# 行政院國家科學委員會專題研究計畫成果報告

## 維生素 E 藉由調控巨噬細胞功能而促進 Th1 型免疫反應

Enhancement of Th1-type immune response via modulation of macrophage functions by vitamin E

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**一、中文摘要**

維生素 E 為一主要的脂溶性細胞膜抗氧化物，可保護細胞免受自由基之破壞。另外，維生素 E 亦可影響細胞內生化反應，但作用機制並不清楚。維生素 E 對於培養的淋巴球有保護與刺激作用，但原因並非是單純的由於減低細胞膜之 lipid peroxidation。有證據指出，維生素 E 亦可能為免疫調控物質 (immunomodulator)，對維持正常的免疫功能很重要。在探討老化的研究發現：年輕時以 Th1 型免疫反應為主，而年老時免疫反應轉而偏向 Th2 型免疫反應。由於維生素 E 有抗老化作用，又因為維生素 E 可促使 IL-2 及 IFN-γ之產生，吾人因而推論維生素 E 之抗老化作用可能是因促進了 Th1 型免疫反應所致。故維生素 E 很可能為 Th1 型免疫反應之一種調控物質。Th1 型免疫反應主要藉由 Th1 細胞分泌 cytokines 執行，如 IL-2、IFN-γ 等。去年的國科會計畫以探討維生素 E 調控淋巴球的反應為主，初步結果支持維生素 E 可刺激淋巴球。但是，另外有證據指出：以維生素 E 處理過之巨噬細胞和淋巴球培養在一起時，可增進淋巴球對 mitogens 及維生素 E 之反應。故維生素 E 可能刺激巨噬細胞釋放出 monokines 如 IL-1，以影響淋巴球的反應。另外已知，巨噬細胞亦可分泌 IL-12，有助於促進 Th1 型免疫反應。吾人因此提出假說：維生素 E 之促進 Th1 型免疫反應，除了可能直接調控淋巴球外，維生素 E 尚可藉由調控巨噬細胞，影響其功能，而促使淋巴球免疫反應趨於 Th1 型。結果證實維生素 E 在 lipopolysaccharide (LPS) 刺激下可增強巨噬細胞之 sarcoma cells 破壞能力，且巨噬細胞以維生素 E 併以適當濃度之 LPS 處理後與淋巴細胞培養可促使後者增生。然而以 enzyme linked immunosorbent assay (ELISA) 卻無法證實 IL-1 分泌有增加。進一步以 enzyme-linked immunospot (ELISPOT) 法亦無法證實維生素 E 及 LPS 處理過的巨噬細胞能刺激以 Th1 型為主的淋巴球免疫反應。

**關鍵詞：維生素 E，免疫調控，巨噬細胞，細胞素**

**Abstract**

Vitamin E (VE) is the major lipid soluble membrane antioxidant which protects the cell from the damage by free radicals. VE can influence intracellular activity, though these mechanisms are not clear. In addition, VE can protect and stimulate cultured lymphocytes and this is not simply due to the decrease of membrane lipid peroxidation. It is evident that VE can act as an immunomodulator which is essential in maintaining normal immunological activity. The investigations on aging show that Th1-type immune response is dominant when one is young and the immune response is switching to Th2 when one is getting old. Because VE has anti-aging effect and also because VE can induce IL-2 and IFN-γ production, we conclude that anti-aging effect of VE is due to it's enhancement of Th1-type immune response. Thus, VE can be an immunomodulator for Th1-type immune response whose effects are due to the release of cytokines: like IL-2, IFN-γ by

Th1 cells. The NSC project last year studied the modulation of lymphocytes by VE. The preliminary results support VE as a lymphocyte stimulator. However, other evidences indicate that the responsiveness of lymphocytes to mitogens and VE is enhanced when lymphocytes are cultured with VE-pretreated macrophages. Therefore, VE may stimulate macrophages to release monokines like IL-1 influencing lymphocyte response. Also, it is known that macrophages can release IL-12 which promotes Th1-type immune response. Therefore, it is hypothesized that in addition to direct lymphocyte modulation, VE can modulate macrophages influencing their functions which guides lymphocyte towards Th1 immune response. The results showed that in the presence of LPS, VE could enhance sarcoma cell-killing ability of macrophages. Also, macrophages, after treatment with VE and LPS, could enhance the proliferation of co-cultured lymphocytes. However, macrophage IL-1 secretion was not proved to be enhanced by ELISA, and lymphocyte responses were not dominated by Th1-type cytokines as measured by ELISPOT.

