

行政院國家科學委員會補助專題研究計畫成果報告

以無性生殖單一小鼠研究其對癌細胞之反應

計畫類別： 個別型計畫 整合型計畫

計畫編號：NSC 89-2313-B-009-002

執行期間：88年8月1日至90年7月31日

計畫主持人：毛仁淡

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一、中文摘要

申請人過去20年致力於研究動脈硬化的致病機轉，其中感到興趣及較迷惑的結果是動脈硬化在血管損傷的形成總是不能一致 (heterogeneous response)。無論在具有遺傳性之 Watanabe 兔或餵食高含量的膽固醇食物之家兔中，皆有二個典型的樣本，有反應嚴重及輕微者。關於這些變化有許多假設被提出來，它可能由於遺傳因素 (genotype) 影響，但有些則認為是受飼食的影響或是其他未知機轉 (phenotype) 的改變所引起。

在我們最近的研究中使用注射 B16 細胞 (一種黑色瘤細胞) 的小鼠，這種腫瘤生長的反應也是變異的及異種的。從 3 次獨立的研究中，有 8~15% 的小鼠皆可對腫瘤產生耐受性而不產生 tumor。這是遺傳基因型(主要的)抑是環境表現型(次要的)? 雖然某些因素伴隨著異種反應並不是容易了解，使用相同的或單一的個體 (複製動物) 來複製腫瘤耐受性動物可以使我們進一步瞭解其耐受機制，相信它可提供我們機會探討這個重要的關鍵點。

依我們研究結果提出下列兩大論點，首先發展複製技術將來可在台灣地區傳授或轉移相關技術給生命科學大眾，其次，我們可以應用無性生殖動物來解答生物醫學上的關鍵問題。

關鍵詞：腫瘤、異致性反應、免疫、動物複製

Abstract

We conclude that the antitumor mice can be randomly selected from balb/c mice.

The tumor resistant mice appear to be authentic, because further challenge with tumor cells (3 times with high dose) did not induce tumors. Thus far, by crossing male and female tumor resistant mice did not reproduce the offsprings (F1) that possessed the ability to resistant tumor growth. Serum antibody shows a single specificity against B-16 cells in which the epitope was located on the cell surface. The immunoreactivity of this serum antibody appeared to be negatively correlated to the size of tumors. Biochemical analysis shows the epitope is a protein with an apparent molecular weight of 120 K. The experiment is now in progress to study the phenotypic modulation on those mice that resistant to B-16 tumor.

All the immunodeficient mice (nude) did not show the protection from the tumor growth suggesting that immunity plays an role in tumor resistant in mice. The occurrence rate of tumore resistance in normal mouse population is about 10%.

Progress has been made in animal cloning as embryos with 8-cell stage have been established following a nucleus transfer. The development of animal cloning technique is now in progress at PRIT.

Keywords: Tumor, Heterogeneous response, Immunity, Animal Cloning

二、緣由與目的

One of the interesting and puzzling events is that the formation of atherosclerotic lesions is always heterogeneous in either Watanabe rabbits or normal rabbits fed with high cholesterol diet

(1-3) . Several hypotheses have suggested that this variation could be due to the genetic effect, but others suggested this could be due to the dietary effect or by chance with some unknown mechanism(s) (4) .

Similarly, in our recent study using mice injected with B16 cells, a tumor melanoma cell line, the response to tumor growth was variable and heterogeneous. From 3 independent studies, there were always 8-15% of mice totally tolerated to the development of tumor. Is this genotypic (primary) or phenotypic (secondary)? Although the factors involved in this heterogeneous response are not readily known, using the identical or duplicated individual to increase the size of tumor-tolerated animals, we think it can provide us the opportunity to answer this important issue.

One major issue remains to be challenged initially is that whether of not the tumor- resistance mice found in our laboratory are genotypic or phenotypic. For this regard, we have inbred those mice and conducted their ability to produce B-16 tumors. We have observed that the humoral antibody activity was related, in part, for the tumor growth.

