台灣鋏蠓過敏原Tropomyosin之分子選殖及免疫特性分析

江啟峰、江主惠、李美芳

E-mail: 322199@mail.dyu.edu.tw

摘 要

研究背景: 台灣鋏蠓是一種體型微小的吸血昆蟲，廣泛分布於台灣各地。如同蚊子叮咬，台灣鋏蠓叮咬後也會造成過敏性反應。已知非脊椎動物的tropomyosin 和甲殼類、塵? B線蟲及許多軟體綱動物之間都具有交互作用。本研究的目的包括二部分: (一)利用分子選殖方式製備台灣鋏蠓重組tropomyosin 及分析其免疫特性；(二)利用人類皮膚纖維母細胞Hs 68 細胞株為平台，進一步探討tropomyosin 刺激人類纖維母細胞後相關的過敏反應。 實驗與方法: 我們萃取台灣鋏蠓RNA ，並利用RT-PCR 方式合成單股cDNA 。根據衣魚tropomyosin 胺基酸序列設計之多變性引子選殖出台灣鋏蠓tropomyosin 基因，之後將PCR 產物接合於TA 載體並定序確定。接著次選殖於pET30a 表現載體，利用E. coli BL21 表現系統大量生產重組蛋白質，並以His-Tag 親和層析方式純化蛋白質。利用西方墨漬法和酵素聯結免疫吸附法進一步確定重組蛋白質具有專一性IgE 結合能力。另一方面，以台灣鋏蠓tropomyosin 刺激人類皮膚纖維母細胞Hs 68 ，並探討相關免疫反應。 結果: 我們利用分子選殖方式製備出台灣鋏蠓tropomyosin 過敏原，命名為For t 4 ，其開放讀架(open reading frame)片段大小為855 bp ，蛋白質分子量為32 kDa。將台灣鋏蠓tropomyosin 胺基酸序列和已知tropomyosin 過敏原比較，發現具有58~67%序列相似性。在酵素免疫吸附法，發現25位對台灣鋏蠓有陽性皮膚試驗之病人血清有6位對台灣鋏蠓tropomyosin 重組蛋白質過敏原有專一性IgE 結合反應，盛行率為24%。而抑制型酵素聯結免疫吸附法，分析rFor t 4 和台灣鋏蠓粗萃取物專一性IgE 結合，具有20~50% 吸附能力。rFor t 4 刺激人類皮膚纖維母細胞確實會造成IL-8、MCP-1、eotaxin 和GM-CSF 趨化激素mRNA 表現。另外，利用ELISA 方法，也偵測到IL-8 趨化激素蛋白質的釋放。 結論: 台灣鋏蠓tropomyosin 在台灣鋏蠓粗萃取物中為次要過敏原，我們希望台灣鋏蠓tropomyosin 重組過敏原可以應用於in vitro 和in vivo 臨床上之檢測工具，並希望可以當作為免疫療法之試劑。

關鍵詞 : 台灣鋏蠓、台灣鋏蠓過敏症、過敏原、台灣鋏蠓原肌凝蛋白質、原肌凝蛋白質

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