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篩選調節過敏反應食材之細胞培養及小鼠評估方法

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已知許多菇菌類食材具有免疫調節的功能，本研究以松杉靈芝、巴西洋菇、茯苓及樟芝等菇菌類為主要篩選材料，採用肥大細胞株RBL-2H3、BALB/c鼠脾臟細胞及DO11.10小鼠 (卵蛋白特異性T細胞受器基因轉殖鼠) 脾臟T細胞等培養方式進行篩選，再以抗原致敏動物餵食模式評估細胞培養篩選方式，建立並評估有效的篩選方法。結果顯示，松杉靈芝及巴西洋菇不影響RBL-2H3肥大細胞分泌組織胺，但茯苓及樟芝處理促進組織胺分泌。菇菌類處理可促進BALB/c鼠脾臟細胞分泌IL-2，並抑制IL-4分泌。以卵蛋白特異性Th1或Th2細胞進行評估，菇菌類顯著抑制Th2細胞激素分泌；並抑制卵蛋白T輔助細胞分化傾向Th2反應。動物試驗結果顯示，松杉靈芝、巴西洋菇及茯苓餵食降低卵蛋白致敏小鼠肺沖洗液中IL-5與嗜伊紅白血球聚集，並抑制Th2細胞激素分泌。綜合本研究結果，菇菌類可顯著減緩致敏小鼠呼吸道發炎，且抑制IL-4及IL-5等Th2細胞激素生成。卵蛋白特異性T輔助細胞與待測樣品共同培養，並分析細胞激素分泌量作為Th1及Th2之分化導向指標，是較為省時且有效的篩選方式。可作為食材調節過敏免疫反應之有效篩選方法。

關鍵字：肥大細胞，T輔助細胞，組織胺，細胞激素，菇菌類。

Evaluation of the Regulatory Effects of Dietary Materials on Allergic Immune Responses by *in vitro* and *in vivo* Assay

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In this study, we like to establish both *in vitro* and *in vivo* systems to study and explore the possible functional foods to regulate the allergic immune responses. It has been well documented that fungi might exert immuneomodulatory effect, and thus the *Ganoderma tsugae*, *Agaricus blazei*, *Poria cocos* and *Antrodia camphorate* were tested for allergic immunomodulatory effect, by using RBL-2H3 mast cell line, primary splenocyte form BALB/c, and OVA-specific $CD4^+$ T cells from T cell receptor (TCR) transgenic DO11.10 mice (naïve OVA-specific T cells). The results showed that ionomycin-stimulated histamine production form RBL-2H3 cells did not affect by *Ganoderma tsugae* and *Agaricus blazei*, but increased by *Poria cocos* and *Antrodia camphorate*. OVA-stimulated IL-2 secretion form Th1 cells were increased, but IL-4 and IL-5 secretion from Th2 cells was decreased by all the fungi samples. Furthermore, fungi treatment increased the IL-2 level and decreased Th2 cytokines production during naïve OVA-specific T cell differentiation *in vitro*. To further investigate the effect of fungi on airway inflammation, the allergen-sensitized mice challenged with aerosol allergen supplemented with *Ganoderma tsugae*, *Agaricus blazei* or *Poria cocos*. The data showed that the percentage of eosinophils, IL-5 and total protein content in bronchoalvelor lavage fluid (BALF), and IL-4, IL-5 levels produced by splenocytes were significantly decreased in mice fed with *Ganoderma tsugae*, *Agaricus blazei* and *Poria cocos*. Overall, these data suggested that the OVA-specific Th cell cultured with OVA plus samples, which could be as a screening method for anti-allergic immune responses substance.

Key words: RBL-2H3 cell, OVA-specific Th cell, Histamine, Cytokines, Mushroom.

前　　言

近年來，世界各國呼吸道過敏疾病(如：氣喘)逐漸增加且倍受重視。在台灣，包括過敏性氣喘在內的過敏疾病流行率高達1/3；台北市7-15歲學童之過敏疾病亦逐年增加，如氣

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喘病自1974年的1.3%到1984年增加為5.1%[1]，到1994年增為10.8%，異位性皮膚炎也自1.43%增為5.82%，過敏性鼻炎自1985年的7.84%增至33.5%，以過敏性氣喘增加之比例最高[2]。過敏性氣喘之發生起因於免疫系統接觸抗原後，啟動一連串的免疫反應。首先，抗原會活化T細胞及B細胞，受到活化的第二型T輔助(Th2)細胞分泌大量IL-4、IL-5等細胞激素，使免疫系統傾向Th2免疫反應。B細胞會生成大量IgE抗體與肥大細胞上之受器結合。當再次接觸特定抗原，與肥大細胞上IgE結合時，肥大細胞即會受活化，促使組織胺等介質釋放[3]，造成血管通透性增加及氣管收縮症狀，同時亦會吸引嗜中性球及嗜伊紅性白血球聚集於肺部，造成呼吸道局部性發炎反應[4,5]。

