

附件：封面格式

# 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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※ 青蔥萃取物調節巨噬細胞 scavenger 受體表現的影響 ※

※ Study of Welsh onion extracts on the scavenger receptor ※

※ expression of macrophages ※

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- 國際合作研究計畫國外研究報告書一份

執行單位：嘉南藥理科技大學 生活應用與保健系

中華民國 91 年 10 月 日

## 一、中文摘要

CD36 是一 88kDa 大小的醣蛋白。已知血小板，巨噬細胞，內皮細胞及脂肪細胞皆可表現 CD36。目前已知 CD36 在巨噬細胞吞噬氧化態低密度脂蛋白過程中，扮演重要角色。在本實驗中，我們探討青蔥萃取液是否可以影響 CD36 蛋白在巨噬細胞中的表現，進而改變細胞內脂質含量。目前結果發現，脂多醣體可以增加巨噬細胞表現 CD36 蛋白，而青蔥萃取液則劑量依賴的抑制此作用。另一方面，在含有氧化態低密度脂蛋白培養液中，脂多醣體可以增加巨噬細胞內膽固醇含量，而青蔥萃取液亦可以抑制此增加作用。更進一步，以染劑對細胞內脂質加以染色，亦有相似結果。

**關鍵詞：**CD36、巨噬細胞、青蔥

## Abstract

CD36 is a glycoprotein with an 88 kDa molecular weight. CD36 is expressed on platelets, macrophages, endothelial cells, and adipocytes. It has been reported that CD36 play an important role in the process of up taking oxidized low-density protein (OxLDL) by macrophages. In this study, we attempt to clarify the effect of Welsh onion extract (WOE) on the expression of CD36 and the intracellular lipid content. The expression of CD36 is increased after stimulated with lipopolysaccharide (LPS) in RAW 264.7 macrophages for 24 h. And, WOE suppress LPS-induced CD36 expression by dose-dependent manner. On the other hand, incubation of macrophages with 50 ug/mL OxLDL and 1 ug/mL LPS for 24 h increase the intracellular cholesterol level. And, WOE also decrease LPS-induced cholesterol accumulation in macrophages by dose-dependent manner. In addition, LPS also promote lipid accumulation in macrophages. And, WOE also inhibit lipid accumulation in macrophages.

**Keywords:** CD36, macrophage, Welsh onion

## 二、緣由與目的

In atherogenesis, elevated plasma levels of LDL have been reported to lead the chronic presence of LDL in the arterial wall. There, LDL is oxidized and these modified lipoproteins activate endothelial cells to attract circulating monocytes. These monocytes enter the arterial wall, differentiate into macrophages, and subject the modified LDL to endocytosis through scavenger receptor pathways that include scavenger receptor A (SR-A), CD-36, and macroscialin (1-3). This unrestricted uptake, which is not limited by serum cholesterol concentration, eventually leads to the formation of lipid-filled foam cells (4). For example, hypercholesterolemic mice in which the gene encoding macrophage chemotactic protein has been disrupted are resistant to the development of atherosclerosis (5, 6). In similarity, disruption of the SR-A and CD-36 genes results in a significant reduction of hypercholesterolemia-induced atherosclerosis in mice (7, 8).

Goldstein et al have first described a receptor on macrophages that mediated the uptake and degradation of oxidized LDL and produced massive intracellular cholesterol deposition (9). Currently, it is known that this receptor belongs to a large family of scavenger receptors, all mediating the uptake of modified LDL (10). It was shown that SR-A is a trimeric transmembrane glycoprotein consisting of 6 distinct domains (11). The collagen-like domain has been shown to be the site for receptor interacting with modified LDL (12, 13). Many different cytokines can influence the expression level of SR-A in macrophages. Both TNF- $\alpha$  and INF- $\gamma$  are produced in atherosclerotic lesions and inhibit SR-A activity on macrophages by transcriptional and posttranscriptional regulation (14, 15). On the other hand, the macrophage colony-stimulating factor and granulocyte colony-stimulating factor enhance the SR-A expression (16, 17).

CD-36 has also been proved to be highly regulated in macrophage during

differentiation (18) and to be present in lipid-loaded macrophages in atherosclerotic lesions (19). This scavenger receptor is enhanced by IL-4 (20), macrophage colony-stimulating factor (18), modified LDL (21), and peroxisome proliferator activated receptor ligands (22). Unlike SR-A, CD-36 is more broadly expressed and has been proved to play an important role in lipoprotein and lipid metabolism (23). Although a role for CD-36 in atherogenesis in humans has not yet been established, multiple line of evidence suggest CD-36 plays a role in lipid accumulation and macrophage foam cell formation in vivo and in vitro.

