

茯苓對於單核球的作用

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一、摘要：

單核球活化所分泌的多種細胞激素，可增強特異及非特異性免疫反應，因而對免疫系統，造血機能及癌細胞的毒殺作用均有相當影響。本研究主要利用補氣藥中的茯苓抽出液，作用於人體血液中之單核球，然後利用免疫學的方法來分析單核球所產生的Tumor necrosis factor(TNF), IL-1, IL-6,及GM-CSF等細胞激素的變化。

我們發現茯苓之酒精萃取液在離體實驗中可抑制人類單核球分泌TNF, IL-1, IL-6及GM-CSF等激素。在含10%茯苓萃取液之培養基顯著抑制這些細胞激素的分泌量。在低含量時(0.62%)其中TNF, IL-1, IL-6分泌增加的現象可被Polymyxin B所抑制，故其分泌增加的現象，應該是由於摻雜在茯苓萃取液中之脂多醣體所造成。又GM-CSF之分泌不受polymyxin B影響，而脂多醣體並不能激GM-CSF之生成，故茯苓中可能另有其他因子可誘發其分泌，不過同樣也會被10%之茯苓酒精萃取液所抑制，所測之四種細胞間質皆與發炎反應有密切關係，由上述結果顯示茯苓在活體中或許具有抑制炎症反應的作用，對於癌症的治療可能幫助不大，但仍須進一步的探討。

二、前言：

癌症的治療，除了利用抗癌藥物或放射綫將癌細胞殺死外，就是免疫治療，包括特異性的免疫治療，如利用腫瘤特異的抗體或腫瘤特異的T細胞來消滅癌細胞，由於這些技術相當費時，也難以建立，因此很難被用於臨床應用。所以非特異性的免疫療法就非常要發展，主要的方法是刺激活化單核球(即吞噬細胞)，Nature Killer Cell等的毒殺腫瘤細胞的能力，其中以單核球的活化最為重要(1-5)，尤其是它所分泌的細胞激素更是影響深遠。在過去的研究已發現

Table 1. Limulus amoebocyte lysate assay for LPS content

LPS concentration* (pg/ml)	Result	Dilution of extract*	Result
200 pg/ml	+	1/1,000	+
20 pg/ml	+	1/10,000	+
2 pg/ml	-	1/100,000	-
0.2 pg/ml	-	1/1,000,000	-

* Both LPS and Fu-Ling extract were diluted to the indicated concentrations by pyrogen-free LAL Reagent Water.

Table 2. Effect of polymyxin B on Fu-Ling extract-mediated monokine secretion

Monokines	Monokine Concentration (pg/ml)*	
	Polymyxin B	Control
TNF α	2324 \pm 481	5958 \pm 788
IL-1 β	3748 \pm 139	5400 \pm 211
IL-6	2920 \pm 728	8508 \pm 2606
GM-CSF	345 \pm 100	395 \pm 32

* Human monocytes were cultured in the medium containing 0.62% of Fu-Ling extract and 1 μ g/ml of polymyxin B. Monokine concentrations were measured as described in "Materials & Methods". Control was the culture without polymyxin B. The data were mean \pm sd. of two similar experiments.

某些物質，如Lactin (1)，細菌的外毒素，或某些中藥可增加某些細胞激素的產量(6-8,10)，但尚無系統性的研究探討，因此本研究主要的目標在於探討人類單核球在經過補氣藥中茯苓的刺激後，其所分泌的細胞激素，TNF, IL-1, IL-6 及 GM-CSF 的變化。

