• 計畫中文名稱	以 shRNA 表達載體擊低貝它一型轉型生長因子的表現探討腦部神經膠質細胞調控神經性犬蛔蟲症發生似阿茲海默症神經退化之分子機轉(I)		
• 計畫英文名稱	Molecular Mechanism of Cerebral Neuroglial Cells Manipulate Neurodegenerative Development of Alzheimer's-Like Changes in Neurotoxocariasis Assessed by Small-Hairpin RNA Expressing Vector for Transforming Growth Factor-B1 Knockdown (I)		
• 系統編號	PC9706-0743	• 研究性質	基礎研究
• 計畫編號	NSC96-2628-B038-004-MY3	• 研究方式	學術補助
• 主管機關	--	• 研究期間	9708 ~ 9807
• 執行機構	臺北醫學大學寄生蟲學科		
• 年度	97 年	• 研究經費	1677 千元
• 研究領域	醫學技術		
• 研究人員	范家？		
• 中文關鍵字	神經性犬蛔蟲症；貝它一型轉型生長因子；shRNA；擊低；發炎細胞激素；神經退化相關因子；		
• 英文關鍵字	Neurotoxocariasis；Transforming growth factor β1；short hairpin RNA；Knockdown；Inflammatory cytokines；Neurodegenerative factors；		
• 中文摘要	主持人國科會 2 年計畫「NSC 94-2320-B-038 -025；NSC 95-2320-B-038-007」初步成果顯示：「犬蛔蟲幼蟲入侵鼷鼠腦部後，似乎可誘使 TGF-β1、GFAP、S100β、TG2、NF-L、 AβPP 與 Tau 等神經退化相關的蛋白質與 mRNA 異常表現，顯示可造成似阿茲海默症神經退化之發生。」(見五年成果說明)。是否 TGF-β1 調控腦部神經膠質細胞導致似阿茲海默症的發生，需要進一步探究。故我們將利用三年時間，以 shRNA vector 擊低 TGF-β1mRNA 的表現，來探討相關的分子機轉。實驗設計略爲：第一年：探究犬蛔蟲幼蟲抗原本身是否可激活神經膠質細胞與神經元細胞株產生發炎細胞激素(IL-1β, TNF-α 與 NO)與神經退化相關因子(TGF-β1、GFAP、S100β、TG2、NF-L、AβPP、Aβ 與 Tau)之蛋白質與基因層次的表現。第二年：搜尋 Ambion web site，委請生技公司合成 4 條 nucleotides，構築成 TGF-β1shRNA vector 後，轉染神經膠質細胞與神經元細胞株，再以最高濃度的蟲體抗原共同培養後，分析 TGF-β1mRNA 的表現，選出最佳擊低 TGF-β1 的 shRNA vector 遞入細胞株 5-6hrs 後，再進行同第一年的實驗。 (三)、第三年：鼷鼠以電鑽於側腦室鑽出一小孔，在電動幫浦輔助下以針筒緩緩送入 10 μg 擊低效果最佳的 shRNA vector 一天後，再進行感染。各組於感染第一天、第一週、第四週與第八週後摘取腦部，進行「病理變化」、「組織免疫化學染色」、「幼蟲回收試驗」、 WB 與 RT- PCR 分析發炎細胞激素與神經退化相關因子的表現情形。		
• 英文摘要	Our preliminary results of 2-year grants (NSC 94-2320-B-038 -025；NSC 95-2320-B-038-007) indicated that protein and mRNA expressions of		

	neurodegenerative biomarkers i.e., TGF-β1、GFAP、S100β、TG2、NF-L、AβPP and Tau were significantly increased in the brain invaded by Toxocara canis larvae in mice that it seemed likely the development of Alzheimer-like diseases in neurotoxocariasis. It needs further studies on whether TGF-β1 played an important role in manipulation of neuroglial cells to development of Alzheimer-like diseases. Based on these results mentioned above, we will apply shRNA vector for TGF-β1 knockdown to investigate underline molecular mechanism. The experimental protocol is briefly described as follows: The first year: To investigate whether Toxocara canis larvae per se and their excretory/secretory antigens (TcES) may trigger or activate neuroglial cells and neurons to produce inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α, and NO) as well as neurodegenerative factors (TGF-β1、GFAP、S100β、TG2、NF-L、AβPP、Aβand Tau) in protein and genic manners. The second year: Search Ambion web site to design 4 sense and anti-sense nucleotides to construct double strand short hairpin RNA for TGF-β1knockdown. They are used to transfect neuroglial cells and neurons and are co-cultured with the highest concentration of TcES to select the most effective TGF-β1knockdown vector by analyzing TGF-β1mRNA expression with RT-PCR. When the best shRNA vector is selected, this vector will be delivered to neuroglial cells and neurons for 5-6 hrs in advance to knock down TGF-β1mRNA expressions. Thereafter, the experimental protocol will be the same as that undertook in the first year experiment. The third year: After punch a small hole in lateral ventricle of the mouse brain, 10 μg shRNA vector selected in the second year experiment will be delivered to the brain for 24 hrs through syringe aided by automatic pump; thereafter, these mice will be orally inoculated with Toxocara canis embryonated eggs. The infected mice will be sacrificed and the brain will be taken and divided to about 4 parts to investigate the pathological changes, immunohistochemical expressions, larval numbers and applyingWB and RT-PCR to assess the inflammatory cytokines and neurodegenerative factors expressions after 1 day, and 1, 4, and 8 weeks post infections.