已知飲食習慣改變具影響過敏疾病的作用，如：高量炸油與飽和脂肪酸的攝取，對於過敏性氣喘小鼠有負面的影響[6]；相反的，單元不飽和脂肪酸、維生素E及松杉靈芝的補充對於人或小鼠呼吸道疾病具有正面的影響[7-8]。顯示飲食內容物足以改善過敏疾病之病情。菇菌類材料普遍使用於傳統飲食及中藥治療，且亦常見使用於保健食品中。已有許多研究證實菇菌類食材，如：靈芝、巴西洋菇等，有活化免疫細胞[9-11]，及抗腫瘤等生理活性[12-14]。在過敏免疫反應研究上，已知靈芝可顯著降低過敏介質生成及減緩過敏免疫反應[15-17]，然而，其餘菇菌類材料對過敏免疫反應調節之研究仍十分缺乏。因此，本研究採用松杉靈芝、巴西洋菇、茯苓及樟芝等菇菌類作為試驗材料，建立初步篩選調節過敏免疫反應食材的方法。

呼吸道發炎、組織胺分泌與Th2反應是過敏性氣喘的主要病理特徵，藉由降低過敏媒介物質與抑制Th2反應之分泌，可以減緩過敏性疾病之病況。因此，本研究欲採用RBL-2H3肥大細胞株作為篩選工具之一，RBL-2H3細胞株在IgE與Fc受器單株抗體或是鈣離子載體(如ionomycin)等刺激下，可以釋放大量組織胺；故可作為篩選減緩過敏反應食材的研究方法[18-22]。在動物致敏模式方面，也可誘發小鼠對過敏原的過度免疫反應，如：抗原特異性IgE抗體生成、呼吸道發炎反應[23,24]。然而，對於過敏免疫反應之試驗模式而言，動物試驗模式雖可整體反應食材對過敏性疾病的影響，但無法迅速且大量的篩選食材。為了建立且評估一有效的篩選方式，本研究以RBL-2H3細胞株與OVA特異性T細胞進行試驗，以篩選出能夠有效減緩過敏免疫反應之飲食材料，並評估篩選方式之效率。

材 料 與 方 法

一、材料與樣品前處理

本研究採用松杉靈芝、巴西洋菇、茯苓及樟芝等菇菌類樣品。巴西洋菇發酵液與茯苓發酵液取自於食品工業發展研究所菌種開發中心(新竹)，發酵液經冷凍乾燥取得固形物後，以PBS緩衝液定量(100 *mg/mL*)回溶，於80℃水浴中放置約30分鐘，取水溶液層進行細胞培養實驗。松杉靈芝(雙鶴集團勇健工業公司，台南)及樟芝(偉翔公司，台北)等粉末樣品，先以PBS定量回溶，配製條件如同巴西洋菇及茯苓，取水溶液部分作為細胞培養試驗材料。茶葉的水或酒精萃物由台大化學所郭悅雄老師提供，分別以PBS與酒精回溶作為試驗材料。

二、肥大細胞株培養

RBL-2H3細胞株是一basophilic leukemia細胞株，性質與肥大細胞相似。RBL-2H3肥大細胞採用15% FBS/MEM medium培養於96孔盤中，先以配製於0.5% FBS/MEM medium之食材樣品預處理24小時，再以500 *ng/mL* ionomycin (Sigam, St Louis, MO)作為刺激劑，與樣品共同培養五小時後，收集上清液進行組織胺濃度分析。組織胺含量以competitive immuno-assay方法，採用Histamine-ELISA kit (IBL, Hamburg, German)進行分析。

三、BALB/c小鼠初代脾臟細胞收集與培養

在無菌操作下取出脾臟，置於含培養基的培養皿內，分離脾臟淋巴細胞，分離方法如文獻所述[25]。5×10^6 *cells/mL*脾臟細胞，加入不同濃度之菇菌類與含裂殖素(10 *μg/mL* PHA，Sigma)培養液共同培養，5% CO_2，37℃之條件下培養48小時後，收取上清液進行細胞激素之分析。

四、脾臟$CD4^+$ T細胞收集與純化

卵蛋白特異性T細胞取自於DO11.10小鼠(OVA-specific TCR基因轉殖鼠)脾臟，並以經放射線處理過(2,700 *rad*, 9 *min*)之BALB/c鼠脾

臟細胞，作為抗原呈獻細胞(APC)。$CD4^+$ T細胞純化是採用SpinSet™ CD4+ T cell kit (StemCell technologise Inc., Vancouver, BC)。將處理好的DO11.10鼠脾臟細胞加入SpinSet™ antibody cocktail混勻反應15分鐘，待反應結束後以2% FBS/PBS buffer洗1次，SpinSet™ Dense Particle加入脾臟細胞液中反應20分鐘，再將細胞液輕輕的平鋪於SpinSet™ Density medium上層，以200 × *g*離心10分鐘，取出$CD4^+$ T細胞層，再以2% FBS/PBS buffer清洗後，加入10% FBS/RPMI-1640培養液即可進行培養。