三、材料及方法：

1. 人體周邊血單核球的製備：(1)抽取30cc健康人之周邊血，放入內含抗凝劑heparin 之管中。(2)加入15cc的Ficoll-Hypaque，在室溫及400g離心30分鐘。(3)分離單核細胞，然後以RPMI 1640 培養於100mm的Petridish中(25×10^6 cells/dish)，在CO₂ incubator中(37°C)培養3小時。(4)將沒有附著的細胞以PBS洗掉，並計算已附著的細胞數。
2. 茯苓的萃取：首先將從中藥房選購的茯苓磨粉，然後將50克的粉狀茯苓放入100cc的75%酒精中，然後經濾紙過濾，再20000g離心20分，再將萃取液乾燥濃縮，再溶於5cc的RPMI 1640 培養液中，離心再通過0.45 μ M的無菌濾膜。
3. 單核球的體外活化：將 2×10^5 cells/ml中加入不同溶度的茯苓的萃取液或脂多醣體(Lipopolysaccharide)。
4. 以ELISA的方法偵測細胞培養液中之TNF, IL-1, IL-6及GM-CSF的濃度(Quantikine, R&D Sytem, MN, USA)。利用Limulus amebocyte lysate test分析脂多醣體(Cape Cod, Inc)。
5. 利用Student's t-test統計及分析結果。