It is a well known fact that vegetables belonging to *Allium* species, especially garlic and onion, have been used in cardiovascular disease prevention and attracted a great deal of attention. In Taiwan, Welsh onion (*Allium fistulosum* L., Alliaceae) is an important flavoring vegetable crop and used for cooking. It has been reported that Welsh onion or garlic extracts can modulate rat aortic vascular tone (24, 25) and inhibit platelet aggregation (26, 27). However, low dose of Welsh onion extract induced vasorelaxation, which was mediated by endothelium-derived nitric oxide. On the other hand, high dose of Welsh onion extract induced endothelium-independent vaso-relaxation. Furthermore, numerous laboratory and clinical investigations have confirmed garlic with the well-known properties (reduction of hyperlipidemia, hypertension; prevention of thrombus formation) that may be useful in the prevention, and treatment of atherosclerotic cardiovascular diseases. It is interesting that garlic and onion oils have been proved to induce differentiation of HL-60 cells (28). However, whether Welsh onion and its derivatives can modulate the expression of macrophage scavenger receptors and the foam cell formation is still unknown. Now, we will investigate whether Welsh onion extract can influence CD36 activity or their expression in macrophages.

### 三、結果與討論

#### **Effect of Welsh Onion Extract on CD36 expression in Macrophages**

To evaluate the changes in CD36 expression on the cell surface, we examined the effect of LPS on the expression of CD36 in RAW 264.7 macrophages by immunoblot. Figure 1 shows the CD36 expression in macrophages after a 24-hour incubation with and without LPS. Macrophages that were incubated in medium containing 1 ug/mL LPS showed a 10-fold higher expression CD36 than did cells incubated in medium without LPS. Next, we compared the magnitude of the effects of Welsh onion extract on CD36 expression on macrophages. Welsh onion extract did not affect CD36 expression in macrophages. But, 1 or 0.5 mg/mL Welsh onion extract suppressed LPS-induced CD36 expression to 30 and 50 %, respectively, of that macrophages cultured with LPS.

#### **Effect of Welsh Onion Extract on Intracellular Cholesterol Accumulation in Macrophages**

To obtain further evidence that Welsh onion extract is involved in lipid metabolism of RAW 264.7 macrophages. Measurement of intracellular cholesterol levels following low-density protein (LDL) treatment by enzymatic assay. We found that 50 ug/mL LDL resulted intracellular cholesterol increasing to a 2-fold of control. And, Welsh onion extract (0.2 - 5 mg/mL) inhibited intracellular cholesterol accumulation by a dose-dependent manner in the presence of LDL (Fig 2).

#### **Effect of Welsh Onion Extract on Intracellular lipid Accumulation in Macrophages**

We then examined the effect of Welsh onion extract on the accumulation of lipid in RAW 264.7 macrophages. Oil red O staining assessed lipid accumulation. Macrophages were treated with 50 ug/mL LDL and 1 ug/mL LPS. Then, a obvious in lipid accumulation was observed (Fig 3B). And, 5 mg/mL Welsh onion extract resulted a marked decrease in intracellular lipid accumulation was observed.


#### 四、計畫成果自評

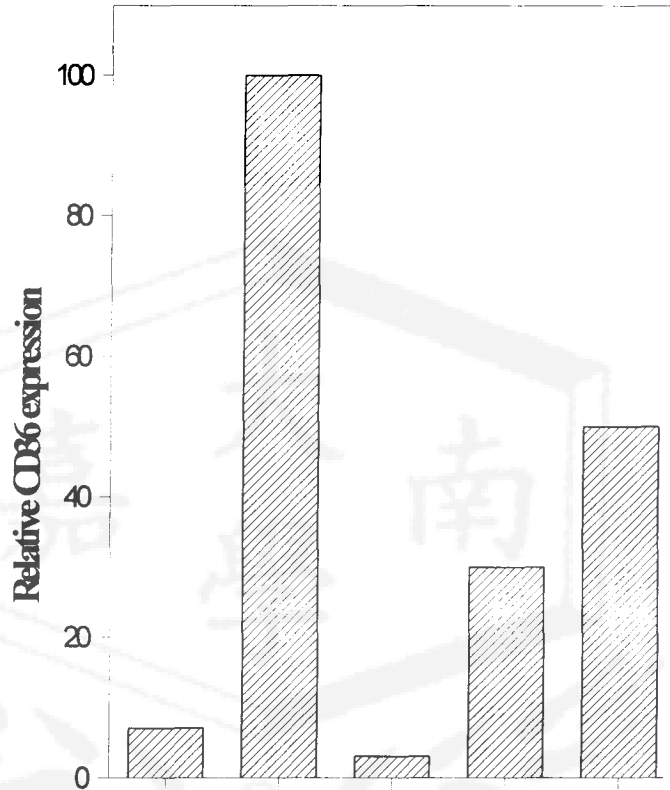
本實驗是個人第一次申請通過的國科會計劃。由於目前實驗所需儀器與空間不足，且校內行政與教學工作繁重，致使進度較為緩慢，數據及結果亦不盡完善。但青蔥為國人日常接觸的食品之一，而國外的相關研究文獻卻不多見。本實驗數據初步證實青蔥可藉由調節巨噬細胞 CD36 蛋白表現及膽固醇代謝，進而影響細胞內脂質量，則是一個新的發現。