五、$CD4^+$ T細胞培養及樣品處理

將純化出之$CD4^+$ T細胞以5×10^5 *cells/mL*培養於48孔盤中，分別加入Th1培養液(含2 *μg/mL* anti-IL-4)或Th2培養液(含1,000 *U/mL* IL-4)，與APC (2×10^6 *cells/mL* irradiated BALB/c splenocytes)、$OVA_{323-339}$ peptide (1 *μg/mL*)及IL-2 (10 *ng/mL*)，培養約7-10天後$CD4^+$ T細胞可分化為Th1或Th2細胞；再分別以OVA peptide作為特異性抗原，與菇菌類處理48小時後，收集細胞培養上清液分析細胞激素含量，以評估卵蛋白特異性T輔助細胞活性之篩選方法。

為評估樣品對OVA特異性T輔助細胞分化導向之影響，DO11.10鼠脾臟純化出之$CD4^+$ T細胞(5×10^5 *cells*)以1 *μg/mL* OVA peptide作為特異性抗原，同時以IL-2及樣品與細胞共同培養72小時後，收集上清液進行細胞激素分析，以Th1與Th2細胞激素分泌量作為T輔助細胞分化導向的指標。

六、致敏動物模式分組及試驗流程

BALB/c雌鼠八週大時依體重隨機分為六組，分別為控制組(OVA/H_2O)、OVA/GT (管餵11.7 *mg/mouse*松杉靈芝)、OVA/AB10 (管餵10 *mg/mouse*巴西洋菇)、OVA/AB50 (管餵50 *mg/mouse*巴西洋菇)、OVA/PC10 (管餵10 *mg/mouse*茯苓)及OVA/PC20 (管餵20 *mg/mouse*茯苓)，不同劑量菇菌類均溶於0.5 *mL*二次水中，每日餵食一次。

致敏動物試驗流程如先前研究所述[8]，於第7天、第17天及第27天分別於腹腔注射卵蛋白(2 *g*, 6 *g*, 6 *g*)為抗原，以含有$Al(OH)_3$之Imject® Alum (Pierce, Rockford, Ill., USA)為佐劑。並給予50 *mg/mL*卵蛋白溶液吸入性致敏二次，誘發呼吸道發炎反應。第二次吸入性致敏後24小時犧牲動物，收集肺沖洗液及脾臟細胞進行培養分析。

七、氣喘小鼠肺沖洗液收集與細胞組成

卵蛋白致敏小鼠窒息後，將靜脈置留管插入其氣管，以saline (0.9% NaCl)沖洗肺臟5次，收取約2.5 *mL*上清液進行IL-5、組織胺及總蛋白質等介質含量分析，肺沖洗細胞以trypan blue dye exclusion法染色，計數存活細胞數目，取2×10^5細胞以cytospin離心製作成細胞玻片，以LiuA及LiuB染劑進行細胞染色，風乾後以阿拉伯膠封片，再以放大1,000倍之油鏡判讀至少五個視野且總數200個以上之細胞數，並依細胞形態標準判讀肺沖洗細胞組成。

八、細胞激素及組織胺檢測

1. 細胞激素含量分析

自菇菌類餵食小鼠取得脾臟細胞，於24孔培養盤中注入5×10^6 *cells/mL*之細胞液，加入培養液或含裂殖素之培養液，培養48小時後收取上清液進行細胞激素之分析。細胞激素含量採用酵素連結免疫分析法(ELISA)，詳細分析方法如先前研究報告所述[25]。

2. 組織胺濃度測定

組織胺含量採用Histamine-ELISA試劑套組進行分析。分析方法為標準品及樣品先甲基化成為N-methyl histamine，以便和peroxidase- conjugated antigen antiserum競爭，與連結在分析盤底的goat anti-rabbit antibody結合。沖洗去未結合的物質後，加入基質p-NPP進行呈色反應。90分鐘後加入2 *N* NaOH終止反應，測450 *nm*吸光值。

九、統計分析

試驗結果是以平均值 ± 標準偏差(mean ± SD)表示，數據之統計分析是採用unpaired Student's t-test之統計方法分析各組間差異之顯著性。以SAS windows version 8.2 (SAS Insitute Inc., Cary, NC, USA.)之統計分析軟體進行數據之統計分析。

結 果 與 討 論

一、肥大細胞分泌組織胺作為篩選方法

已知肥大細胞活化且分泌組織胺是過敏病

症的重要反應，飲食補充可抑制肥大細胞分泌組織胺的食材，可能具減緩病情的作用。本研究首先採用多種菇菌類作為材料，結果如表一所示，松杉靈芝、巴西洋菇不影響ionomycin刺激下，RBL-2H3肥大細胞分泌組織胺，然而茯苓處理有促進組織胺分泌的現象，但未達顯著性差異，樟芝處理顯著增加組織胺分泌。由此結果推測，樟芝對過敏免疫反應可能較不具有減緩的效用。已有研究指出，靈芝固醇類及三萜類區分物顯著抑制大鼠週邊血液肥大細胞分泌組織胺[22,15]；松杉靈芝補充可減緩過敏免疫反應[8]，且松杉靈芝三萜類萃物補充可減緩過敏性氣喘小鼠呼吸道過度反應及發炎反應[17]。本試驗結果顯示，松杉靈芝及巴西洋菇等菇菌類水溶液，對肥大細胞分泌組織胺無顯著的抑制作用，推測可能是因為採用菇菌樣品水溶液處理細胞，水溶液中不包含固醇類及三萜類等抑制組織胺分泌之成分。