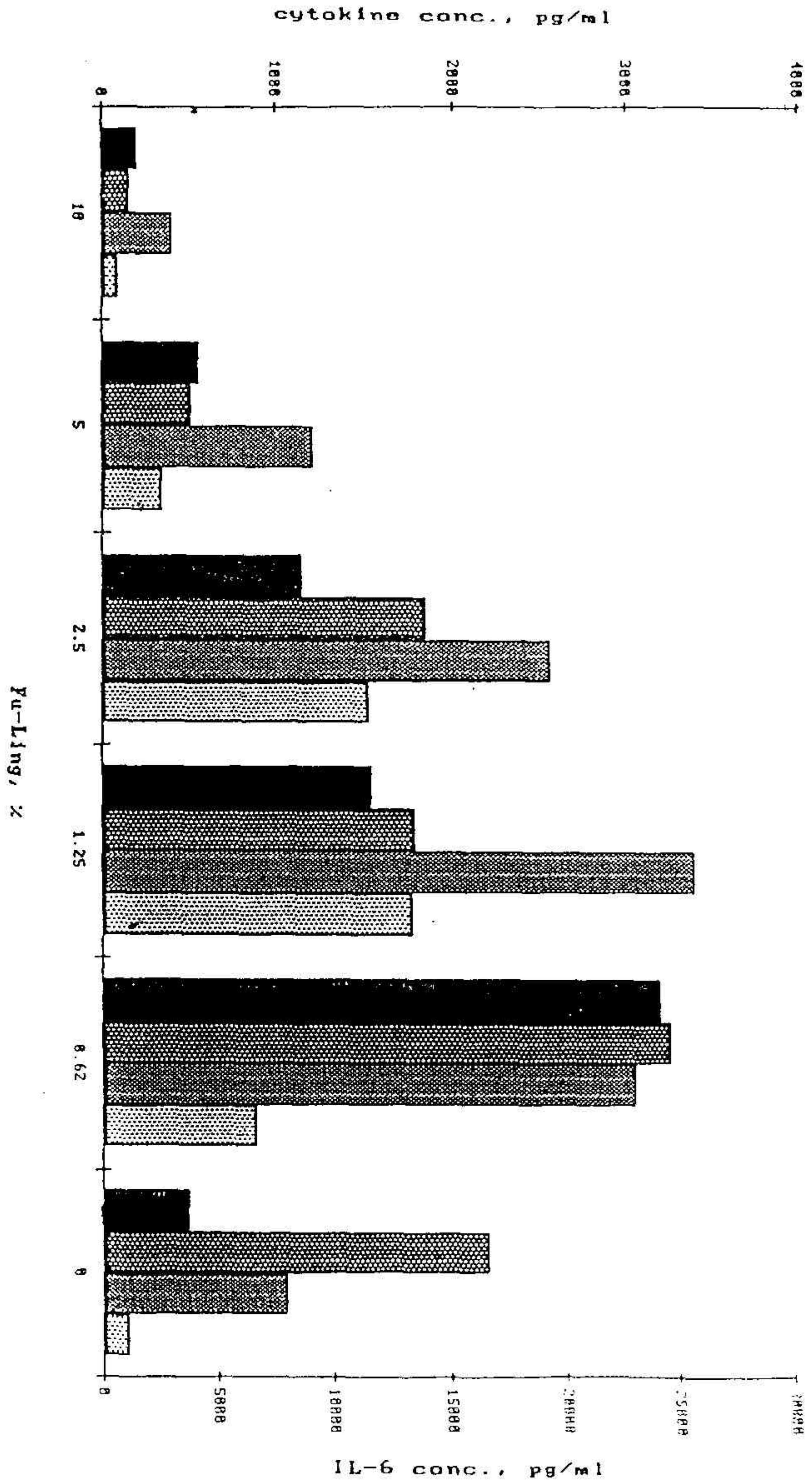
四、結果：

圖 1 顯示茯苓對於單核球分泌TNF- α , IL-1b, IL-6及GM-CSF的作用：當濃度為10%時，則4種激素皆明顯的被抑制，其減少的幅度分別為93.8%，95.4%，86.9%及90.3%。

表 1 顯示大約有200ng/ml的脂多醣體(LPS)存在於茯苓萃取液中，而這些LPS的存在就是造成茯苓在低溶度(0.62%)時對單核球刺激生成TNF- α , IL-1b, 及IL-6的結果。此點可由加入Polymyxin B後得到證實(表 2)，至於對GM-CSF的影響可能另有其它因子。

圖 2 及圖 3 表示，0.62%茯苓萃取液及1ng/ml的LPS對單核球產生TNF- α , IL-1b及IL-6產生時間的影響，由此可見，LPS及茯苓之作用差異並不大，但GM-CSF似乎不受LPS的刺激。

Figure 1. Induction of TNF- α , IL-1 β , IL-6, and GM-CSF secretion by Fu-Ling extract. The monolayers of human peripheral monocytes were cultured with the medium containing various percentages of Fu-Ling extract, ranging from 10% to 0.62%. The monocytes were incubated in CO₂ incubator for 24 hours, and then the media were collected for quantitative determination of individual cytokines. Control were the monocytes cultured with reagent-free medium. The left Y-axis represented the concentration of TNF- α (■), IL-1 β (▨), and GM-CSF (▩). The right Y-axis represented the concentration of IL-6 (□). Data were mean of four similar experiments.