**Keywords**: vitamin E, immunomodulation, macrophage, cytokine

## 二、緣由與目的

維生素 E 為一主要的脂溶性細胞膜抗氧化物 (Bendich, 1990; Mahan & Escott-Stump, 1996)，可保護細胞免受自由基之破壞 (Packer, 1991; Meydani et al., 1992; Nachbar & Korting, 1995)。因此，維生素 E 對維持正常的細胞形態、構造、與細胞內活性很重要 (Rozewicka et al., 1991; Rubino et al., 1995; Wang et al., 1996; Karasu et al., 1997; Kuo et al., 1997)。除了抗氧化功能外，有證據指出，維生素 E 亦可能為免疫調控物質 (immunomodulator)，對維持正常的免疫功能很重要 (Bendich, 1990; Meydani et al., 1990; Penn et al., 1991; Oonishi et al., 1995; Traber & Packer, 1995)。另外，維生素 E 亦可影響細胞內生化活性 (Packer & Suzuki, 1993; Suzuki & Packer, 1993)。這些例子皆不能單純的以維生素 E 之抗氧化功能解釋，然而其作用機制 (mechanisms) 也並不是很清楚。另外，維生素 E 可為免疫調控物質 (Moriguchi et al., 1993; Langweiler et al. 1981)。維生素 E 除了可增進淋巴球對 mitogens 的反應 (Corwin & Shloss, 1980)，對於 in vitro 培養的淋巴球亦直接有保護與刺激作用，但原因並非是單純的由於維生素 E 減低細胞膜之 lipid peroxidation (Calder & Newsholme, 1993; Tu et al., 1995)。

在探討老化的研究發現：哺乳類及人類之免疫反應，出生之後以產生 IL-4、IL-10 之 Th2 型反應為主；到青壯年時以產生 IL-2、IFN-γ 之 Th1 型反應為主；而至年老時又以 Th2 型反應為主 (Pahlavani & Richardson, 1996; Lesourd, 1997; Shearer, 1997)。因此有人提出促進 IL-2 活性可返老還童之假說 (McCarty, 1997)。筆者亦曾發現，貓由 19 週齡步入 20 週齡時，其受刺激之淋巴球，所釋放之 IL-2 有顯著上升 (Lin, 1992)。由於維生素 E 有抗老化作用，又因為維生素 E 可促使 IL-2 之產生，並可提高 concanavalin A 刺激 T 淋巴球之作用 (Meydani et al., 1990; Anonymous, 1992; Sakai & Moriguchi, 1997)，同時也促進 IFN-γ之釋放 (Moriguchi et al., 1990)。吾人因而推論維生素 E 之抗老化作用可能是因促進了 Th1 型免疫反應所致，即維生素 E 有助年老者免疫反應由 Th2 型趨向 Th1 型。換言之，維生素 E 很可能為 Th1 型免疫反應之一種調控物質。最近在雞的一項研究指出，高劑量維生素 E 可提升 $CD4^+CD8^-$ T cells 數目 (Erf et al., 1998)，很可能此細胞族群即為 Th1 cells，惟作者沒進一步確認。

另外有證據指出：維生素 E 可抑制受 LPS 刺激的 Kupffer cells (肝的吞噬細胞) 釋放 tumor necrosis factor (TNF)-α，此 cytokine 可在肝造成病變；然而在移除維生素 E 之後，再以 LPS 刺激之仍舊無效，故維生素 E 的作用並非只是抗氧化 (Bellezzo et al., 1998)。以維生素 E 處理過之巨噬細