另採用茶葉酒精及水萃物作為對照(表一)，茶葉酒精萃物對組織胺分泌有減緩的現象，但與控制組無顯著差異。相反的，隨著茶葉水萃物處理劑量增加，組織胺濃度顯著上升。已有研究證實，gallic acid與EGCG等多酚類是綠茶中的抗發炎成分，可顯著降低肥大細胞活化與組織胺分泌[26,27]。但Maeda-Yamamoto等人亦指出，不同的茶葉品種或製程所得之茶湯，對肥大細胞分泌組織胺的影響不盡相同[28]。得知茶葉萃取物對過敏免疫反應的影響機制仍需更進一步的研究加以證實。此評估模式之試驗結果顯示，RBL-2H3細胞株分泌組織胺，可作為食材影響過敏免疫反應的篩選方法。

二、BALB/c鼠脾臟細胞活性作為篩選方法

T細胞是調節免疫反應的主要細胞群，促進Th1或抑制Th2免疫反應，對於過敏免疫反應均具有減緩的效用。以PHA刺激由BALB/c小鼠分離之脾臟細胞激素分泌量為100%，如表二所示，松杉靈芝與脾臟細胞共同培養，顯著促進IL-2並抑制IL-4分泌，但不影響IFNγ與IL-5分泌。高劑量巴西洋菇顯著增加IL-2分泌，但不影響IFNγ、IL-4及IL-5的分泌。高劑量茯苓發酵液顯著促進小鼠脾臟細胞生成IL-2，IFNγ分泌也有增加的趨勢($0.05 < p < 0.1$)；Th2細胞激素生成方面，茯苓處理顯著抑制IL-4分泌，不影響IL-5生成。樟芝顯著促進IL-2分泌，且有抑制IFNγ分泌的現象($p = 0.065$)，樟芝有抑制IL-4分泌的作用($p = 0.07$)，不影響IL-5分泌。

以上試驗結果顯示，菇菌類顯著促進IL-2，且抑制IL-4分泌，可調節Th1與Th2免疫反應，具有調節過敏疾病中T細胞免疫反應之潛能，尤以茯苓最為顯著。以裂殖素刺激BALB/c鼠脾臟細胞，T細胞雖被活化，但細胞激素分泌量低；因此，本研究接著採用對卵蛋白高度反應性的T輔助細胞進行樣品評估。

三、特異性T輔助細胞活性及分化導向作為篩選方法

取自DO11.10基因轉殖小鼠(OVA-specific TCR transgenic mice)之$CD4^+$ T細胞(T輔助細胞)，約有70%原始T輔助細胞對卵蛋白胜 片段($OVA_{323-339}$ peptide)具高度的專一性[29]；以卵蛋白、IL-2及適當之單株抗體共同培養，約

表一　食材對RBL-2H3肥大細胞株分泌組織胺之影響[1]

Table 1. The effects of food materials on histamine secretion from RBL-2H3 mast cell line

	Histamine[3] (*ng/mL*)					
(*μg/mL*)	*G. tsugae*	*A. blazei*	*P. cocos*	*A. camphorate*	TEA-EtOH[2]	TEA-H_2O[2]
0	275 ± 61	275 ± 61	275 ± 61	275 ± 61	249 ± 59	249 ± 59
50	290 ± 7	314 ± 68	345 ± 52	349 ± 36	233 ± 41	453 ± 124[a]
100	304 ± 38	316 ± 41	397 ± 19	376 ± 21[a]	225 ± 22	568 ± 49[a]
200	305 ± 50	332 ± 74	430 ± 138	359 ± 26[a]	208 ± 28	623 ± 43[a]
500	278 ± 65	300 ± 36	438 ± 123	329 ± 63	193 ± 52	660 ± 32[a]

1 RBL-2H3 mast cells (5×10^4 cells) were individually pretreated with each mushroom material for 24 *h*, and then stimulated with 500 *ng/mL* ionomycin for 5 *h*.

2 TEA-EtOH: the ethanol extracts of tea, TEA-H_2O: the water extracts of tea.

3 The data are representative for three independent experiments. Data with a superscript reveal significant difference at the 0.05 level compared with control group (0 *μg/mL*) by Student's t-test.

表二　菇菌類對BALB/c小鼠脾臟細胞分泌細胞激素之影響

Table 2. The effects of various mushroom on cytokine secretion by PHA-stimulated splenocytes from BALB/c mice

