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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※　　使用去氧核糖核酸微陣列分析敗血症小鼠胰臟細胞的基因表現　　※
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計畫主持人：林芳郁教授

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□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

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行政院國家科學委員會專題研究計畫成果報告

國科會專題研究計畫成果報告

Preparation of NSC Project Reports

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一、中文摘要

目的:使用 cDNA 微陣列在敗血症老鼠的肝中分析動態的基因表現形式。

背景:敗血症在外科的加護病房中的患者中是死亡的最主要的因素之一。基因層次的基因表現形式分析將會提供對敗血症的分子的變化的觀察。

方法:mRNA 從大腸桿菌或金黃色葡萄球菌引起的敗血症老鼠的肝萃取。在六次的靜脈注射後（0h，2h，8h，24h，48h 和 72h）。以 6,144 個基因的老鼠 cDNA 微陣列用色度法檢視敗血症老鼠肝的基因的動態的變化。

結果:在敗血症發生的時候， 有 5.3% 的基因有兩倍以上的變化，其中 184 個基因是增加，而有 144 個基因減少。有改變的大部份基因是普遍出現在革蘭氏陰性和陽性 的敗血症中，但是有一些基因的表現在細菌感染之後在特別的時間點在兩者的類型 敗血症之間是不同的。以北方墨點法和免疫染色法來確定微陣列資料的準確度。

結論: 微陣列結果支持革蘭氏陽性和陰性敗血症都分享最後的總的路徑。這些結果可能提供對敗血症的致病原理的洞察也提供一些改變的基因或可以為診斷的工具和治療性的策略的新目標基因。

關鍵詞：微陣列、敗血症、基因表現

Abstract

To use cDNA microarray to analyze the dynamic gene expression patterns in liver of septic mice. Sepsis is the most common cause of death in patients in the surgical intensive care unit. Genome-wide gene expression analysis will provide insights into molecular alterations of sepsis. The mRNAs were extracted from livers of septic mice induced by live *E. coli* and *S. aureus* intravenously at six time points (0h, 2h, 8h, 24h, 48h and 72h) after injection. The mouse cDNA microarray containing 6,144 genes with colorimetric detection system was used to monitor the dynamic changes of tested genes. During sepsis progression, 5.3% of assessed genes were altered with a more than two-fold change in which the expression of 184 genes was increased and that of 144 genes was decreased. Most of the genes with altered expression were commonly present in Gram-Negative and Gram-positive Sepses, but the expressions of some genes were different between both types of sepses at particular time points after bacterial infection. Northern blotting and immunohistochemistry were used to confirm the accuracy of microarray data at RNA and protein levels. The microarray results support the hypothesis that both gram-positive and gram-negative sepses share the final common pathway involving in the pathogenesis of sepsis. These results may provide insights into the pathogenesis of sepsis and might also help to identify some altered genes that could serve as new targets for diagnostic tools and therapeutic strategies.

Keywords: microarray, sepsis, gene expression

二、Introduction:

Sepsis is the most common cause of death in patients in the surgical intensive care unit that often leads to the development of the multiple organ dysfunction syndrome (MODS). Although there have been many attempts to avert the development of MODS, mortality estimates for sepsis are still consistently in the range of 40-60% with approximately 400,000 new cases per year in the United State (1). In modern intensive care unit, gram-positive bacteria account for up to 50% of severe sepsis, yet the pathogenesis of gram-positive sepsis is poorly understood than that of gram-negative sepsis (2). There is increasing experimental evidence that fundamental differences exist in the host response to gram-positive bacterial pathogens compared with that to gram-negative organisms (3). The liver is thought to be a major organ responsible for the initiation of MODS during sepsis, as it plays a central in metabolism and host defense mechanisms (6,7). However, the complicated interactions among hepatic cells and the global changes of gene expression in liver during sepsis are still not completely understood. An understanding of the biological and molecular mechanisms underlying sepsis induced by Gram-positive and Gram-negative organisms may facilitate the development of new diagnostic

and therapeutic strategies in sepsis. To achieve this goal, identification and characterization of genes differentially expressed in liver during sepsis are essential. Systematic studies of gene expression patterns using cDNA microarray provide a powerful approach to molecular dissection of cells and tissues by comparing expression levels of thousands of genes simultaneously (4-6). Therefore, we analyzed alterations in the gene expression patterns in various time points of liver during sepsis using the mice cDNA microarray (6,144 genes, including known regulatory genes and expressed sequence tags, mouse ESTs) with colorimetric detection system (4,7).