胞和淋巴球培養在一起時，可增進淋巴球對 mitogens 及維生素 E 之反應 (Oonishi et al., 1995)。巨噬細胞為抗原活化 T 細胞所須之抗原呈獻細胞 (antigen-presenting cell)，亦可分泌 monokines 如 IL-1 刺激 T 細胞。目前的研究顯示，雖然高劑量維生素 E 不影響體內巨噬細胞的數目 (Erf et al., 1998)，維生素 E 對維持與促進巨噬細胞功能卻很重要，如抑制 prostaglandin $E_2$ 產生 (Sakamoto et al., 1991; Romach et al., 1993)，增進吞噬作用 (Moriguchi et al., 1990)，與 IL-1 之分泌 (Romach et al., 1993; Politis et al., 1995)。故維生素 E 可能刺激巨噬細胞釋放出 IL-1，以影響淋巴球的反應。另外已知，巨噬細胞亦可分泌 IL-12，有助於促進 Th1 型免疫反應 (Janeway & Travers, 1997; Kuby, 1997)。最近的研究尚發現，IL-12 加上 IL-18 可進而再刺激巨噬細胞釋放出 IFN-γ (Munder et al., 1998)，這使得巨噬細胞和 Th1 型免疫反應更加密切。根據前面所述，吾人因此提出假說：維生素 E 之促進 Th1 型免疫反應，除了可能藉由直接調控淋巴球外，維生素 E 尚可能藉調控巨噬細胞，影響其功能，使免疫反應趨於 Th1 型。故本研究的重要性乃有助吾人了解維生素 E 如何藉由調控巨噬細胞功能而促進 Th1 型免疫反應，進而了解維生素 E 調控 Th1 型免疫反應之機制。

## 三、結果與討論

使用 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) 法來衡量細胞被刺激進而活化、分裂的程度。MTT 是種 tetrazolium salt，活細胞的粒線體可將 MTT 轉變成紫色結晶，4 小時後加入 sodium dodecyl sulfate 及 HCl 之 solvent 以溶解紫色結晶，置於 37°C incubator。隔日以 ELISA reader (wavelength：570 nm/690 nm) 讀其 absorbance (optical density) 值。活細胞的數目和液體顏色強度成正比關係。維生素 E 溶在 1% ethanol 成 5μM。結果證實維生素 E 在 LPS 刺激下可增強巨噬細胞之 sarcoma cells 破壞能力，且巨噬細胞以維生素 E 併以適當濃度之 LPS 處理後與淋巴細胞培養可促使後者增生。然而以 ELISA 卻無法證實 IL-1 分泌有增加。進一步以 ELISPOT 法了解維生素 E 及 LPS 處理過的巨噬細胞是否能刺激以 Th1 型為主的淋巴球免疫反應。使用孔內有 nitrocellulose base 之 96 孔盤 (Milititer HA; Millipore)。加入 50 μl 之抗 Th1-cytokine (IL-2, IFN-γ) 或抗 Th2-cytokine (IL-4, IL-10) 之 monoclonal antibody [10 μg/ml in phosphate- buffered saline (PBS), pH 7.4]，4°C，overnight。以無菌 PBS 洗二次，加入 200μl 含有 fetal calf serum 之 RPMI 1640 culture medium，置於室溫 1 hr 之後，然後加入待測之細胞 ($1 \times 10^5$/100μl/well)。培養 20 hr 後，以含有 0.05% Tween 20 之 PBS 洗二次，然後加入 50 μl 連有 biotin 之抗各種 cytokines 之 monoclonal antibodies。培養 37°C，90 分後，再洗四次，然後加入 poly-horse radish peroxidase conjugated avidin。培養 37°C，90 分後，洗四次，最後加入 substrate (4mg 3-amino-9-ethyl carbazole + 1ml dimethylformamide + 14ml acetic buffer, pH 5)。若細胞有 cytokines 分泌，則有呈色反應。用水沖洗以中止反應，乾燥後，以解剖顯微鏡計算呈色點數 (此即代表 cytokines 分泌細胞數目)。結果發現，以 LPS 刺激過的巨噬細胞在有無維生素 E 或 ethanol 存在下，其上清液培養過的淋巴細胞之各種 cytokines 之 ELISPOT 點數皆沒顯著不同。

## 四、計畫結果自評

結果證實維生素 E 在 LPS 刺激下可增強巨噬細胞之 sarcoma cells 破壞能力，且巨噬細胞以維生素 E 併以適當濃度之 LPS 處理後與淋巴細胞培養可促使後者增生。然而以 ELISA 卻無法證實 IL-1 分泌有增加。進一步以 ELISPOT 法亦無法證實維生素 E 及 LPS 處理過的巨噬細胞能刺激以 Th1 型為主的淋巴球免疫反應。可能所用 tests 靈敏度之問題或有其他干擾或參與因子如 prostaglandin，必須再近一步加以測試。

## 五、參考文獻

Anonymous. 1992. Vitamin E supplementation enhances immune response in the elderly. Nutr. Rev. 50:85-87.