	Cytokine release effects (% of control)[1]			
(*μg/mL*)	IL-2[2]	IFNγ[2]	IL-4[2]	IL-5[2]
	G. tsugae			
0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
100	88 ± 4	101 ± 16	85 ± 7	80 ± 21
200	105 ± 1	106 ± 3	84 ± 9	68 ± 26
400	148 ± 8[a]	114 ± 17	73 ± 7[a]	72 ± 32
500	183 ± 26[a]	136 ± 39	84 ± 10	68 ± 28
	A. blazei			
0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
100	101 ± 8	89 ± 3	106 ± 5	87 ± 17
200	109 ± 15	93 ± 18	102 ± 6	97 ± 21
400	114 ± 12	89 ± 5	87 ± 12	92 ± 21
500	127 ± 17[a]	102 ± 11	100 ± 1	92 ± 30
	P. cocos			
0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
100	108 ± 21	115 ± 6	74 ± 1[a]	88 ± 11
200	123 ± 10	140 ± 17	59 ± 13[a]	97 ± 2
400	160 ± 32[a]	147 ± 1	53 ± 10[a]	89 ± 17
500	162 ± 26[a]	153 ± 51	54 ± 6[a]	85 ± 21
	A. camphorate			
0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
100	88 ± 21	67 ± 5	81 ± 9	89 ± 13
200	93 ± 1	71 ± 15	73 ± 11[a]	78 ± 34
400	117 ± 1	62 ± 22	68 ± 13	80 ± 28
500	146 ± 29[a]	62 ± 29	65 ± 9	88 ± 27

1 The data are representative for three independent experiments. Data with a superscript reveal significant difference at the 0.05 level compared with control group (0 *μg/mL*) by Student's t-test.

2 Cytokines level during Th cell differentiation were 174-300 *pg/mL* for IL-2, 182-789 *pg/mL* for IFNγ, 6-64 *pg/mL* for IL-4 and 0.13-1.85 *ng/mL* for IL-5.

7-10天分別分化為Th1或Th2細胞。

Th1細胞經卵蛋白刺激培養48小時後，可分泌大量的IL-2 (1-15 *ng/mL*)與IFNγ (38-66 *ng/mL*)；相對的，Th2細胞會分泌大量IL-4 (2-10 *ng/mL*)與IL-5 (2.5-7.5 *ng/mL*)；分泌量均顯著高於受PHA刺激BALB/c鼠脾臟細胞激素分泌濃度。如表三所示，松杉靈芝與茯苓顯著促進Th1細胞分泌IL-2，且降低IFNγ分泌。在Th2細胞活性方面，巴西洋菇及高劑量茯苓與樟芝處理顯著抑制IL-4及IL-5分泌。

上述結果顯示，Th1及Th2細胞分泌細胞激素，可分別受菇菌類處理影響，但細胞分化耗費時間長，為簡化試驗步驟與時程，並進一步探討菇菌類是否影響T輔助細胞的分化導向，因此採用卵蛋白特異性T輔助細胞與$OVA_{323-339}$ peptide及菇菌類共同培養72小時。如表四所示，松杉靈芝、巴西洋菇、茯苓與樟芝對T輔助細胞分化過程中，有增加IL-2分泌的現象，並顯著降低IFNγ、IL-4與IL-5生成。

本研究室另採用免疫奶粉純化蛋白進行試驗。已知免疫奶粉是一營養強化的奶類製品，利用疫苗在母牛身上接種，使牛隻的乳汁中富含特殊抗體，得以幫助胎牛對抗環境感染源。以免疫奶粉純化蛋白處理卵蛋白特異性T輔助細胞，IFNγ分泌量顯著高於未處理組[30]。以上結果顯示，OVA特異性T細胞培養模式下，並非所有受測食材均抑制IFNγ分泌，免疫奶粉純化蛋白質可促進T輔助細胞分泌IFNγ，菇菌類具抑制作用。

IFNγ是Th1細胞激素，可以活化巨噬細胞參與細胞性免疫反應；但Hansen等人[31]指出，IFNγ對Th2免疫反應傾向的過敏性呼吸道過度反應，無減緩的作用。亦有研究指出，眼睛過敏患者的淚液中，IL-4與IFNγ濃度均顯著高

表三　菇菌食材對OVA特異性第一型或第二型T輔助細胞分泌細胞激素之影響[1]
Table 3. The effects of various mushroom on cytokine release from Th1 or Th2 cells

	Cytokine release effects (% of control)[2]			
	Th1 cells		Th2 cells	
(*μg/mL*)	IL-2	IFNγ	IL-4	IL-5
	G. tsugae			
0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
100	153 ± 39	78 ± 9[a]	93 ± 9	102 ± 10
200	164 ± 29[a]	71 ± 13[a]	99 ± 13	103 ± 9
400	195 ± 69[a]	59 ± 10[a]	75 ± 13	80 ± 13
500	146 ± 36	47 ± 14[a]	74 ± 15	71 ± 22
	A. blazei			
0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
100	177 ± 85	85 ± 8[a]	91 ± 8[a]	80 ± 10[a]
200	181 ± 81	68 ± 6[a]	89 ± 6[a]	71 ± 5[a]
400	171 ± 103	64 ± 8[a]	79 ± 6[a]	49 ± 14[a]
500	135 ± 48	58 ± 10[a]	66 ± 9[a]	39 ± 12[a]
	P. cocos			
0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
100	196 ± 61	98 ± 15	103 ± 12	104 ± 36
200	233 ± 88[a]	86 ± 10	102 ± 6	128 ± 16
400	218 ± 99[a]	69 ± 10[a]	82 ± 11[a]	91 ± 12
500	171 ± 89	54 ± 11[a]	61 ± 18[a]	69 ± 27[a]
	A. camphorate			
0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
100	153 ± 41	68 ± 17[a]	88 ± 15	88 ± 6
200	135 ± 56	49 ± 10[a]	78 ± 21	66 ± 14[a]
400	105 ± 65	24 ± 8[a]	56 ± 25[a]	42 ± 22[a]
500	80 ± 44	11 ± 4[a]	44 ± 25[a]	31 ± 15[a]

1 The OVA-specific Th1 and Th2 cell were cultured as described in materials and methods.

2 The data are expressed as a relative cytokine production of control group values, which are representative for three independent experiments. Data with a superscript reveal significant difference at the 0.05 level compared with control group (0 *μg/mL*) by Student's t-test.