三、RESULTS and DISCUSSION:

Two dominant pathogens, *E. coli* and *S. aureus*, which cause sepsis induced by gram-negative and gram-positive bacteria clinically in intensive care unit of surgery in the United State and Taiwan were used to establish mouse sepsis model (8). The serum GOT and GPT levels were immediately increased 2 h after both pathogen injections and remained elevated through the whole study period. The infiltrated leukocytes among hepatic parenchyma were significantly increased in mice with sepsis induced by *E. coli* and *S. aureus*.

To understand and identify hepatic mediators involved in the progression of gram-negative and gram-positive induced sepsis, mice were divided into 6 time-point groups (0, 2, 8, 24, 48 and 72 h after bacterial challenges) and the cDNA microarray was used to assess the expression profile of 6,144 mouse genes in whole liver simultaneously. The great majority of affected genes performed similar profiles in both gram-negative sepsis and gram-positive sepsis. According to the dynamic expression profiles, genes were divided into 2 groups in which 184 genes were activated and 144 genes were inhibited after bacteria challenges. Thus, there was altered expression of 5.3% of analyzed genes during sepsis progression. To further analyze the altered genes, those were grouped on the basis of their cellular functions. Genes affected in bacteria infection belonged to several different groups. The first group included genes involved in inflammation, cell defense and stress response. The second group included cell structural proteins and genes encoding for proteins involved in motility, nutrition transportation and vesicle trafficking. The third group included cell surface receptors, growth factors, transcription factors and genes encoding proteins involved in signal transduction. The fourth group included metabolic enzymes and genes encoding proteins involved in protein synthesis, protein modification and protein turnover.

To confirm the validity of microarray, Northern blot analyses were performed. Five up-regulated genes (Inhibitor of NF-κB α chain (I-κB), TYRO binding protein (TBP), fibrinogen γ-A chain, H-2 class II β chain, and proteasome 26S subunit) and four down-regulated genes (endothelin-converting enzyme 1 (ECE), Ras-related protein 6 (RAB), glutathione-S-transferase θ2 (GST), and NADH-ubiquinone oxidoreductase ashi subunit (NQO)) were randomly selected from each of four functional gene groups. The expression of I-κ B α chain was increased with 5.8 and 3.4-fold at 2 h after *E. coli* or *S. aureus* challenges compared with that of normal liver control and rapidly returned to normal level 24 h later. We found that maximally increased expression of TBP presented at 48h after bacterial injection, and the maximally increased expression of fibrinogen γ-A chain, H-2 class II β chain, and proteasome 26S subunit presented at 72h after bacterial injection. The expression of ECE-1, RAB6, GST θ2, and NQO ashi subunit was decreased throughout the whole duration of infection. Interestingly, we found 23 genes those exhibited differential expression profiles between *E. coli* and *S. aureus* challenges at some particular time-points during study period. The expressions of Monocarboxylate transporter 1 (MCT1) and RAB4A were easy to distinguish between *E.coli* infected mice and *S. aureus* infected mice at 8h after infection and the expressions of Apolipoprotein A-IV (Apo A-VI), solute carrier family 3 (SLC3), and guanylate binding protein (GBP-2) were also easy to distinguish at 48h after infection.