Bellezzo, J.M., K.A. Leingang, G.A. Bulla, R.S. Britton, B.R. Bacon & E.S. Fox. 1998. Modulation of lipopolysaccharide-mediated activation in rat Kupffer cells by antioxidants. J. Lab. Clin. Med. 131:36-44.

Bendich A. 1990. Antioxidant micronutrients and immune responses. Ann. N.Y. Acad. Sci. 587:168-180.

Calder, P.C. & E.A. Newsholme. 1993. Influence of antioxidant vitamins on fatty acid inhibition of lymphocyte proliferation. Biochem. Mol. Biol. Int. 29:175-183.

Corwin L.M. & J. Shloss. 1980. Influence of vitamin E on the mitogenic response of murine lymphoid cells. J. Nutr. 110:916-923.

Erf G.F., W.G. Bottje, T.K. Bersi, M.D. Headrick & C.A. Fritts. 1998. Effects of dietary vitamin E on the immune system in broilers: altered proportions of CD4 T cells in the thymus and spleen. Poul. Sci. 77:529-37.

Janeway, C.A. & P. Travers. 1997. Immunobiology 3rd. Current Biology Ld., New York.

Karasu C., G. Ozansoy, O. Bozkurt, D. Erdogan & S. Omeroglu. 1997. Antioxidant and triglyceride-lowering effects of vitamin E associated with the prevention of abnormalities in the reactivity and morphology of aorta from streptozotocin-diabetic rats. Antioxidants in Diabetes-Induced Complications (ADIC) Study Group. Metabolism: Clin. Exp. 46:872-879.

Kuby, J. 1997. Immunology 3rd. W.H. Freeman and Company, New York.

Kuo J.H., H.W. Chen, R.G. Chou & C.K. Lii. 1997. Vitamin E protection of cell morphology under oxidative stress is related to cytoskeletal proteins in rat hepatocytes. Arch. Toxicol. 71:231-237.

Langweiler M., R.D. Schultz & B.E. Sheffy. 1981. Effect of vitamin E deficiency on the proliferative response of canine lymphocytes. Am. J. Vet. Res. 42:1681-1685.

Lesourd B.M. 1997. Nutrition and immunity in the elderly: modification of immune responses with nutritional treatments. Am. J. Clin. Nutr. 66:478S-484S.

Lin D.-S. 1992. Change in interleukin 2 production by lymphocytes during maturation of young cats. Comp. Immunol. Microbiol. Infect. Dis. 15:301-304.

Mahan, L.K. & S. Escott-Stump. 1996. Krause's Food, Nutrition, & Diet Therapy 9th Ed.

McCarty, M.F. 1997. Promotion of interleukin-2 activity as a strategy for 'rejuvenating' geriatric immune function. Med. Hypotheses 48:47-54.

Meydani, S.N., M.P. Barklund, S. Liu, M. Meydani, R.A. Miller, J.G. Cannon, F.D. Morrow, R. Rocklin & J.B. Blumberg. 1990. Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects. Am. J. Clin. Nutr. 52:557-563.

Meydani, M., W. Evans, G. Handelman, R.A. Fielding, S.N. Meydani, M.A. Fiatarone, J.B. Blumberg & J.G. Cannon. 1992. Antioxidant response to exercise-induced oxidative stress and protection by vitamin E. Ann. N.Y. Acad. Sci. 669:363-364.

Moriguchi, S., N. Kobayashi & Y. Kishino. 1990. High dietary intakes of vitamin E and cellular immune functions in rats. J. Nutr. 120:1096-1102.