於正常人，且取自患者的纖維母細胞以IFNγ處理，ICAM-1表現量顯著增加，並促進發炎性細胞聚集[32]。因此推論，菇菌類降低IFNγ、IL-4與IL-5分泌，有助於減緩過敏性疾病。

由以上細胞培養試驗結果得知，樣品與T輔助細胞共同培養72小時，並測量細胞激素的分泌量反應T輔助細胞的分化導向的試驗結果，與Th1或Th2細胞各別處理之試驗結果相同，松杉靈芝、巴西洋菇、茯苓與樟芝等菇菌類可促進IL-2且抑制IFNγ、IL-4與IL-5分泌，且卵蛋白特異性T輔助細胞分化導向試驗模式是較佳的食材篩選方法。

四、菇菌類對卵蛋白致敏小鼠之免疫調節作用

Th2免疫反應與呼吸道發炎反應為過敏性氣喘之特徵。本研究在菇菌類食材補充的同時，採用卵蛋白致敏誘發過敏免疫反應與呼吸道發炎反應。呼吸道發炎包含免疫細胞聚集與發炎介質分泌，如表五所示，茯苓餵食顯著降低肺沖洗液中細胞數量，但松杉靈芝與巴西洋菇不影響肺沖洗液免疫細胞數量。松杉靈芝、巴西洋菇與茯苓餵食，顯著降低肺沖液 伊紅白血球比例，並顯著抑制肺沖洗液IL-5濃度。此外，組織胺及蛋白質亦是重要的發炎介質，菇菌類補充不影響肺沖洗液組織胺含量，但巴西洋菇與茯苓餵食顯著降低肺沖液中蛋白質濃度。已知嗜伊紅白血球是過敏性氣喘病症重要的細胞群，且嗜伊紅白血球聚集與IL-5濃度呈現正相關性[33]。亦有研究指出，在過敏原致敏下或是肺炎病患，肺沖洗液中蛋白質濃度會急劇上升[34,35]。推論菇菌類可能藉由降低IL-5生成，進而減少嗜伊紅白血球聚集，並降低蛋白質生成，達到減緩呼吸道發炎的作用。

脾臟細胞分泌細胞激素能力方面，如表六

表四　菇菌食材對OVA特異性T輔助細胞分化導向之影響

Table 4. The effect of various mushroom on Th1 or Th2 cytokine productions during OVA-specific naïve $CD4^+$ T cells differentiation

	Cytokine release effects (% of control)[1]			
(*μg/mL*)	IL-2[2]	IFNγ[2]	L-4[2]	IL-5[2]
	G. tsugae			
0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
100	204 ± 59	79 ± 20	91 ± 11	105 ± 35
200	203 ± 41	60 ± 13[a]	64 ± 19[a]	66 ± 12[a]
400	175 ± 97	48 ± 14[a]	38 ± 5[a]	55 ± 14[a]
500	176 ± 111	33 ± 10[a]	25 ± 6[a]	27 ± 16[a]
	A. blazei			
0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
100	162 ± 38	66 ± 12[a]	74 ± 7[a]	72 ± 6[a]
200	139 ± 36	57 ± 10[a]	48 ± 6[a]	32 ± 12[a]
400	126 ± 51	26 ± 4[a]	25 ± 6[a]	31 ± 18[a]
500	124 ± 70	18 ± 5[a]	23 ± 7[a]	33 ± 40[a]
	P. cocos			
0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
100	128 ± 9	86 ± 6[a]	91 ± 26	125 ± 33
200	156 ± 10	64 ± 6[a]	75 ± 19[a]	83 ± 14
400	157 ± 59	34 ± 12[a]	35 ± 12[a]	41 ± 12[a]
500	155 ± 94	25 ± 8[a]	26 ± 7[a]	20 ± 12[a]
	A. camphorate			
0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
100	161 ± 53	75 ± 20[a]	98 ± 11	94 ± 15
200	177 ± 60	51 ± 16[a]	75 ± 12[a]	69 ± 13[a]
400	217 ± 177	34 ± 7[a]	40 ± 8[a]	39 ± 13[a]
500	242 ± 135[a]	19 ± 8[a]	21 ± 1[a]	19 ± 4[a]

1 The results are expressed as the mean ± SD of four independent experiments. Data with a superscript reveal significant difference at the 0.05 level compared with control group (0 *μg/mL*) by Student's t-test.

2 Cytokines level during Th cell differentiation were 7.7-41.7 *ng/mL* for IL-2, 7.9-14.5 *ng/mL* for IFNγ, 0.25-0.94 *ng/mL* for IL-4 and 0.59-3.68 *ng/mL* for IL-5.

