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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慢性B型肝炎之新模式：以腺相關病毒為載體轉染B型肝炎病毒於細胞或小鼠

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計畫類別：個別型計畫

計畫編號：NSC 89－2320－B－002－218－

執行期間：89 年 08 月 01 日至 90 年 07 月 31 日

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本成果報告包括以下應繳交之附件：（證書，較之編目，如最後一頁說明）

- □赴國外出差或研習心得報告一份
- □赴大陸地區出差或研習心得報告一份
- □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：國立台灣大學醫學院臨床醫學研究所

中　華　民　國　90 年　10 月　30 日

一、中文摘要

B 型肝炎病毒是引發慢性或急性肝炎的主要病原之一，初步估計全球至少有三億人為 B 型肝炎帶原者，其中每年約有一百萬人死於 B 型肝炎相關疾病。B 型肝炎病毒是一個不會直接引發感染細胞死亡的病毒，其引發肝病的主要原因在於宿主體內的免疫系統會攻擊受感染的細胞，卻無法有效的清除掉病毒，造成肝臟慢性的發炎，多次但無效的免疫反應在肝臟發生，使肝臟產生無法回復的病變，如肝硬化，甚至使肝癌產生的機率增高。

目前沒有一個好的小鼠動物模式用於研究 B 型肝炎病毒所引發的免疫反應與致病機制間的關係。雖然 B 型肝炎病毒基因轉殖鼠被用於研究該病毒之致病機制已有一段時間，這些研究為 B 型肝炎的研究找出一些新的方向，例如 cytokine 抑制病毒的機制等。但基因轉殖鼠幼年即接觸到 B 型肝炎抗原，其對 B 型肝炎病毒的免疫反應並不正常，對 B 型肝炎病毒無法產生類似人類慢性肝炎時的免疫反應，所以不適合用於研究免疫反應所造成的 B 型肝炎病變。故我們採用以成鼠感染 B 型肝炎病毒，追蹤其病毒表現情形、在老鼠肝臟所造成的病變及造成病變的因子。

由於老鼠無法自然感染 B 型肝炎病毒，故我們採用線相關病毒包裹 B 型肝炎病毒的基因體後，以此病毒感染老鼠，可成功於老鼠體內偵測到病毒抗原的表現，

二、英文摘要

The hepatitis B virus is a noncytopathic, enveloped virus with a circular, double-stranded DNA genome that cause acute and chronic necroinflammatory liver disease and hepatocellular carcinoma. HBV infection acquired in adult life is usually clinically inapparent and the majority of infected adults recover completely from the disease and clear the virus. Neonatally transmitted HBV infection, however, is rarely cleared, and over 90% of such children become chronically infected. While a highly effective preventative vaccine is now available, this is of no assistance for the estimated 300 million people who are already infected worldwide. There are at least one million infected people die annually of HBV-induced liver diseases. It has been difficult to examine the immunological mechanisms involved in HBV pathogenesis because of the lack of an inbred mouse model with well-defined immune system. Although the HBV transgenic mouse models have been developed for long periods, it is not appropriate to use transgenic mice as HBV infection models because most of these mice show immunological tolerance rather than chronic liver diseases. HBV infection is species-specific and it is impossible to infect mouse by direct inoculation with HBV particles. We tried to seek a more rational experimental system as HBV-infected animal model to elucidate the immunological mechanisms.

三、計劃緣由及目的

The hepatitis B virus is a noncytopathic, enveloped virus with a circular, double-stranded DNA genome that cause acute and chronic necroinflammatory liver disease and hepatocellular carcinoma. HBV infection acquired in adult life is usually clinically inapparent and the majority of infected adults recover completely from the disease and clear the virus. Neonatally transmitted HBV infection, however, is rarely cleared, and over 90% of such children become chronically infected. While a highly effective preventative vaccine is now available, this is of no assistance for the estimated 300 million people who are already infected worldwide. There are at least one million infected people die annually of HBV-induced liver diseases.

