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探討趨化因子促使感染廣東住血線蟲之嗜酸性白血球通過 血腦障壁之研究 研究成果報告(精簡版)

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計畫主持人：李秀雄
共同主持人：賴世展
計畫參與人員：此計畫無其他參與人員：
此計畫無其他參與人員：

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Chemokines mediates enhanced transmigration of *Angiostrongylus cantonensis*-infected eosinophils across the blood-brain barrier

探討趨化因子促使感染廣東住血線蟲之嗜酸性白血球通過血腦障壁之研究

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中文摘要

血液-中樞神經系統障壁(blood-central nervous system barrier, blood-CNS barrier)的限制(或選擇)，此屏障主要分成血-腦屏障 (blood-brain barrier, BBB) 及血-腦脊髓液障壁 (blood-cerebrospinal fluid barrier, blood-CSF barrier)兩個細胞性屏障 (cellular barrier)。BBB結構是由腦和脊髓內毛細血管(capillary)之單層內皮細胞(endothelial cells)緊密連接(tight junction)組成，使大分子不能通過，但水和某些離子仍能通過。Blood-CSF barrier結構主要是脈絡叢(choroids

plexus)之單層上皮細胞之間緊密連接。一些分解酵素參與腦障壁(brain barrier)的破壞，其中包含基質金屬蛋白酶(matrix metalloproteinases, MMPs)，會分解細胞外質(extracellular matrix, ECM)，增加BBB的通透性，媒介腦部的發炎反應，引起神經發炎。本研究顯示以廣東住血線蟲感染 BALB/c小白鼠，誘發嗜伊紅性腦膜炎或嗜伊紅性腦膜腦炎(eosinophilic meningoencephalitis)的模式，趨化因子 CCL-2 與細胞外質fibronectin分解有密切關係。

關鍵詞：廣東住血線蟲、腦膜炎、腦

膜腦炎、趨化因子

英文摘要

Blood-central nervous system barrier (blood-CNS barrier) consist of two cellular barriers, blood-brain barrier (BBB) and blood-cerebrospinal fluid (blood-CSF) barrier is built up by a monolayer of endothelial cells lining the brain capillaries and restricting the blood and the brain interstitial fluid. The second barrier, blood-CSF barrier is built up by a monolayer of epithelial cells of the choroids plexus separating the blood from the CSF. Some proteolytic enzymes are involved in the disruption of brain barriers. Matrix metalloproteinase-9 (MMP-9) has been identified an essential role in the breakdown of the extracellular matrix

(ECM) macromolecules、BBB damage、as a mediator of brain inflammatory reaction and induce neuro-inflammatory diseases. Our study showed that use the BALB/c mice infected with *Angiostrongylus cantonensis* induced-meningitis as a model. We demonstrate the correlation between chemokines CCL-2 and fibronectin degradation.
Keywords: *Angiostrongylus cantonensis*; meningitis; meningoencephalitis; chemokines

前言

廣東住血線蟲(*Angiostrongylus cantonensis*)是一種寄生在大鼠心臟

及肺動脈血管的線蟲，屬於人畜共通的寄生蟲(zoonotic parasites)。主要分佈在東南亞和南太平洋一帶，台灣整個島嶼幾乎都有此寄生蟲的存在，每年都有因飲食不當而感染廣東住血線蟲的病例報告出現(Yii, 1976; Tsai et al., 2004)。人類的感染主要是由於吃蝸牛肉而意外感染(Alicata, 1965)，此寄生蟲發育中的幼蟲會侵入人類的 CNS，造成神經細胞脫髓鞘(demyelination) (Hwang et al., 1993)，小腦浦金氏細胞(Purkinje cells)喪失、損傷及空泡化(Perez et al., 1989; Yoshimura, 1993)，嗜伊紅性腦膜炎(eosinophilic meningitis) (Hsu et al., 1990; Ismail and Arsura, 1993)或嗜伊紅性腦膜腦炎(eosinophilic meningoencephalitis) (Gardiner et al., 1990)等病理現象。

廣東住血線蟲感染非適當宿主(如人類或鼯鼠)，發育中的幼蟲會侵入中樞神經系統(central nervous system, CNS)中並發育至未成熟的成蟲(immature adult)，約在感染後三週，CSF 中的嗜伊紅性白血球(eosinophil)會達到高峰，並伴隨嗜伊紅性腦膜炎的病徵(Sugaya and Yoshimura, 1988; Sasaki et al., 1993)。

趨化因子(chemokines)是由不同類型的細胞分泌的低分子量(8-10 kD)的細胞因子，趨化因子和其受體(receptor)的相互作用參與細胞的生長、發育、分化、凋亡和分佈等多種生理功能，並在多種病理過程中發揮重要作用，如發炎過程中淋巴細胞的移行、人類免疫缺乏病毒(human immunodeficiency virus，