表五　菇菌類食材對OVA致敏小鼠肺沖洗液中細胞數量及媒介物生成之影響

Table 5. Effects of various mushroom on airway inflammatory mediator in BALF from OVA-sensitized and challenged BALB/c mice

	Cell no. (× 10^5 /*mice*)	Eosinophils (*%*)	IL-5 (*ng/mice*)	Histamine (*ng/mice*)	Protein (*mg/mice*)
OVA/H_2O	14.4 ± 4.4	8.57 ± 2.73	1.52 ± 0.44	4.75 ± 4.90	0.98 ± 0.53
OVA/GT	9.71 ± 7.86	3.33 ± 1.82[a]	0.92 ± 0.59[a]	4.22 ± 3.32	0.71 ± 0.31
OVA/AB10	10.0 ± 7.6	4.83 ± 1.25[a]	0.78 ± 0.52[a]	4.23 ± 4.62	0.58 ± 0.18[a]
OVA/AB50	11.3 ± 7.0	4.92 ± 2.00[a]	0.68 ± 0.29[a]	3.60 ± 2.75	0.60 ± 0.22[a]
OVA/PC10	6.9 ± 2.1[a]	4.64 ± 0.91[a]	0.52 ± 0.26[a]	3.65 ± 2.61	0.47 ± 0.18[a]
OVA/PC20	4.4 ± 1.6[a]	3.14 ± 0.91[a]	0.53 ± 0.26[a]	2.93 ± 3.28	0.32 ± 0.08[a]

1 BALF was collected 24 *h* after the last OVA inhalations. Cytospin preparations were examined for cellular content.

2 OVA/GT means that BALB/c mice were immunized and challenged with OVA, and supplemented with *G. tsugae*. OVA/AB and OVA/PC means that OVA-immunized mice supplemented with *A. blazei* and *P. cocos*, respectively.

3 The inflammatory mediators in BALF were assayed as materials and methods, and data were expressed as the total amount in each BALF from each mouse. [a] significant difference at the 0.05 level compared with OVA/H_2O group (0 *μg/mL*) by Student's t-test.

表六　菇菌類食材對卵蛋白致敏BALB/c小鼠脾臟細胞分泌細胞激素的影響[1]

Table 6. Effects of various mushroom on cytokine secretion from OVA-sensitized and challenged BALB/c mice splenocytes

Group	Mitogen	Non-specific		Specific
		Spontaneous	PHA	OVA
	N	IFNγ (*pg/mL*)[2]		
OVA/H_2O	9	55 ± 40	430 ± 158	65 ± 74
OVA/GT	8	27 ± 39	401 ± 266	41 ± 54
OVA/AB10	8	9 ± 17	759 ± 312	175 ± 84
OVA/AB50	8	18 ± 37	1057 ± 908	197 ± 204
OVA/PC10	7	4 ± 11	851 ± 419	171 ± 131
OVA/PC20	4	124 ± 204	2077 ± 1799[a]	454 ± 330[a]
		IL-4 (*pg/mL*)[2]		
OVA/H_2O	9	2.47 ± 2.33	11.21 ± 9.49	12.10 ± 4.23
OVA/GT	8	2.03 ± 1.67	9.74 ± 3.90	13.82 ± 4.20
OVA/AB10	8	0.47 ± 0.74[a]	9.64 ± 4.06	12.95 ± 7.83
OVA/AB50	8	0.22 ± 0.44[a]	7.84 ± 4.22	10.36 ± 6.64
OVA/PC10	7	0.18 ± 0.47[a]	11.50 ± 2.97	6.52 ± 4.10[a]
OVA/PC20	4	0.21 ± 0.42[a]	9.03 ± 3.47	1.97 ± 0.59[a]
		IL-5 (*pg/mL*)[2]		
OVA/H_2O	8	49 ± 16	130 ± 61	121 ± 55
OVA/GT	8	39 ± 8	138 ± 61	105 ± 48
OVA/AB10	8	33 ± 8[a]	136 ± 69	95 ± 33
OVA/AB50	7	29 ± 6[a]	112 ± 45	109 ± 48
OVA/PC10	7	34 ± 9[a]	120 ± 73	81 ± 18
OVA/PC20	3	25 ± 9[a]	57 ± 19	50 ± 6[a]

1 The 5×10^6 *cells/mL* splenocytes were cultured with the medium described earlier in the absence or presence of PHA (10 *μg/mL*) or OVA (25 *μg/mL*) for 48 hours. IFNγ, IL-4 and IL-5 levels were determined by ELISA method.

2 The detection limits for IFNγ and IL-5 are 75 *pg/mL*, and 3.9 *pg/mL* for IL-4.

3 The inflammatory mediators in BALF were assayed as materials and methods, and data were expressed as the total amount in each BALF from each mouse. Data with a superscript reveal significant difference at the 0.05 level compared with OVA/H_2O group (0 *μg/mL*) by Student's t-test.