It is generally thought that HBV is not directly cytopathic for the hepatocyte and the associated liver diseases are cause by immune response to viral antigen expressed by infected hepatocyte. It has been difficult to examine the immunological mechanisms involved in HBV pathogenesis because of

the lack of an inbred animal model with well-defined immune system. Although the HBV transgenic mouse models have been developed for long periods, it is not appropriate to use transgenic mice as HBV infection models because most of these mice show immunological tolerance rather than chronic liver diseases. Moreover, HBV infection is species-specific and it is impossible to infect mouse by direct inoculation with HBV particles. We tried to seek a more rational experimental system as HBV-infected animal model to elucidate the immunological mechanisms involved in HBV pathogenesis and even to develop an effective immunotherapy to chronic HBV infection.

Adeno-associated virus (AAV) is a defective member of parvovirus family. AAV can be propagated as a lytic virus or maintained as a provirus integrated into the host cell genome. The development of gene transfer vector from AAV has provided an efficient and effective way of delivering genes into mammalian cells, both in vitro and in vivo. AAV has several unique properties which distinguish it from other viral vectors. Its advantages include stable and efficient integration of recombinant viral DNA into the host genome, lack of any associated human disease, a broad host range, the ability to infect nondividing cells and the ability to carry nonviral regulatory sequences without interference from the viral genome. In addition, there is no evidence so far that AAV vectors may elicit immune response in vivo. We have cloned the whole HBV genome into AAV vector and produced recombinant HBV-AAV by tranfecting the above vector plus two other helper plasmids. The recombinant virus infected cells or mice could produce HBsAg and HbeAg in vitro and in vivo respectively. We also could detect HBV DNA in the serum of recombinant virus infected mice. We will do the immunohistochemical examination to detect the expression of HBeAg and HBcAg in the livers of HBV infected mice and detect the HBV specific antibodies production in the serum of the infected mice by ELISA. These HBV-infected mice will also be monitored for the progress of their liver diseases if any symptoms appear in them.

四、結果與討論

This conctruction and virus preparation could generate recombinant viruses that have AAV capsid but HBV genome. The viruses could infect wild range of host and could not produce AAV particles after infection. The HBV particles could be produced after HBV-AAV infection in mice(figure1, 4). The recombinant HBV-AAV could infect HuH-7 cells and induce high level of HBsAg and HBeAg expression in vitro(figure2a, 2b). The recombinant HBV-AAV could infect mice and induce high level of HBsAg production in the serum of these mice. The peak of HBsAg expression was at day 35 after infection(figure 3). Only intrahepatic injection of recombinant HBV-AAV into mice could induce high-level expression of HBsAg in the serum of the mice. We could not detect HBsAg in the serum of i.v. injected B6 mice(figure3) but could detect low HBsAg in the of the i.v. injected Balb/c mice(data not shown). There was detectable HBV DNA in the serum of infected mice at day 40 after injection(both in the groups of i.v.and intrahepatic injection of HBV-AAV). This meant that there were some HBV viral particles produced after injection but we could not rule out this was the amplication of injected HBV-AAV genome. More precise PCR primers sets should be design to distinguish those two viral genomes. There were more HBsAg produced in the serum of mice after intrahepatic injection of HBV-AAV than iv. injection. The possible explanation was that HBV-AAV would be trapped in other organs after i.v. injection.

五、參考文獻

1. Xiao,X., Li,J. and Samulski,R.J. (1998) Production of High-Titer Recombinant Adeno-Associated Virus Vectors in the Absence of Helper Adenovirus. J. Virol.