HIV，俗稱愛滋病毒)感染、移植排斥、類風濕疾病等(Lane et al., 2006; Stamatovic et al, 2006)。炎症性趨化因子是在前炎症性細胞因子或病原因子的刺激下，由發炎部位的固有細胞和滲出的細胞所產生的，它們的主要任務是作用細胞(包括單核細胞、顆粒細胞和作用細胞)的募集。內環境穩定性趨化因子是由分散的淋巴或非淋巴組織微環境(如皮膚、黏膜)所產生，它們主要參與血細胞生成、抗原處理、免疫監視過程中細胞的生理性移行和定位(Loetscher et al., 2000; Sallusto et al., 2000; Campbell and Butcher, 2000)。趨化因子中 CC 家族的趨化因子 CCL2 (過去稱為 monocyte chemoattractant protein-1, MCP-1，現在歸類在 CC subfamily)會刺激單

核球釋出基質金屬蛋白酶-9(matrix metalloproteinase-9, MMP-9)，促使白血球通過基底膜移行至作用部位，而抑制 TNF- α 則會抑制趨化因子減少 MMP-9 蛋白的分泌(Robinson et al., 2002)。

目前已知 BBB 的損壞常伴隨基底膜上細胞外質(extracellular matrix, ECM)的分解，即 ECM 的分解會導致 BBB 被破壞形成缺口(Rosenberg et al., 1993; Robert and Robert, 1998)。ECM 是由三類生物大分子組成：(一) 膠原(collagen)：是富含脯氨酸(proline)和甘氨酸(glycine)的蛋白分子，由三條 α 肽鏈組成的螺旋狀結構，然後再相互交聯形成纖維狀(間質性膠原)或網狀(基底膜膠原)結構，而膠原的降解大部分在細胞外進行(大約 15%在細胞內進行)(Timpl et al., 1981;

Butkowski et al., 1987; Yurchenco and Schittny, 1990)。(二) 非膠原糖蛋白(non-collagenous glycoprotein)：包括纖維連結蛋白(fibronectin)、層粘連蛋白(laminin)及細胞粘合素(tenascin)、副層連蛋白(entactin)、粗纖維調節素(undulin)、血栓粘合素(thrombospondin)等。它們的分子具有多個功能區，能與其他 ECM 及多種細胞受體結合，從而對細胞的生長、分化及代謝產生重要調控作用，它們可由肝細胞、間質細胞(主要是貯脂細胞)所產生(Paulsson et al., 1987a; Yurchenco and Schittny, 1990)。(三) 蛋白多糖(proteoglycan)：是一類由蛋白質做骨架側鏈為糖胺多糖(glycosaminoglycan)的分子。依側鏈不同可分為硫酸乙酰肝素(heparan sulfate) (Paulsson et al., 1987b)、硫酸

皮膚素(dermatan sulfate)、硫酸軟骨素(chondroitin sulfate)及硫酸角質素(keratan sulfate) (Yurchenco and Schittny, 1990; Ard and Faissner, 1991)。

本研究擬以廣東住血線蟲感染嗜酸性白血球細胞株，誘發 BBB 損傷的模式進行研究。將探討趨化因子與細胞外質(collagen、laminin and fibronectin)分解的關係。

材料與方法

一. 廣東住血線蟲第三期幼蟲(AcL₃)

之收集：

大白鼠感染廣東住血線蟲後，糞便中會含廣東住血線蟲的第一期幼蟲(AcL₁)，收集糞便中的AcL₁感染水螺(*Biomphalaria glabrata*)，待

AcL₁發育至第三期幼蟲(AcL₃)時，將水螺外殼碾碎，取其組織，剁碎。用組織均質器絞碎，以 1:30 (組織:消化液)比例加入人工胃蛋白酶消化液(pepsin, Sigma, USA)，以磁性攪拌子於 37°C 之恆溫箱中，均勻攪拌消化 2 小時。以雙層紗布濾去雜質，加入生理食鹽水稀釋並靜置，每隔 30 分鐘倒去約一半的上清液，再加入生理食鹽水稀釋靜置，重複上述步驟至完全清澈為止。以滴管吸取下層之沈澱物，置於玻璃皿中，在解剖顯微鏡下，觀察並吸取AcL₃。每 50 隻AcL₃為一單位，置於玻璃培養皿中。

依照本實驗室先前的研究成果 (Chen et al., 2004)。簡述如下，將檢體(小白鼠腦部組織均質液或細胞培養液)在 4°C，12,000 g 離心 10 分鐘，取上清液並進行定量。將萃取出之新鮮蛋白質，填入 SDS 電泳膠片進行電泳。電泳結束後，將膠片上之蛋白轉移至 nitrocellulose membrane。利用 primary antibody 及 horse radish peroxidase (HRP)-conjugated secondary antibody 進行反應，以 ECL (enhanced chemiluminescence)檢測蛋白質。

結果

二、西方點墨法(Western blotting)

