行政院國家科學委員會專題研究計畫成果報告

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應用 S1 表現蛋白偵測雞傳染性支氣管炎病毒抗體

(Using different fragments of the expressed S1 protein as antigens to detect infectious bronchitis viral antibody)

一、中文摘要

依照已定序完成之台灣雞傳染性支氣管炎病毒分離株 A1171 的 S1 醣蛋白基因序列設計引子 IBVc1-IBVc2, 利用此引子進行反轉錄聚合酶連鎖反應，增幅出 539bp 的 PCR 產物, 其序列位置包括不具病毒中和能力的抗原決定位 S1-F、S1 和 S2 之間的裂解訊號及一小部份 S2 的 N 端。將 PCR 產物與 pRSET A,B,C 載體進行接合作用後，轉化至大腸桿菌勝任細胞內，再利用 IPTG 在原核系統中誘導台灣雞傳染性支氣管炎病毒分離株 S1 醣蛋白基因的表現。表現得到的 S1-F 融合蛋白質分子量約 27.5 kD，可與兔子抗 IBV 的多源抗血清反應。將 S1-F 融合蛋白當作酵素連結免疫吸附法的抗原，以 I-ELISA 的方法測定血清抗體，証實 S1 基因表現蛋白可有效測定不同雞傳染性支氣管炎病毒分離株抗血清的抗體。

關鍵詞：雞傳染性支氣管炎、S1 基因、酵素連結免疫吸附法

Abstract

Enzyme-linked immnunosorbent assay (ELISA) using an expression S protein fragment as an antigen was developed for detection of antibodies against IBV. The primers (IBVc1-IBVc2) were designed according to the published entire S1 nucleotide sequence of Taiwan IBV isolate Tw/1171/92. The F frgament of S1 gene was amplified using primers IBVc1-IBVc2, cloned into the pRSET vectors and transformed into competent *E. coli* BL21 (DE3). A 27.5 kD S1-F fusion protein was successfully expressed, affinity-purified and used as a coated antigen for serum antibody detection by ELISA. Although there was little cross-reactivity in the serum neutralization (SN) test, cross-reactivity was observed in this ELISA system among different IBV strains, H120, Taiwan Group I and Taiwan Group II. The ELISA technique using expression S1-F protein is able to detect antibodies from different IBV serotypes and appears to be suitable for large-scale serological surveys.

Keywords: ELISA, expression S1 protein, infectious bronchitis

Introduction

Infectious bronchitis viruses (IBV) infect chickens of all ages causing drops in egg production, retardation in growth, and heavy mortality. In spite of widespread use of vaccines, outbreaks caused by IBV occur frequently and many strains of IBV have been isolated in different countries [3]. IBV contains three structural proteins, spike glycoproteins, membrane glycoprotein and nucleocapsid protein. The spike glycoprotein is post-translationally cleaved into S1 and S2 sub-units [2]. S1 glycoprotein has been demonstrated to be correlated to the viral infectivity and able to induce virus neutralization [2, 7]. Location and function of epitopes on spike protein had been reported [6, 7, 10, 11]. There are eight antigenic sites on S protein, six on S1 (overlapped S1-A/B/C, S1-D, S1-E, and S1-F) and two on S2 (S2-G and S2-H). S1-A/B/C, S1-E, and S2-G are able to induce SN antibody but not S1-F [8].

Among the various serological tests for IBV, the enzyme-linked immunosorbent assay (ELISA) is the most sensitive and popular serological technique for flock monitoring. Although it lacks serotype specificity [12, 13], it is convenient for evaluating vaccination response. Using commercial kits for antibody detection by

ELISA is a routine work in Taiwan. More than 92,396 blood samples were tested for IBV antibody by ELISA in the whole year of 1998 [1]. The ELISA kits were commercially available and coated with the whole virus. It detects antibody against S protein as well as other proteins of IBV. Spike glycoprotein is responsible for viral infectivity, virus-neutralizing or haemaglutination-inhibiting antibody induction, membrane fusion and cell attachment [6, 9, 10]. Since S protein relating to viral infectivity, using it as a coated antigen for antibody detection is more close to protection and much easier than the whole virus in ELISA. In the present study, an expression S protein fragment was used as a coated antigen for IBV antibody detection in ELISA. This protein could catch the antibodies from different IBV serotypes.