72(3):2224-2232

2. Xiao,X., Li,J., McCown,T.J. and Samulski,R.J. (1997). Gene Transfer by Adeno-associated Virus Vectors into Central Nervous System. Exp. Neurol. 144:113-124

3. Patijn,G.A. and Kay,M.A. (1999) Hepatic gene therapy using adeno-associated virus vectors. Seminar in Liver Disease. 19(1):61-69

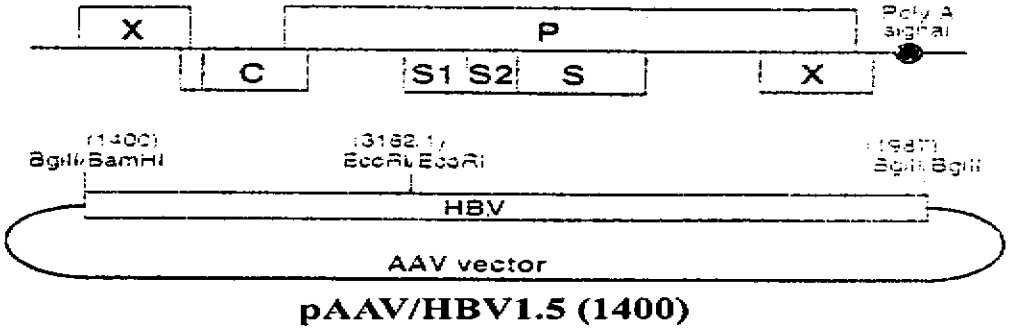
4. Guidotti,L.G., Matzke,B., Schaller,H. and Chisari,F.V. (1995) High Level Hepatitis B Virus Replication in Transgenic Mice. J. Virol. 69(10):6158-6169

5. Milich,D.R., Jones,J.E., Hughes,J.L., Price,J., Raney,A.K. and McLachlan,A. (1990) Is a function of the secreted hepatitis B e antigen to induce immunologic tolerance in utero? Proc. Natl. Acad. Sci. 87:6599-6603

6. Guidotti,L.G., Ishikawa,T., Hobbs,M.V., Matzke,B., Schreiber,R. and Chisari,F.V. (1996) Intracellular Inactivation of the Hepatitis B virus by Cytotoxic T Lymphocytes. Immunity. 4:25-36

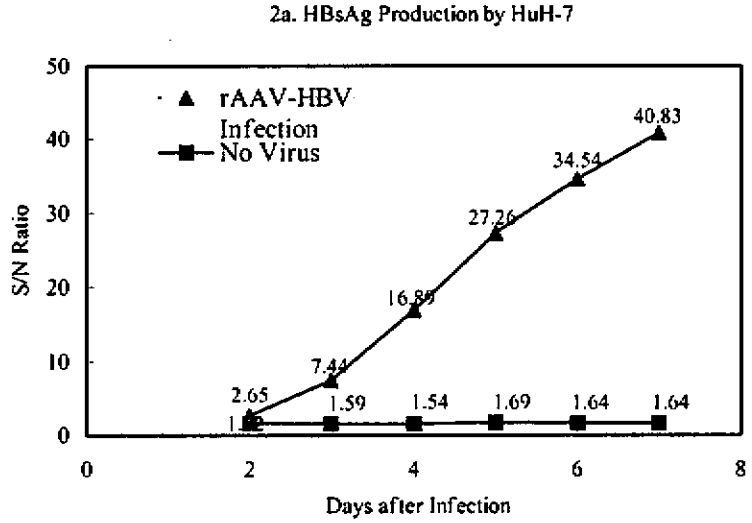
六、圖表

Figure 1 Construction of recombinant HBV-AAV



pAAV/HBV1.5 (1400)