蛋白質(chemokines、collagen、laminin and fibronectin)的檢測方法

趨化因子 CCL-2 的變化

本研究以廣東住血線蟲感染鼯鼠，誘發寄生蟲性腦膜腦炎，藉由觀

察鼯鼠發炎細胞滲入蜘蛛膜下腔中的發炎反應與趨化因子 CCL-2 的關係，結果顯示廣東住血線蟲造成的嗜伊紅性白血球增多症 CCL-2 表現會增加 (Fig. 1)。

細胞外質 fibronectin 的變化

細胞外質在廣東住血線蟲引起寄生蟲性腦膜腦炎過程中發生改變，感染廣東住血線蟲第 15 天 fibronectin 開始分解，fibronectin monomer 在第 15 至 25 天明顯的增加 ($P < 0.05$) (Fig. 2)。

討論

廣東住血線蟲是引起嗜伊紅性腦膜炎與嗜伊紅性腦膜腦炎的主要原因。在非適當宿主(例如鼯鼠或人類)感染此線蟲都伴隨有神經系統的傷害，此乃蟲體僅在腦部發育成第四或第五期未成熟幼蟲，無法進一步發育

為成蟲，並在腦部造成機械式及細胞毒性之傷害引起。根據近年來之研究，感染廣東住血線蟲後發現鼯鼠腦部組織有顯著的 NF- κ B 及 iNOS 大量表現 (Lee *et al.*, 2000)。NF- κ B 蛋白活化表現調節中樞神經系統的發炎反應 (Grilli and Memo, 1997)，已知它媒介活化免疫及發炎反應的標的基因，包含 cytokines、chemokines、MHC class I、iNOS、COX-2、P53 及 IAP (Grilli *et al.*, 1993; Baldwin, 1996; Wu and Lozano, 1994; Uberti *et al.*, 1998)。本研究證實廣東住血線蟲感染後趨化因子明顯增加。

寄生蟲生活史中有不同的階段(期)，在生活史的特定階段表現特定的抗原，產生特異性反應，所以在寄生蟲感染中，免疫反應在不同的寄生蟲及不同的感染期是不同的，加上寄生

蟲抗原成分多並經常發生抗原變異，
 由此可知，寄生蟲感染誘發宿主產生
 免疫反應的機制相當複雜。細胞激素
 可做為免疫反應的介質，既參與抗體
 媒介的免疫也參與細胞媒介的免疫，
 在免疫反應過程中有關鍵作用。

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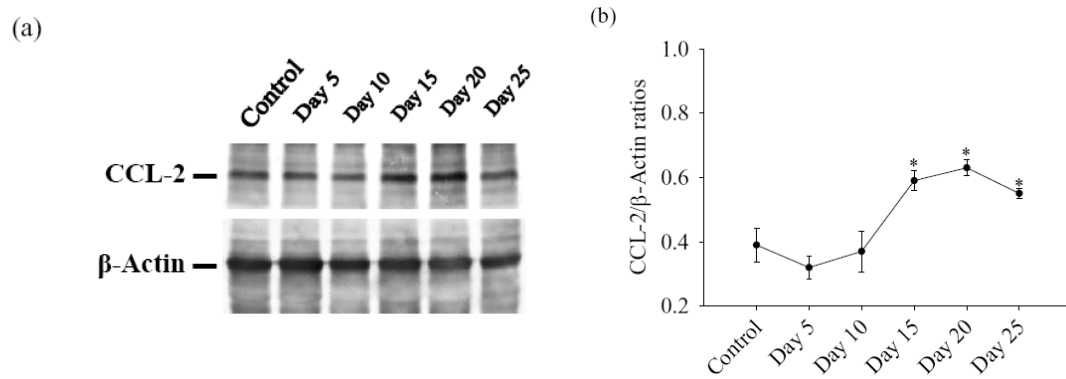


Fig 1. The protein levels of CCL-2 in the brain. (a) The CCL-2 was present at all time points, showing significant degradation on days 15, 20, and 25 PI. β -actin was used as a loading control. (b) Quantitative analysis of the bands were performed with a computer-assisted imaging densitometer system.

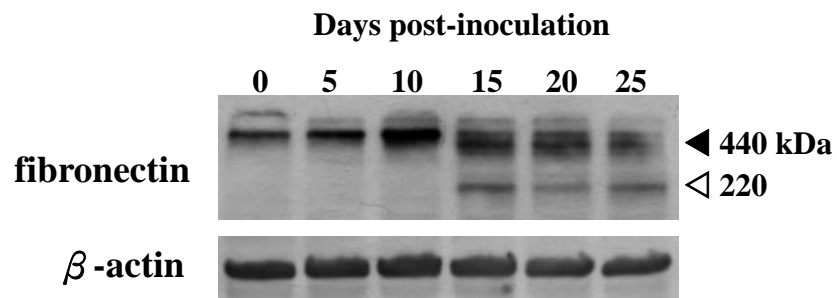


Fig 2. The protein levels of fibronectin in the brain. The 440 kDa fibronectin dimer was present at all time points, showing significant degradation on days 15, 20, and 25 PI. The 220 kDa fibronectin monomer was increased on days 15, 20, and 25 PI. β -actin was used as a loading control.