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<b>LPS(1ug/mL)</b>	-	+	-	+	+
<b>WOE(mg/mL)</b>	-	-	1	1	0.5
<b>CD36</b> →					



**Fig. 1. Effect of Welsh onion extract on CD36 expression in RAW 264.7 macrophages.** Cells were treated with LPS (1 ug/mL) for 24 h and analyzed for CD36 protein expression by immunoblot. Welsh onion extract (WOE) inhibit CD36 expression in macrophages cultured with LPS.

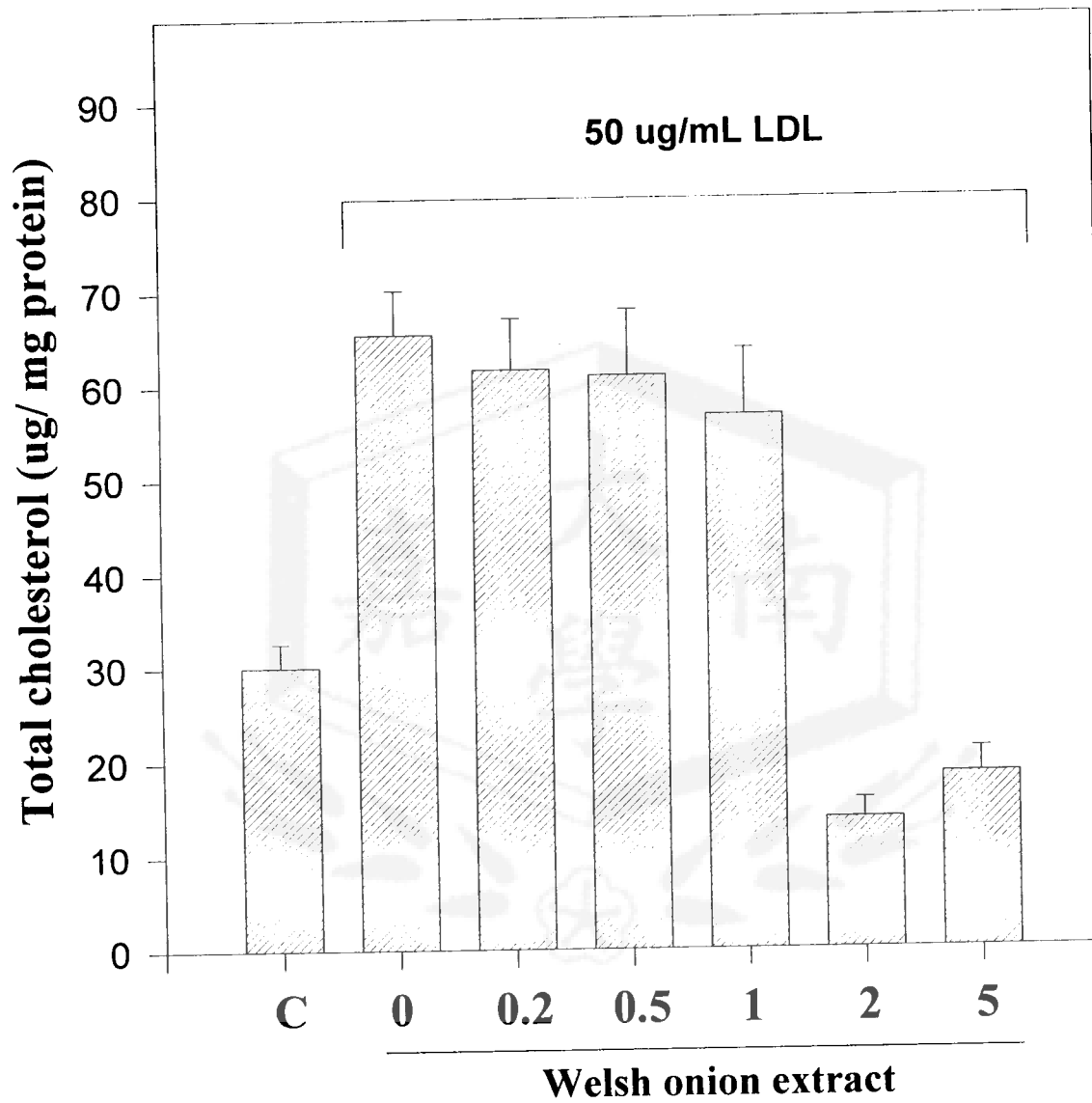
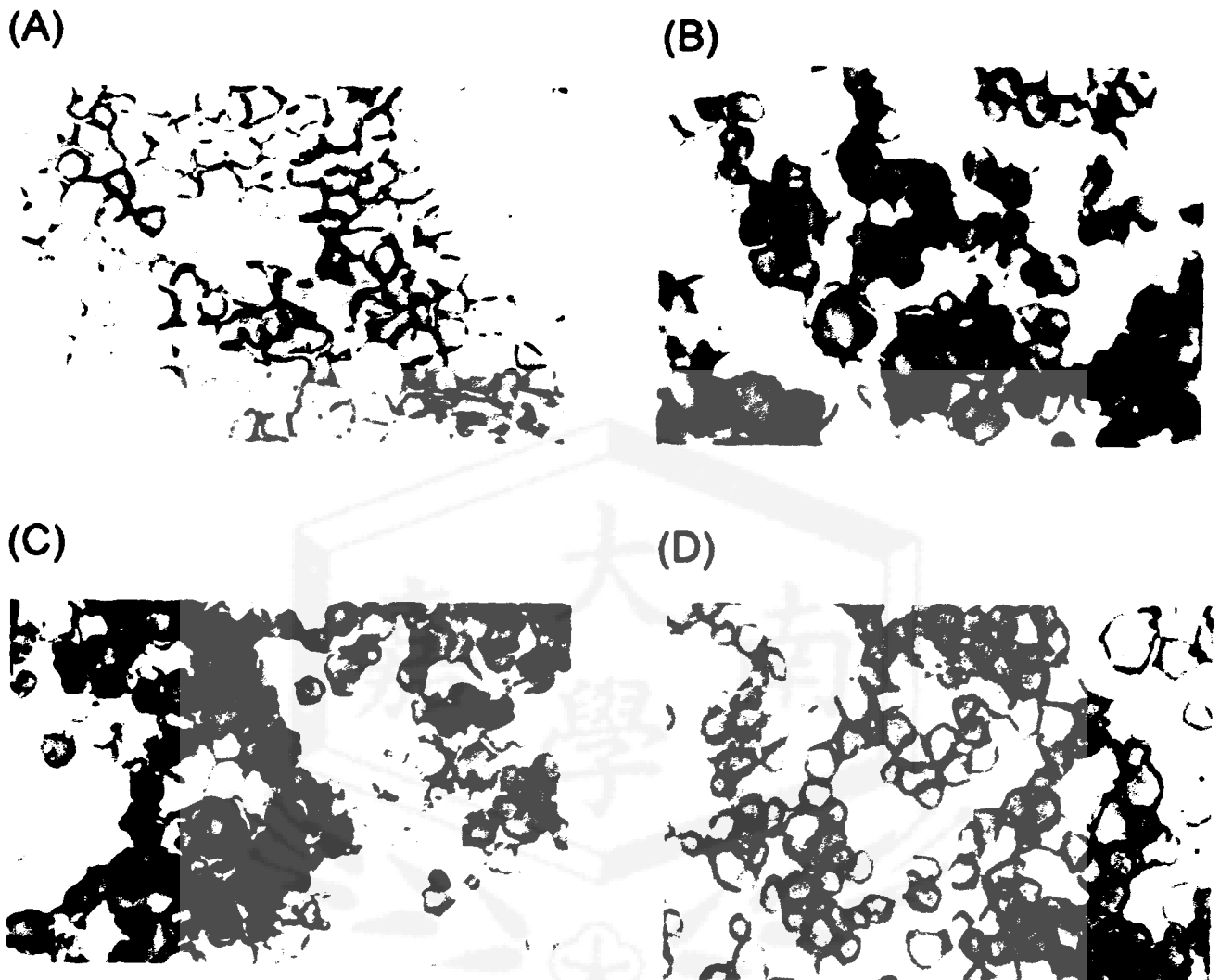


Fig. 2. Effect of Welsh onion extract on intracellular cholesterol accumulation in macrophage. Cells were incubated with Welsh onion extract (0.2 - 5 mg/ml) in the presence of LDL (50 ug/mL) for 24 h.



**Fig. 3. Welsh onion extract inhibits lipid accumulation in RAW 264.7 macrophages. (B) Cells were treated with LDL (50 ug/mL) for 24 h and then assessed by Oil red O staining. (C) Macrophages were stimulated with LPS (1 ug/mL) for 24 h in the presence of LDL. (D) Welsh onion extract (5 mg/mL) inhibit LDL-stimulated lipid accumulation in macrophages cultured with LPS.**