行政院國家科學委員會補助專題研究計畫成果報告

靈芝對腸免疫系統功能之影響：第一部份 IgA 濃度
Effects of *Ganoderma lucidum* mycelium on gastrointestional immune function: Part I. IgA concentration

計畫類別：■個別型計畫　　□整合型計畫

計畫編號：NSC 89-2312-B-041-001

執行期間：88 年 8 月 1 日至 90 年 1 月 31 日

計畫主持人：夏彩蘭

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執行單位：嘉南藥理科技大學　保健營養系

中　華　民　國　90 年　4　月　31　日

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一、中文摘要

19 天大 C57BL/6J 雌性小鼠飼以含 0、0.5、1、3 和 5% 靈芝 (*Ganoderma lucidum*) 菌絲體之 AIN-93G 飼料 4 週。小鼠的生長情形和飼料攝取量並不受靈芝菌絲體之影響。以 ELISA 測定小腸內容物、血清及糞便中 IgA 含量，只有餵食含 3%靈芝菌絲體之AIN-93G飼料小鼠，其小腸內容物之IgA含量與對照組比較有顯著增加。靈芝菌絲體並不影響糞便、血清 IgA 和血清 IgG 的濃度。在特異性抗體反應方面，靈芝菌絲體卻有抑制的效果。五週大雌性小鼠在飼以含 0,、0.5、1、3 和 5%靈芝菌絲體之 AIN-93G 飼料四週期間，在第七天及第二十一天以管餵之方式給予 5 μg cholera toxin (CT)。餵以含靈芝菌絲體飼料之小鼠糞便、小腸內容物和血清的 anti-CT-IgA 抗體與對照組比較均顯著減少。同時血清總 IgA 的濃度也顯著低於對照組。血清中 anti-CT-IgG 濃度只有飼以含 3%靈芝菌絲體之小鼠顯著低於對照組，但血清總 IgG 的濃度則不受靈芝菌絲體之影響。實驗結果顯示，靈芝對小腸內容物 IgA 含量有增加的作用，但降低特異性抗體反應。

關鍵詞：IgA，靈芝菌絲體，腸道免疫

Abstract

19-day old female C57BL/6J mice were fed AIN-93G diet containing 0, 0.5, 1, 3 and 5% (wt/wt) mycelium of *Ganoderma lucidum* for 4 weeks. Mice fed the AIN-93G diet containing different levels of *Ganoderma lucidum* mycelium did not affect the food intake and showed growth comparable with that for the control mice fed AIN-93G diet only. The IgA level in luminal washes of small intestine, serum and fecal pellets as well as serum IgG were determined by ELISA. The small intestine luminal IgA level was significantly increased in mice fed diet containing 3% mycelium of *Ganoderma lucidum*. However, the concentration of serum IgA, serum IgG and fecal pellets IgA were not different among groups. The specific antibody response was also determined. Five-week-old female C57BL/6J mice were orally immunized with 5 μg cholera toxin (CT) on day 7 and 21 of the 4 week feeding period. The specfic anti-CT-IgA antibody responses were decreased in luminal washes, serum and fecal pellets of mice fed mycelium of *Ganoderma lucidum*. In addition, the concentration of total serum IgA was also decreased in all group of mice fed the mycelium of *Ganoderma lucidum*. The anti-CT-IgG response was only decreased in mice fed 3% mycelium of *Ganoderma lucidum*. However, the total serum IgG was not affected by the *Ganoderma lucidum* mycelium. The results indicate that the mycelium of *Ganoderma lucidum* may elevate the IgA level in the luminal washes of small intestine in non-immunized mice, but reduce the specific antibody response of mice orally immunized with CT.

Keywords：*Ganoderma lucidum*，IgA，Gut immunity

二、緣由與目的

靈芝對免疫功能的研究包括自然殺手細胞的活性(1)，人類血中淋巴球的

mitogenic effect (2)，經γ射線處理小鼠脾臟 T cell subset 和細胞免疫功能的回復(3,4)，和抗腫瘤的作用 (5,6,7)。其中研究靈芝抗腫瘤為最多，特別是靈芝中多醣體的抗腫瘤作用。研究方法普遍以菌絲體或子實體的抽出物對實驗動物給予腹腔注射，結果顯示靈芝抽出物可增強免疫功能。腸免疫系統以 IgA 為主要的作用分子，因此 IgA 為腸黏膜的後天免疫防衛的主要屏障。它的功能包括抑制微生物在腸黏膜表面拓展繁殖 (8)，減少抗原通過腸黏膜(9)。更有學者提出 IgA 不但能防止病毒的吸附和黏著腸黏膜，還可抑制病毒在細胞內複製(10)。另一方面，polymeric IgA 可能在 lamina propria 與抗原結合，使抗原以 polymeric immunoglobulin receptor-mediated 的方式通過鄰近的上皮細胞而被移入腸腔(11)。目前靈芝對腸免疫系統功能之影響研究甚為缺乏，由於靈芝為口服的保健食品，因此靈芝對腸道免疫功能之影響值得吾人研究。