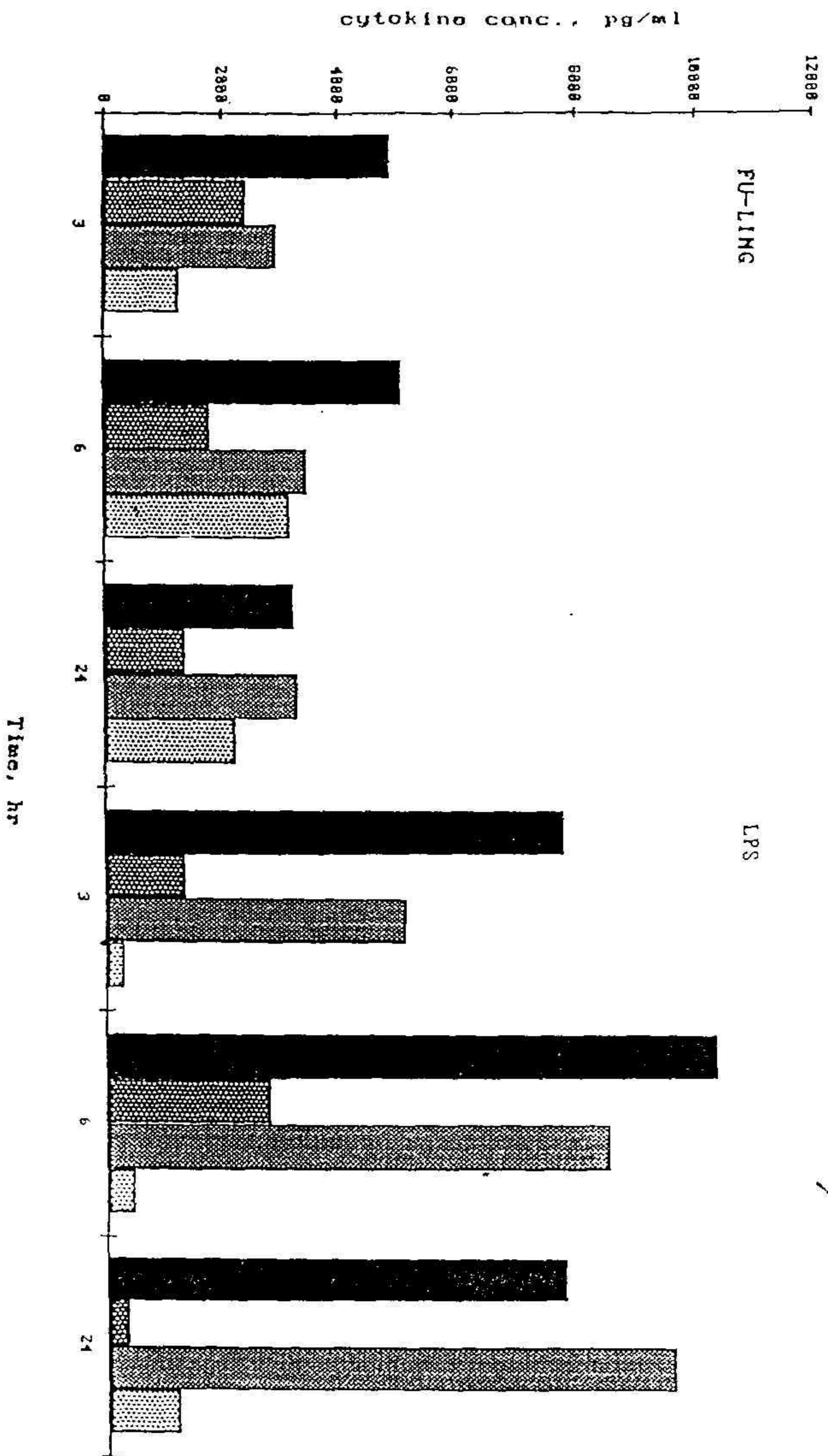


Figure 2. The time course of TNF- α and IL-1 β secretion from either Fu-Ling extract-stimulated or LPS-stimulated monocytes. Monocyte monolayers were cultured with the media containing either 0.62% of Fu-Ling extract (left half) or 1 ng/ml of LPS (right half). The culture media were collected at 3, 6, and 24 hours of period, and were then assayed for TNF- α (■) and IL-1 β (◻). Control for both TNF- α (▨) and IL-1 β (◻) secretion were the culture without any treatment. Data were the mean of three similar experiments.