Figure 2 HBsAg and HBeAg expression by HuH-7



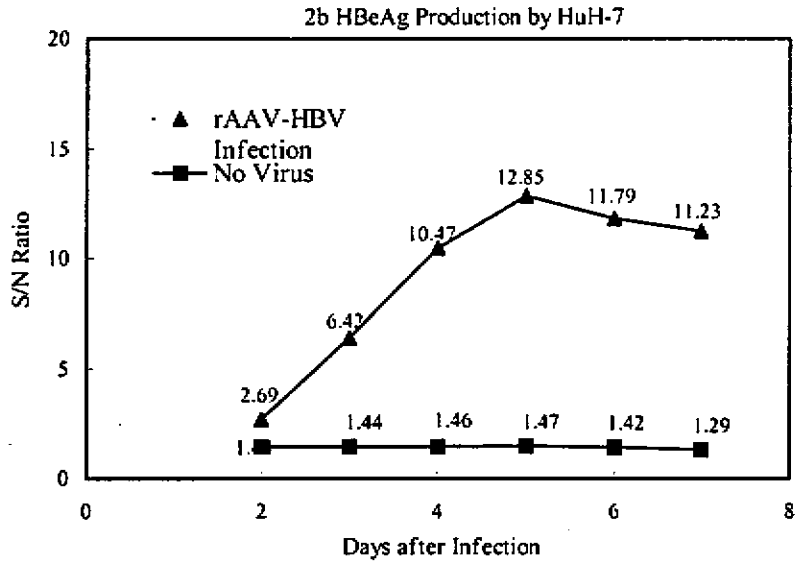


Figure 3 HBsAg expression in the serum of HBV-AAV -infected mice

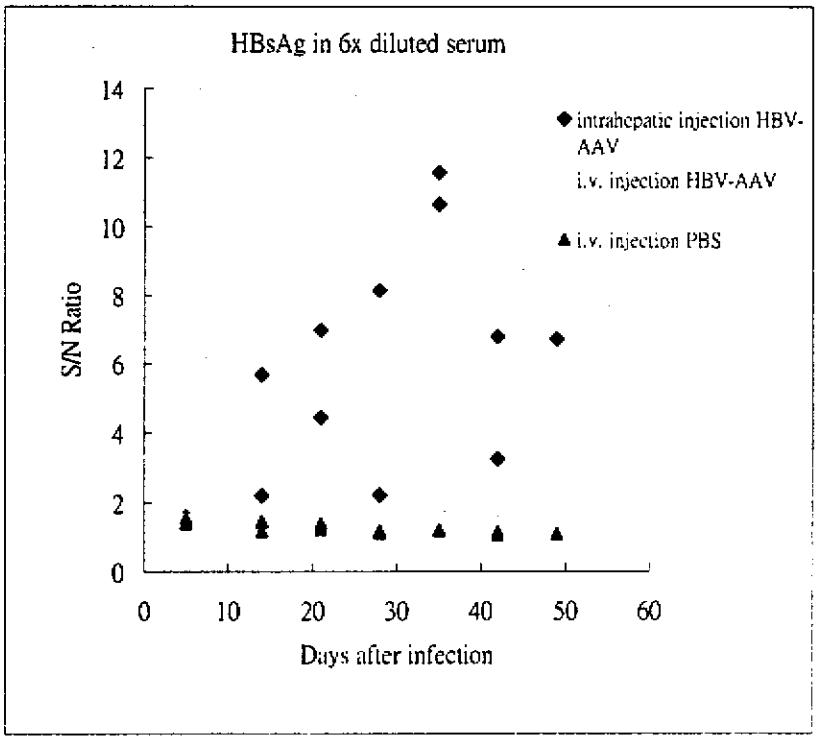
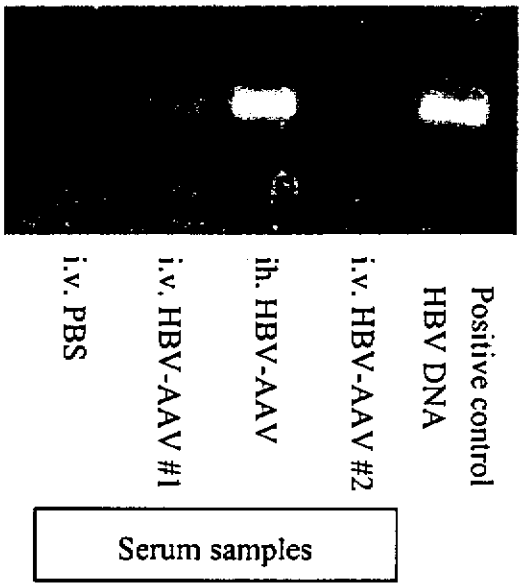


Figure 4 HBV DNA expression in the serum of HBV-AAV infected mice



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計畫編號 NSC89-2320-B002-218

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90年11月9日