本研究計畫之目的是評估靈芝對小鼠腸道體液免疫功能之影響，以小腸內容物之 IgA 含量和糞便中 IgA 的濃度及腸道的特定 anti-CT 抗體反應作為評估指標，藉此評估靈芝在生體中的效應。

三、結果與討論

實驗一：靈芝菌絲體對小鼠腸 IgA 含量之影響

靈芝菌絲體對小鼠的生長 (Fig.1) 和飼料總攝取量 (C=70.6±4.1g、G0.5=70.1±6.3g、G1=70.9±4.0g、G3=69.7±3.0g、G5=72.3±6.0g) 並無影響。Fig 2.是靈芝菌絲體對小腸內容物、血清和糞便中 IgA 含量的影響。只有飼以 3%靈芝菌絲體之小鼠小腸內容物 IgA 含量與對照組比較有顯著增加。血清和糞便中 IgA 的濃度則不受影響 (Fig. 2)。各組小鼠之血清 IgG 的濃度並無顯著差異(data not shown).

實驗二：靈芝菌絲體對腸道特異性抗體反應之影響

小鼠的生長 (Fig. 3) 和飼料總攝取量 (C=92.6±16.6、T=89.1±8.8g、G0.5=90.2±17.3g、G1=84.8±.12.0g、G3=86.4±14.3g、G5=92.8±8.9g) 在各組間並無差異。不論飼料中靈芝菌絲體含量多寡，均對小腸內容物、糞便和血清中 anti-CT-IgA 抗體反應有抑制的作用 (Fig. 4)。只有飼以 3%靈芝菌絲體之小鼠血清中 anti-CT-IgG 抗體的濃度顯著低於對照組 (Fig. 5)。血清總 IgA 和 IgG 濃度方面，靈芝菌絲體減少血清總 IgA 濃度(Fig. 6)，但血清總 IgG 濃度(Fig. 7)則不受靈芝菌絲體之影響。

由以上結果顯示，靈芝菌絲體有調控腸體液免疫反應的作用。靈芝菌絲體對腸免疫系統之影響可能是藉由調控細胞激素(cytokine)分泌而達成。口服 CT 在腸主要是引起 Th2 反應（12）。靈芝菌絲體可能抑制 Th2 細胞激素 IL-4、IL-5、IL-6、IL-10 的分泌而降低腸 anti-CT-IgA 反應。

四、成果自評

本研究工作大致按原計劃進行，為實驗二原使用的小鼠年齡由 19 天大改為五週大，因 19 天大小鼠進行 CT 免疫後有死亡現象。實驗結果與原先預期有出入之處，靈芝菌絲體確有調控腸免疫功能之作用，雖然能增加小腸內容物 IgA 含量，卻抑制腸特異性抗體反應。靈芝菌絲體調控腸免疫功能之機制有待進一步探討。

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六、圖表

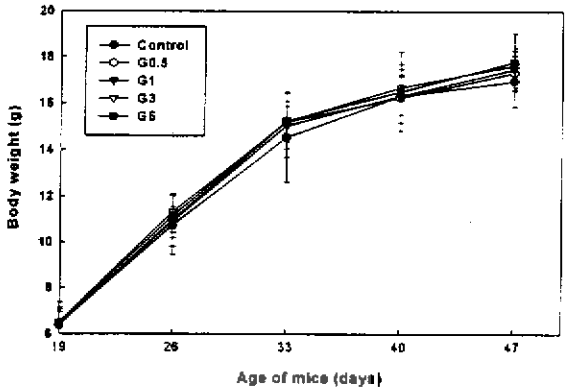

Figure 1. The weight gain for mice fed control or control diet containing 0.5 (G0.5), 1 (G1), 3 (G3) and 5% (G5) mycelium of *Ganoderma lucidum* for 4 weeks. Data represent mean±SD, n=9 except 10 for G0.5, G5 and 11 for control.

