetic tree 分析台灣 B19 病毒株和韓國 B19 病毒株為相同 strain 所演化而來的。

關鍵字: 人類微小病毒 B19, 全身性紅斑性狼瘡, B 型肝炎, C 型肝炎

二. 英文摘要:

It has been previously reported that parvovirus B19 can be detected in sera of patients with SLE and hepatitis. In order to assess whether differences in the parvovirus B19 genome can be found in these diseases, we have partially sequenced the genomes of parvovirus B19 derived from serum samples from patients with erythema infectiosum (EI), systemic lupus erythematosus (SLE), chronic hepatitis B virus (HBV), and hepatitis C virus (HCV) infection. Sera were examined for parvovirus B19 infection by nested PCR, southern blotting and direct nucleotide sequencing. The region of the genome between nucleotide (nt) 2429 and 2751 that includes parts of the coding regions of NS1 and VP1 unique region were amplified by nested PCR and sequenced. Fifty-three parvovirus B19 isolates derived from four patients with EI, 17 patients with SLE, 20 patients with HBV and 12 patients with HCV infection were partially sequenced for the genotypes. The nucleotide sequence showed that there were 12 nucleotide changes and 7 amino acid substitutions in comparison with Au strain employed as a standard strain. They were classified into nine types named from TW-1 to TW-9 according to the nucleotide variations. The nucleotide variability of all samples ranged from 0.9% to 2.2 % and the amino acid variability ranged from 0.9% to 2.8%. Of the 17 serum samples with parvovirus B19 DNA from patients with SLE, six were TW-5 and five were TW-1. Of the 20 serum samples with parvovirus B19 DNA from patients with chronic HBV infection, 19 were TW-3. Of the 12 serum samples with parvovirus B19 DNA from patients with chronic HCV infection, all were TW-9 genotype. The results of the phylogenetic tree analysis indicated that the common epidemiological origins of the viruses were from Korea and Taiwan.

Keywords: human parvovirus B19, systemic lupus erythematosus, hepatitis B virus, hepatitis C virus, direct nucleotide sequencing

三. 計畫緣由及目的:

Human parvovirus B19 (B19) has been associated with a variety of clinical

manifestations including rash, thrombocytopenia, leukopenia, fetal wastage, hypocomplementemia, autoimmune hemolytic anemia, arthritis, hepatitis, and vasculitis [1-8]. It is also the causative agent of erythema infectiosum. B19 is a small, nonenveloped virus containing a single-stranded DNA of 5,600 nucleotides and composed of two capsid proteins, VP1 {554 amino acids(aa)} and VP2 (781 aa), and a nonstructural protein, NS1[9-11]. The two capsid proteins have the same open reading frame and VP1 is identical to VP2 except for an additional 227 amino acids at the NH2-terminus. These additional amino acids are referred as the VP1 unique region [9,12]. Over 95% of capsid proteins are VP2, while VP1 accounts for less than 5%. These B19 structural proteins are known to determine the virus tropism and elicit neutralizing antibody responses [13-14]. Most of the VP1 unique region is on the capsid surface, which contains multiple linear epitopes [15], genetic heterogeneity [16-17] and is thought to be important in the immune response for neutralizing B19 [18-21]. The non-structural protein NS1 is known as a trans-activator protein for the viral DNA replication [22]. NS1 may play some roles in cytotoxicity related to apoptosis [23-24].

Chronic hepatitis B virus (HBV) and HCV virus (HCV) infection occur worldwide. They can cause acute or chronic hepatitis, cirrhosis of the liver and hepatocellular carcinoma. It has been suggested that chronic HBV and HCV infection may act as a trigger mechanism for the development of autoimmune rheumatic diseases. Taiwan is a hyper-endemic area for chronic HBV and HCV infection. The hepatitis B surface antigen (HBsAg) carrier rate in its general population is about 10% to 20% [25], and anti-HCV seroprevalence is about 1.6% to 19.6% [26].