To demonstrate the protein expression of identified gene was also consistent with the microarray analysis, four antibodies (specifically against C/EBP α, C/EBP β, I-κ B α chain, and adaptin C) were used to carry out immunohistochemical analysis at 6 sampling time-points after bacterial challenges. The protein level of C/EBP β was increased since 2h after infection and the

maximal expression was present at 8h after bacterial injection. The highest expression of I-κ B was present at 72h and 8h post infection for *E. coli* and S. *aureus*. Respectively. Whereas C/EBP α and adaptin C were down-regulated and minimal expression was present between 24h and 48h after bacterial injection. Furthermore, apoptotic cells were evaluated in liver biopsy specimens using TUNEL assay in mice injected with *E. coli* or *S. aureus*. In both experimental groups, the number of apoptotic cells was increased, reaching maximal levels at 24h postinjection. At this time-point, the number of apoptotic cells form *E. coli* infected specimen was 37.6 ± 6.9 versus 4.0 ± 0.5 in the control group ($P < 0.01$, n=5) and that from *S. aureus* infected specimen was 10.2 ± 1.5 versus 4.0 ± 0.5 in the control group ($P < 0.02$, n=5). Regardless of infection time, *E. coli* infection elicited much severer cell apoptosis than did *S. aureus* infection. Moreover, we found that the major cell type of apoptotic cells was Kupffer cells rather than hepatocytes morphologically.

Sepsis is not a single disease, but a complex condition comprising a large variety of local and systemic inflammatory responses. The understanding of the complex nature of sepsis is critical to the development of rational strategies to prevent, treat or prognose the process. Recently, a powerful new technique, cDNA microarray, was developed that allows the simultaneous analysis of the expression of multiple genes. The main application of cDNA microarrays is comparative expression profiling of different tissues or cells. The present study is the first, to our knowledge, that investigates changes in the expression patterns of 6,144 mouse genes using cDNA microarrays to determine molecular events associated with sepsis induced by either *E. coli* or *S. aureus* in mouse liver.

In our study, the elevated serum GOT and GPT levels and the increasingly infiltrated leukocytes among hepatic parenchyma indicated that there were great injury and inflammatory reactions in liver after administrations with *E. coli* and *S. aureus*. We used cDNA microarrays to profile the gene expression patterns of liver in septic mice induced by *E. coli* and *S. aureus*. We found that the great majority of affected genes presented similar profiles in both Gram-negative and Gram-positive sepses and we identified a more than twofold alteration in the expression of 328 genes in liver obtained from septic mice. Consistent with previous studies (9-11), our microarrays data also demonstrated that the expression of six acute phase proteins (fibrinogen, lipopolysaccharide binding protein, lysophospholipase I, hemopexin, amyloid β A4 and 2'-5' oligoadenylate synthetase I) was up-regulated and the expression of three negative acute phase proteins (α-albumin, retinal binding protein.2 and transthyretin) was down-regulated in both *E. coli* sepsis and *S. aureus* sepsis. It confirmed the accuracy of our microarray analysis. In these septic mice, 5.3% of the genes monitored were differentially expressed and could be divided into different functional classes: 1. Immunity, Inflammation, and Coagulation, 2. Antioxidant Defense and Detoxification 3. Vesicle Trafficking and Transporter 4. Cell signaling, Cell cycle and Apoptosis. There were 23 genes whose expressions were differently between *E. coli* and *S. aureus* induced sepsis. Although the roles of these genes between Gram-positive and Gram-negative sepsies are unknown yet, this data provides clues to investigate the possible differences for pathogenesis, prognosis, diagnosis and therapy between Gram-positive sepsis and Gram-negative sepsis. In conclusion, we examined the dynamic gene expression profiles between *E. coli* induced sepsis and *S. aureus* induced sepsis. There are complex alterations of genes involved in different cellular functions during sepsis progression, and a number of these genes represent novel observations deserving further investigation to elucidate whose roles in sepsis. Most of the changes observed are generally present in both types of sepsis, but the expressions of some genes are distinguishable between sepses caused by different pathogens at particular time points after bacterial infection. These findings are in line with the hypothesis that both gram-positive and gram-negative sepses share the final common pathway involved in the pathogenesis of sepsis. These results may provide wider insights into the pathogenesis of sepsis and might also help to identify some altered genes that could serve as new markers for diagnosis and potential targets for therapies in sepsis.

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