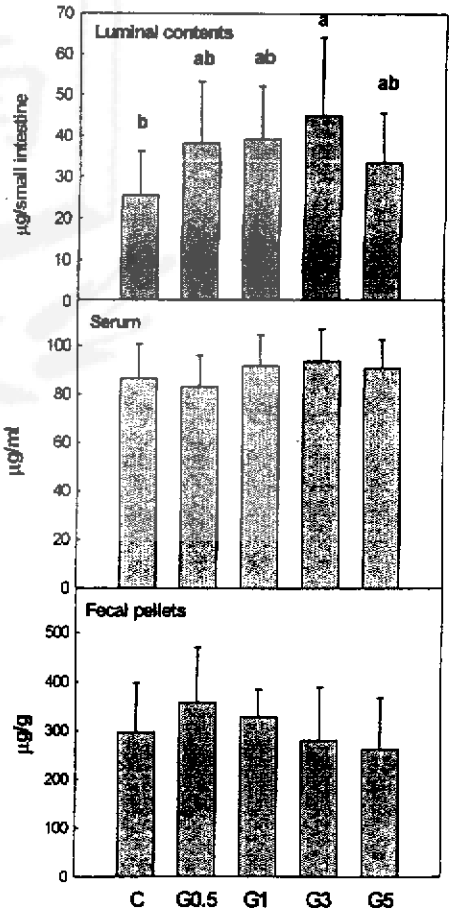

Figure 2. Immunoglobulin A in luminal contents of small intestine, serum and fecal pellets in mice feeding control (C) or control diet containing 0.5 (G0.5), 1 (G1), 3 (G3) or 5% (G 5) mycelium of *Ganoderma lucidum* for 4 weeks. Bars represent mean±SD; n=9 except 10 for G0.5 and 11 for C. Within each graph, bars not sharing a letter are different (P<0.05) according to Duncan's Multiple Range test.

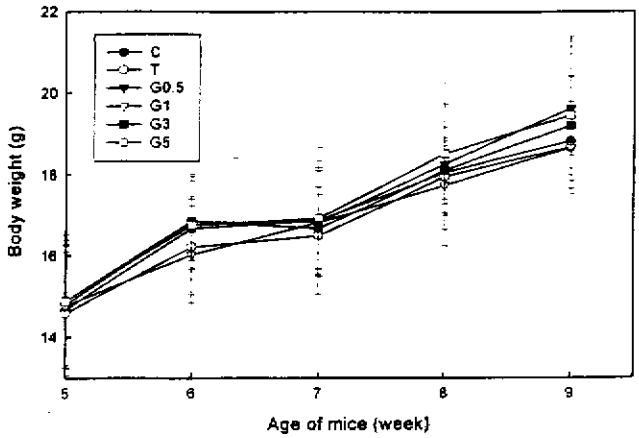

Figure 3. The growth curve for mice fed control (C and T) or control diet containing 0.5 (G0.5), 1 (G1), 3 (G3) and 5% (G5) mycelium of *Ganoderma lucidum* for 4 weeks. Mice were orally immunized with 0.1ml of 0.2M $NaHCO_3$ containing 5 μg cholera toxin on day 7 and 21. Control mice (C) were only received 0.1ml $NaHCO_3$ buffer twice. Data represent mean+SD, n=10 except 9 for T and G1.

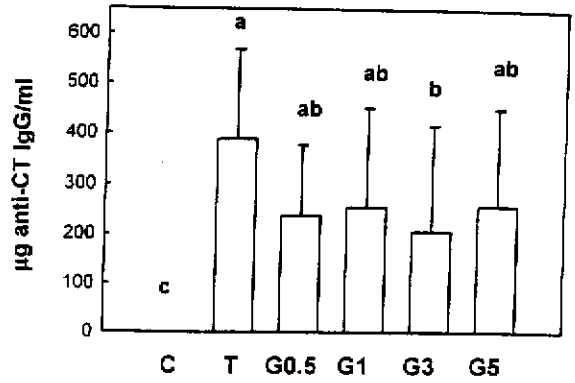

Figure 5. The anti-cholera toxin (CT) immunoglobulin G in serum in mice feeding control (C) or control diet containing 0.5 (G0.5), 1 (G1), 3 (G3) or 5% (G5) mycelium of *Ganoderma lucidum* for 4 weeks. Mice were orally immunized with 0.1ml of 0.2M $NaHCO_3$ containing 5 ug CT on day 7 and 21. Bar represent mean±SD; n=10 except 9 for C and G1. Within each group, bars not sharing a letter are different (P<0.05) according to Duncan's Multiple Range test.