B19 infection has been found to be the cause of acute hepatitis [5]. Although distinct genetic types have been described [27-30], the significance of these differences to the clinical features of B19 has yet to be determined. The aim of this study was to investigate the incidence and genotypes of B19 infection in patients with SLE and chronic hepatitis with HBV and HCV infection.

四. 結果與討論:

We analyzed the presence of B19 DNA in serum of patients with erythema infectiosum (EI), SLE, HBV and HCV infection. B19 DNA was detected in all four patients with erythema infectiosum (EI), 17 of 72 patients with SLE, 20 of 54 serum samples from patients with HBV infection, and 12 of 51 patients with HCV infection by nested PCR and confirmed by southern blotting. Blood samples taken from the same patients were also studied for B19 antibodies. B19 IgM and IgG antibodies were detected in all four (100%) serum samples from patients with EI; 9 (12.5%) and 63 (87.5%) of 72 serum samples from patients with SLE; 1 (1.9 %) and 54 (100 %) of 54 serum samples from patients with HBV infection; and 1 (2%) and 36 (70.6 %) of 51 serum samples from patients with HCV infection, respectively. Fifty-three parvovirus B19 isolates derived from four patients with EI, 17 SLE, 20 HBV, and 12 HCV infection were partially sequenced and the nucleotide sequences were compared with Au strain which is used as a standard strain.[9] These sequences were classified into nine types named from TW-1 to TW-9 according to the nucleotide variations. There were 12 nucleotide changes and 7 amino acid substitutions in

the regions between nt 2429 and 2751. The nucleotide variability of all samples ranged from 0.9 to 2.2% and the amino acid variability ranged from 0.9% to 2.8%. For serum samples collected from EI patients, one patient's genotype remained unchanged, TW1, and the other changed from TW-2 to TW-1. Of the 17 serum samples from patients with SLE, six were TW-5, five were TW-1, two were TW-3, one was for TW-2, TW-4, TW-6, and TW-7. TW-1 and TW-5 genotypes were the most common types in SLE patients. Of the 20 serum samples from patients with HBV infection, 19 were TW-3 and only one was TW-8. Of the 12 serum samples from patients with HCV infection, all samples were TW-9. TW-1 and TW-5, TW-3 and TW-9 were the dominant genotypes of B19 in patients with SLE, HBV, and HCV infection. To further assess the genetic relationship among B19 virus strains, the phylogenetic tree analysis was conducted based on nucleotide sequences. The phylogenetic tree were resolved by the software, MEGA® based on Kimura-2 parameter and neighbor-joining methods. On the basis of the derived topology, the 35 B19 isolates from the worldwide could be diverged into three clusters. A distance of 0.005 means a genetic distance or divergence of 5 nucleotide substitutions per 1000 nucleotide sites. Nine TW isolates were closely related with one of the clusters (i.e. USA2, KOR2, USA5, VEN1, KOR1), and far away from the other two clusters (i.e. Au, USA1, USA4, USA6 to 15, BRZ1, IRE1, and UK1, USA3, Wi, JAP1, CHI1/Xian, and CHI2/Xian) (sequences available in GeneBank) [9,16]. This finding showed that TW strains were different from other B19 isolates. Kerr *et al.* suggested VP1 unique region might be appropriate for studying genome variability and clinical correlation [27]. In our study, we found that B19 DNA was detected in four patients with EI, 17 of 72 patients with SLE, 20 of 54 serum samples from patients with chronic HBV infection and 12 of 51 patients with chronic HCV infection. This is not in agreement with the findings of Cacoub *et al* that none of 36 patients with HCV infection had B19 DNA [28]. Erdman *et al* reported that no particular B19 genotype was associated with a common clinical presentation [16]. The discrepancy can be explained by patients selection bias. All of our samples were selected to the patients with specific diseases such as EI, SLE, HBV and HCV infection. The report of Erdman *et al* mostly included patients with various clinical manifestations of human parvovirus B19 infection. Our data indicated that distinct genotypes of human parvovirus B19 were associated with specific diseases. However, the underlying mechanism for it is still obscure. It is possible that point mutations in a viral coding region might alter virus epitopes and host-cell tropism, thereby causing different immune responses.

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