≡、 METHOD, RESULT AND DISCUSSION

METHOD

The standard established analytical procedures are performed in the present study, but are not described in details:

ELISA

SDS-PAGE

Preparation of antiserum

Immunocytochemistry

Southern and Northern blot

PCR and nucleotide sequencing

HPLC

Histology

Nucleus transfer and in vitro fertilization

(5-7)

RESULT AND DISCUSSION

Heterogeneous response in animal models:

As indicated previously, the PI of this proposal as well as the others have experienced a biological variation in cholesterol-diet induced atherosclerotic lesions(1-4). It hampers the investigation in new drug intervention, since the lesions are so heterogeneous making the data difficult to interpret and to statistical evaluation. Similarly, inoculation of B-16, a melanoma cell line, into peritoneal cavity of mice in our recent study attempting to relate the immunity and development of melanoma. The response to tumor development was also heterogeneous. There were about 8-15% of mice that totally tolerated for the development in a 150-day study. From three independent studies, the melanoma cells were evenly diffused through out in the cavity in unresponsive mice. The medium survival time (MST) of mice (n=12) for each experiment was about 25-30 days. The survivals had lived for greater than 150 days before the sacrifice. As judging from the pattern of melanoma cells in hypo-responsive mice, it is unlikely that it was from the experimental variation produced from the inoculation. This is because of the fast growth of the tumor, solid tumor can be formed even with the cell number reduced to 1/3 during the inoculation. We re-inoculated (second challenge) the B-16 cells into two hypo-responsive mice. Again, the same mice was resistant to the tumor development. Experiment using large number of mice is currently underway to further confirm this result. From this preliminary study, we plan to test the hypothesis that genotypic (or phenotypic) modulation may play a role in those mice that do not produce tumor.

B-16 melanoma cell culture and inoculation:

Preparation of B-16 in culture is relatively simple, the cell line is originally derived from the Balb/c mouse. It is now maintained in DMEM with the addition of

FCS in a quite stable condition. Interestingly, the tumor could also be formed in the peritoneal cavity of ICR mice. We think that it was not species dependent, although B-16 cells are originated from a Balb/c mouse. The procedure for inoculation is now standardized and unlikely to “miss” during the injection of the cells.

In vivo and ex vivo immunity test:

Whether the presence of hyporesponsive mice owing to the immune modulation is not known and has not been reported. One of the systems that can be used in testing the in vivo immunity. Briefly, the spleen tissue enriched with B cell is obtained from the mice to be tested. Following the homogenization, spleen cells are cultured and then inoculated (IP injection) into normal mice that are capable of developing tumors. Finally, the medium survival time in each testing group is determined. Otherwise, the size of tumor can be determined at certain period of time. This system is now established in PI's laboratory. One advantage using B-16 melanoma cell line is the color intensity of the cells, once the tumor is formed it can be easily identified. Using 96 microtiter plates, we have also developed an ex vivo approach to study the natural killer (NK) cell activity cocultured with T cell. The cytotoxicity is based on the release of lactose dehydrogenase (LDH) or the release of Cr-51 in B-16.

We further tested the tumor growth in immunodeficient mice, these mice were obtained from the medical school of Chung-Kong University. Unlike the normal population of mice (in which the occurrence rate was about 10% in tumor resistance), all of the immunodeficient mice inoculated with tumor cells exhibited malignant tumors. The medium survival time was about 10 days (n=14) as compared to that 20 days in normal mice. Thus our data suggest that immunity plays a key role, at least in part, in the tumor resistance.

In vivo humoral immunity:

To test whether or not injection of B-16 cell extract and membrane components may produce plasma antibody against B-16 cells, 6 mice were immunized with killed B-16 cell components. No serum antibody was detected using either Westernblot or ELISA. The animals were then given intraperitoneal inoculation of B-16 cells for producing solid tumor, during which time the production of serum antibody was examined. Again, there was no antibody activity detected, however, the antibody was found when whole cells were used in a solution ELISA. Using immunocytochemical (ICC) technique, the antigen epitope was found to be located on the cell membrane. On Westernblot, an antibody with a single specificity against a molecular weight 120 K was identified. Meanwhile, the tumor size was negatively correlated with the immunoreactivity of this antibody. When weight of tumors was greater than 4.0 grams, the immunoreactivity was low. Whether or not this antibody could be used as a marker for screening the immunity remains to be investigated.

No antibody activity was observed when immunodeficient mice were used in the study.

Animal cloning:

We have made a great effort in developing the technique for animal cloning. First, one permanent research assistant (Mr. Chin-Jen Chang, graduated from the Department of Animal Science, Chung-Chin University) supported by Pig Research Institute Taiwan was recruited. During the past 2 years, we have conducted nucleus transfer from the somatic cells to oocytes in the Department of Applied Biology, PRIT, where the PI of this proposal was the Department chairman. The embryo has been formed in vitro and developed into 8-cell stage. Second, with this grant support, we have purchased a new microinjector (now set up in PRIT) that can smoothly transfer the nucleus. Third, we

had sent Dr. Chin-Chi Wu to the Laboratory of Dr. Jerry Yang, University of Connecticut, to learn the animal cloning technique for 6 months. This technique is now established in PRIT. We anticipate the cloned animal be produced in the next 1-2 year

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