Results

The of SN titers in specific antisera against homologous and heterologous IBV strains are shown in Table 1. As expected homologous titers were higher than heterologous, especial between different serotypes, e.g. between Tw/1171/92 and H120 or Tw/2296/95. Conjugate diluted 1:1000 or less produced non-specific reactivity, and 1:4000 dilution or more obtained P/N ratios about 1. Appropriate antigen concentration used in indirect ELISA was determined by using chicken polyclonal antisera diluted 1:100 and conjugate diluted 1:2500. High OD values were seen by increasing the protein content of the antigen used to coat the wells. Antigen concentration of the range between 500 ng to 125 ng detected greatest P/N ratio (positive/negative ratio). As little as 125 ng/well of S1-F fusion protein consistently detected IBV antisera in indirect ELISA, with lower background and higher antibody titers. The antigen concentration was adjusted at 125 ng/well for the following tests.

The OD values of different sera reacted to different antigen in ELISA are shown in Table 1. The S1-F protein gave less background against SPF chicken serum than H120 antigen. It was highly cross-reactive among various distinct IBV serotypes, including Mass, Taiwan Group I (Tw/1171/92) and Taiwan Group II (Tw/2296/95), similar as the results obtained by ELISA coated with the whole virus, H120 and Tw/2296/95. In addition, detection for antibody titers of field sera using S1-F fusion protein was significantly greater than those obtained by ELISA with H120 or Tw/2296/95, indicated the usefulness for monitoring vaccination response of chickens under field conditions.

Discussion

The sensitivity of the ELISA test can be altered greatly and linearly by adjusting the protein content of the antigen. Extremely high antiserum titers can be obtained by increasing the protein content of the viral antigens or expression protein used to coat the plates. Using any concentration falling in the linear range of the well should be acceptable, but the lower concentration, 125 ng/well, were chosen in order to obtain acceptable values with minimal background. Generally, the whole virus antigens were not so pure as expression antigen. One might not compare the absolute OD values even the concentration all the antigens used was 125 ng/well. However, one can compare the OD values of different antiserum with the same antigen.

The epitope involved in neutralization and hemagglutination is conformation dependent [14]. The epitope of the fragment F shows antigen-antibody reaction but neither neutralization nor hemagglutionation ability [9]. It is well known serotype of IBV is determined by neutralization test [3]. Since the S1-F has no neutralization ability, the fusion protein is highly cross-reactive among various IBV serotypes (including Massachusetts, Taiwan Group I and Taiwan Group II). As the fusion proteins produced in prokaryotes were not glycosylated, the broad antigen spectrum of S1-F fusion protein might due to the conformation-independent antigenicity and lacking of

neutralization and haemagglutination-inhibition ability of antigenic site S1-F. That the higher OD values in ELIAS using S1-F protein than those using the whole virus indicated the ideal usefulness for monitoring vaccination response of chickens under field conditions. As little as 125 ng/well of S1-F fusion protein consistently detected IBV-specific sera in indirect ELISA, with less non-specific reactivity but higher antibody titers. In addition, the similarity scores of the amino acids in the F fragment of S1 protein are mostly more than 1 [4]. This highly conserved region provides a common epitope in antibody detection for all IBV serotypes.

Conventional virus propagation and purification is relatively time-consuming and labor-intensive, while cloned F fragment of IBV S1 gene is inexpensively expressed in quantities in prokaryotic expression system with IPTG induction. Although S1-F fusion protein cannot distinguish IBV serotypes, it should be very useful as a rapid aid to evaluate the immune status of a vaccinated flock. To establish a serotype-specific ELISA system, one should clone and express the neutralization epitopes, such as S1-A, B, C , S1-D, or S1-E as a coated antigen for ELISA test.