Moriguchi, S., H. Miwa, M. Okamura, K. Maekawa, Y. Kishino & K. Maeda. 1993. Vitamin E is an important factor in T cell differentiation in thymus of F344 rats. J. Nutr. Sci. Vitaminol. 39:451-463.

Munder M., M. Mallo, K. Eichmann & M. Modolell. 1998. Murine macrophages secrete interferon γ upon combined stimulation with interleukin-12 and IL-18: a novel pathway of autocrine macrophage activation. J. Exp. Med. 187:2103-2108.

Nachbar, F. & H.C. Korting. 1995. The role of vitamin E in normal and damaged skin. J. Mol. Med. 73:7-17.

Oonishi, K., S. Moriguchi & Y. Kishino. 1995. The role of macrophages in increased mitogen response of rat splenic lymphocytes following in vitro incubation with vitamin E. J. Nutr. Sci. Vitaminol. 41:445-453.

Packer L. 1991. Protective role of vitamin E in biological system. Am. J. Clin. Nutr. 53:1050S-1055S.

Packer L. & Y.J. Suzuki. 1993. Vitamin E and alpha-lipoate: role in antioxidant recycling and activation of the NF-kappa B transcription factor. Mol. Asp. Med. 14:229-239.

Pahlavani, M.A. & A. Richardson. 1996. The effect of age on the expression of interleukin-2. Mech. Ageing Dev. 89:125-154.

Penn N.D., L. Purkins, J. Kelleher, R.V. Heatley, B.H. Mascie-Taylor & P.W. Belfield. 1991. The effect of dietary supplementation with vitamins A, C, and E on cell-mediated immune function in elderly long-stay patients: a randomized controlled trial. Age Ageing 20:169-174.

Politis, I., M. Hidiroglou, T.R. Batra, J.A. Gilmore, R.C. Gorewit & H. Scherf. 1995. Effect of vitamin E on immune function of dairy cows. Am. J. Vet. Res. 56:179-184.

Romach E.H., S. Kidao, B.G. Sanders & K. Kline. 1993. Effects of RRR-α-tocopheryl succinate on IL-1 and $PGE_2$ production by macrophages. Nutr. Cancer 20:205-214.

Rozewicka L., B. Barcew-Wiszniewska, J. Wojcicki, L. Samochowiec & B. Krasowska. 1991. Protective effect of selenium and vitamin E against changes induced in heart vessels of rabbits fed chronically on a high-fat diet. Kitasato Arch. Exp. Med. 64:183-192.

Rubino A., A. Loesch, M.A. Goss-Sampson, P. Milla & G. Burnstock. 1995. Effects of vitamin E deficiency on vasomotor activity and ultrastructural organisation of rat thoracic aorta. Br. J. Pharmacol. 115:415-420.

Sakai S. & S. Moriguchi. 1997.Long-term feeding of high vitamin E diet improves the decreased mitogen response of rat splenic lymphocytes with aging. J. Nutr. Sci. Vitaminol. 43:113-22.

Sakamoto W., K. Fujie, H. Handa, J. Nishihira & M. Mino. 1991. Vitamin E inhibits $PGE_2$ and $O_2^-$ production in rat peritoneal macrophages. Bioch. Biophy. Acta 1074:251-255.

Shearer G.M. 1997. Th1/Th2 changes in aging. Mech. Ageing Dev. 94:1-5.

Suzuki Y.J. & L. Packer. 1993. Inhibition of NF-kappa B activation by vitamin E derivatives. Biochem. Biophy. Res. Commun. 193:277-283.

Traber M.G. & L. Packer. 1995. Vitamin E: beyond antioxidant function. Am. J. Clin. Nutr. 62(6 Suppl):1501S-1509S.

Tu K.Y., R. Matthews & K.S. Matthews. 1995. Protective effect of vitamin E on lymphocyte growth capacity during incubation in vitro. Mech. Ageing Dev. 82:129-148.

Wang S.T., J.H. Kuo, R.G. Chou & C.K. Lii. 1996. Vitamin E protection of cell morphology and protein thiols in rat hepatocytes treated with tert-butyl hydroperoxide. Toxicol. Letters 89:91-98.