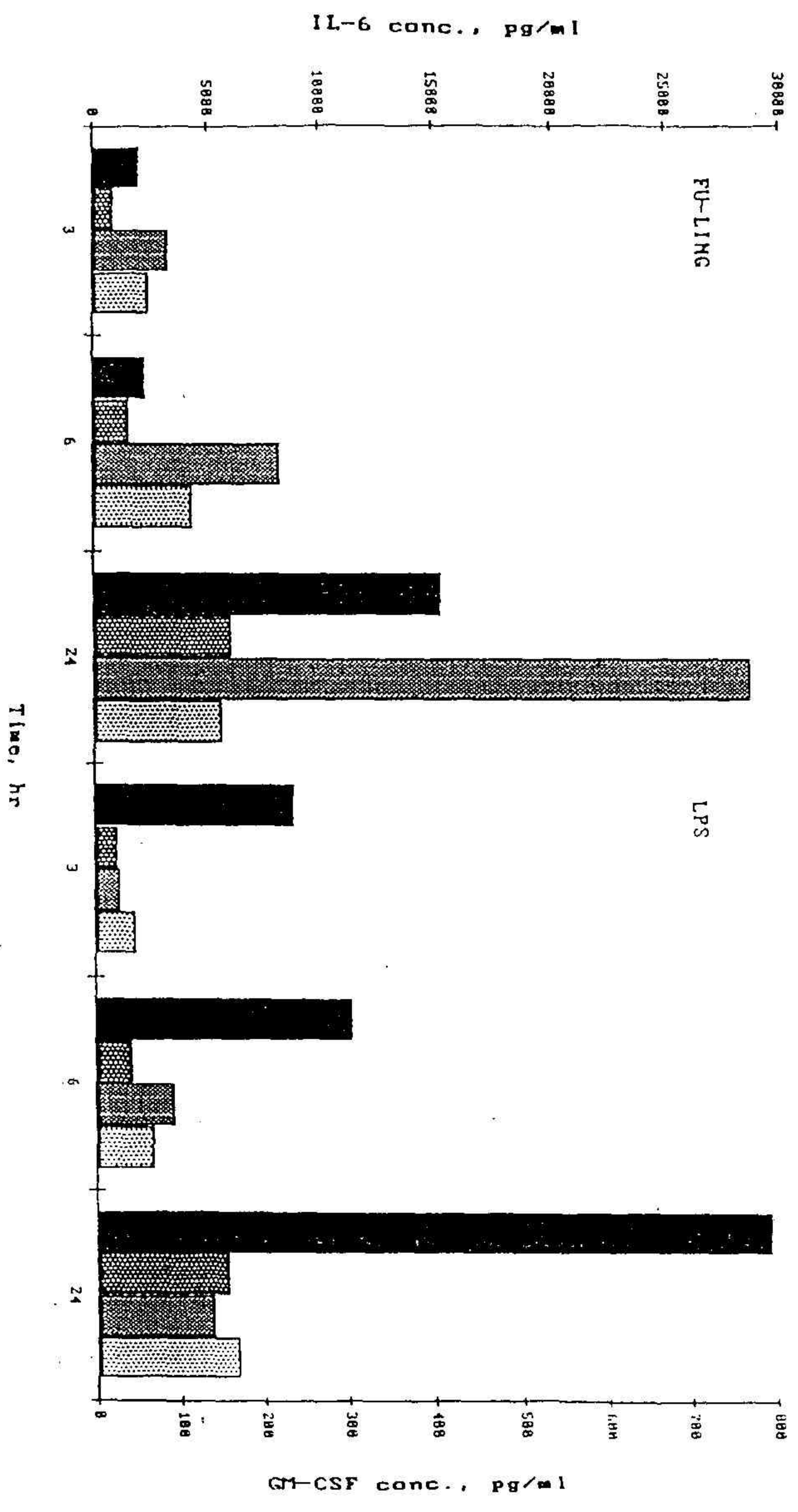


Figure 3. The time course of IL-6 and GM-CSF secretion from either Fu-Ling extract-stimulated or LPS-stimulated monocytes. Monocyte monolayers were cultured with the media containing either 0.62% of Fu-Ling extract or 1 ng/ml of LPS. The culture media were collected at 3, 6, and 24 hours of period, and were then assayed for both IL-6 (■) and GM-CSF (▨). Controls for IL-6 (▩) and GM-CSF (▧) secretion were the cultures without any treatment. Data were mean of three similar experiments.

五、討論

在過去茯苓一直被當做補氣藥的成員之一，它有利水，安神，健脾等作用，本研究發現，茯苓在低溶度時的免疫促進作用，其實是因污染的細菌脂多醣體造成，因此未來關於中藥對免疫機能的研究，一定必須考慮此因子的影響。由本研究顯示茯苓在高濃度時，對4種細胞激素有抑制的作用，因此它或許有抑制炎症反應的作用，至於對於癌症治療的效果可能幫助不大，但仍須進一步探討。