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(因篇幅故以下略)

計畫成果自評部份

研究內容與原計畫相符合、達成預期目標、研究成果有學術及應用價值、適合在優良學術期刊發表。

(出國報告於成果報告附件繳交。)

行政院國家科學委員會補助國內專家學者出席國際學術會議報告

報告人姓名	王金和	服務機構及職稱	國立台灣大學獸醫學系教授
會議 時間	1999/07/10 ~ 1999/07/14	本會核定補助文號	NSC88-2313-B-002-075 應用S1表現蛋白偵測定傳染性支氣管炎病毒抗體
會議 地點	New Orleans, LA, USA		
會議名稱	(中文) 美國獸醫學會1999年學術研討會 (英文) 1999 Annual meeting of American Veterinary Medical Association		
發表論文題目	(中文) 以S1表現蛋白質抗原酵素聯連結免疫吸附法測定傳染性支氣管炎抗體 (英文) Using expression S1 protein as a coated antigen for IBV antibody detectio by ELISA		

報告內容應包括下列各項：

一、參加會議經過

由台灣搭乘飛機經Los Angles轉Atlanta再轉New Orleans共約24小時，頗為疲憊，經一天的休息後回復體力。報到後與來自世界各地的學者見面招呼、談話並交換研究心得，獲益良多。研討會第一天為家禽福利問題，會場有保護動物團體的代表與演講德的獸醫學者針鋒相對，會場氣氛火爆，美國對家禽福利的重視由此可見。第二天開始學術研討，分禽病及火雞疾病，本人都在禽病組聆聽，並提問題討論。本人此次準備看板論文，於規定時間內立於板邊說明。會後順道拜訪University of Georgia禽病系及美國農部東南禽病中心 (Southest Poultry Research Laboratory, Agricultural Research Service, USDA)。

二、與會心得

美國禽病獸醫師協會為美國獸醫學會的一個分會，每年在美國各地舉辦一次學術研討會，與會學者將一年來的研究成果在此會議發表，可謂禽病界的盛會。此次研討會的題目有關家禽白血病的研究口頭報告有18篇，看板論文5篇，可見本病受禽病學者的重視，此與本人的研究計畫相同，了解最新研究情形對個人從事本病的研究很有助益。本次研討會有一個大缺點，即美國禽病獸醫師協會沒有印出研討內容，僅於美國獸醫學會大會的手冊上印出題目，此事經本人向大會提出質問，但並無滿意答案，令本人頗為失望，應引以為戒。

三、考察參觀活動(無是項活動者省略)

因New Orleans離美國禽病研究的重鎮University of Georgia不遠，因此會後搭飛機至Athens，拜訪該大學的禽病系。該系系主任Dr. Kleven熱情介紹該系的教學、研究及服務，其制度很多值得我們學習，令我印象最深刻者為其Master of Avian Medicine (MAM)訓練，此為訓練臨床禽病獸醫師的計畫。在該校禽病系對面即美國農部的東南禽病研究所，該所有level 2及level 3的隔離室，設備非常完善，由所內有自己的工廠及設計工程師專門為研究人員設計特別用途的雞籠可見其財源及設備之雄厚。該所所長Dr. David E. Swayne除了詳細介紹之外，並表示與我合作研究。

四、建議

暑假期間機票費甚貴，建議暑假開會的機票費補助

五、攜回資料名稱及內容

1. Convention program, American Veterinary M
(July 10-14, 1999). 內容包括Advances in vete
prevention, aquaculture, bovine, companion a
alternative medicine, computer classroom,
professional development, poultry, public an
small ruminants, swine, veterinary technicia
2. Symposium on poultry management and pr
Pathologists. 2本
3. 1999 [illegible] 136th AVMA TM,multimedia CD-ROM.1片

六、其他

資深研究人員前去國外開會，可以在短期吸收許多
究工作。在會場所得到的知識對今後傳染性支氣
(發表之論文即為國科會專題研究報告)。