所示，高劑量茯苓餵食顯著促進IFNγ分泌，松杉靈芝與巴西洋菇餵食不影響IFNγ分泌；Th2細胞激素分泌量方面，巴西洋菇與茯苓補充可顯著降低自發性IL-4與IL-5分泌量，且茯苓餵食亦顯著減少卵蛋白特異性IL-4與IL-5的分泌。先前的研究已指出，松杉靈芝餵食可促進BALB/c小鼠脾臟細胞分泌IL-2，並抑制IL-4生成[25]；且松杉靈芝補充對卵蛋白致敏BALB/c小鼠，亦具有促進IL-2且抑制IL-4自發性的分泌[8]，得知松杉靈芝作為保健用食材具有調節Th1/Th2平衡的效用。由以上結果得知，飲食中巴西洋菇與茯苓補充可抑制Th2細胞激素分泌，對於Th2免疫反應傾向的過敏性疾病具有顯著的減緩效果。

整合本研究體外細胞培養與卵蛋白致敏動物餵食之結果於表七，松杉靈芝、巴西洋菇及茯苓水溶液處理可促進IL-2分泌，且抑制Th2細胞分泌細胞激素，與Th2細胞分化導向，並減緩卵蛋白致敏小鼠呼吸道發炎反應。顯示松杉靈芝、巴西洋菇及茯苓等菇菌類食材，雖不顯著影響RBL-2H3肥大細胞分泌組織胺，菇菌類食材可降低Th2細胞活化與分化。已有研究指出，Th2細胞激素處理可誘發氣喘患者呼吸道平滑肌細胞分泌趨化激素，並促使肥大細胞聚集[36]。推測菇菌樣品對過敏性疾病具減緩病情的作用，可能是透過抑制Th2細胞激素分泌。此外，卵蛋白特異性T輔助細胞培養試驗中，樟芝處理可促使IL-2分泌，與抑制Th2細胞分化導向及活性，推測樟芝對於抗原特異性T輔助細胞也具有調節之作用。本研究結果顯示，食藥用菇菌類材料除了抗腫瘤[12,37-39]、活化單核細胞[40-42]及抗氧化[43-46]等生理活性外，菇菌類對於T輔助細胞亦具有調節的作用。

由表七之統整檢測各種評估方法之一致性與靈敏度，由動物試驗之結果顯示，組織胺釋放量未受影響並不代表呼吸道中IL-5與嗜伊紅

表七　細胞培養及小鼠評估過敏免疫反應結果
Table 7. Summary of allergic immune responses by *in vitro* and *in vivo* assay

	G. tsugae	*A. blazei*	*P. cocos*	*A. camphorate*
Cell culture models				
RBL-2H3 mast cell line	NS	NS	NS	↑
BALB/c primary splenocytes	↑IL-2 ↓ IL-4	↑IL-2	↑IL-2 ↓IL-4	↑IL-2 ↓IL-4
OVA-SpecificTh1 cells	↑IL-2 ↓IFNγ	↓ IFNγ	↑IL-2 ↓ IFNγ	↓ IFNγ
OVA-SpecificTh2 cells	NS	↓ IL-4, IL-5	↓ IL-4, IL-5	↓ IL-4, IL-5
OVA-Specific T cell differentiation	↓ IFNγ ↓ IL-4, IL-5	↓ IFNγ ↓ IL-4, IL-5	↓ IFNγ ↓ IL-4, IL-5	↑IL-2、↓IFNγ ↓ IL-4, IL-5
OVA-sensitized and challenged BALB/c mice				
BALF	↓ EO% ↓ IL-5 Histamine (NS) ↓ protein	↓ EO% ↓ IL-5 Histamine (NS) ↓ protein	↓ EO% ↓ IL-5 Histamine (NS) ↓ protein	*No investigation*
Splenocytes	Th1: NS Th2: NS	Th1: NS Th2: ↓ IL-4, 5	Th1:↑IFNγ Th2: ↓ IL-4, 5	*No investigation*

1 The symbol "↓" means decreased, "↑" means increased, NS means not significantly different and EO% indicated that the percentage of eosinophil in BALF.

白血球等發炎指標均不受影響，由此得知，單純由組織胺的釋放能力無法全面性的評估食材對於過敏性疾病之影響。松杉靈芝、巴西洋菇、茯苓及樟芝處理，可促進BALB/c脾臟細胞與卵蛋白特異性T細胞分泌IL-2，並降低IL-4分泌；二方法相較之下，卵蛋白特異性T細胞分泌之細胞激素濃度，及Th2細胞激素分泌受抑制之百分比較為顯著。Th1/Th2細胞培養與T輔助細胞分化導向二種評估方式，均反應動物試驗模式脾臟細胞激素分泌所呈現之結果，但以T輔助細胞分化導向之試驗方法較為省時且花費較低，因此作為調節過敏免疫反應食材篩選為佳。

結　　論

OVA特異性T細胞與待測樣品共同培養，並分析Th1及Th2細胞激素作為分化導向之指標，可以得知待測樣品是否可以顯著的藉由調節T細胞，進而達到減緩過敏免疫反應，是一省時且靈敏性高之篩選方法。由本研究之結果得知，菇菌類具有調節過敏免疫反應中T細胞的活性，可顯著的抑制Th2免疫反應且減緩呼吸道發炎之病症，是具有調節過敏免疫反應潛能之食材。

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