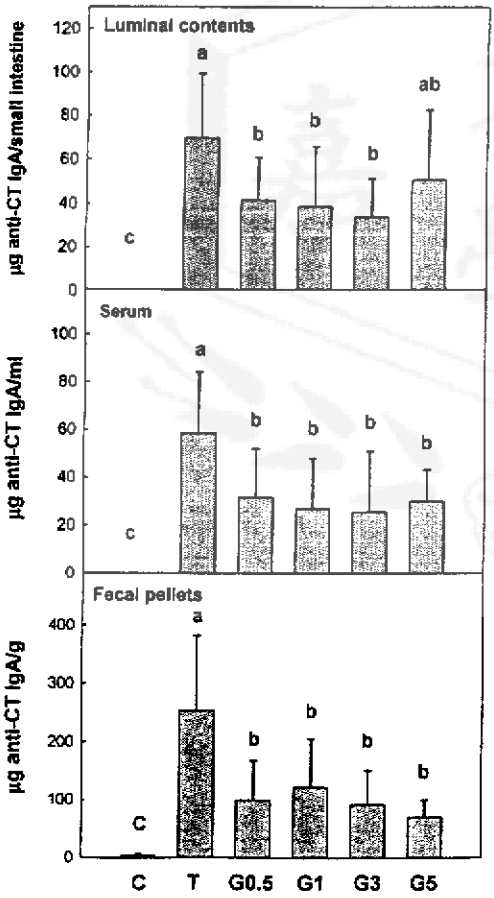

Figure 4. The anti-cholera toxin (CT) immunoglobulin A in luminal contents of small intestine, serum and fecal pellets in mice feeding control (C and T) or control diet containing 0.5 (G0.5), 1 (G1), 3 (G3) or 5% (G5) mycelium of *Ganoderma lucidum* for 4 weeks. Mice were orally immunized with 0.1ml of 0.2M $NaHCO_3$ containing 5 μg CT on day 7 and 21 of feeding period. Control mice were only received 0.1ml of 0.2M $NaHCO_3$ twice. Bars represent mean±SD; n=10 except 9 for T and G1. Within each group, bars not sharing a letter are different (P<0.05) according to Duncan's Multiple Range test.

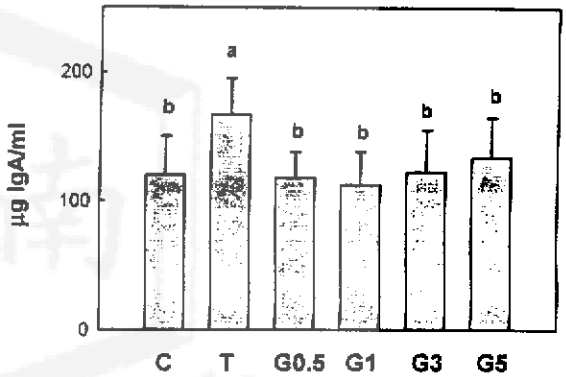

Figure 6. The serum total IgA in mice feeding contol (C and T) or control diet containing 0.5 (G0.5), 1 (G1), 3 (G3) or 5% (G5) mycelium of Ganoderma lucidum for 4 weeks. Mice were orally immunized with 0.1ml of 0.2M $NaHCO_3$ containing 5μg CT on day 7 and 21. Bar represent mean±SD; n=10 except 9 for C and G1. Within each group, bars not sharing a letter are different (P>0.05) according to Duncan's Multiple Range test.

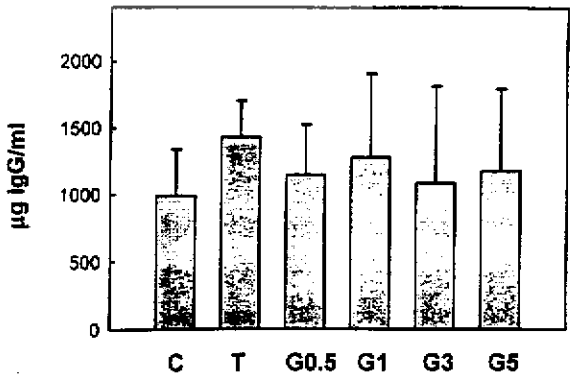

Figure 7. The serum total IgG in mice feeding control (C and T) or control diet containing 0.5 (G0.5), 1 (G1), 3 (G3) or 5% (G5) mycelium of Ganoderma lucidum for 4 weeks. Mice were orally immunized with 0.1ml of 0.2M $NaHCO_3$ containing 5μg CT on day 7 and 21. Bar represent mean±SD; n=10 except 9 for C and G1. Within each group, bars not sharing a letter are different (P>0.05) according to Duncan's Multiple Range test.