山藥塊莖主要儲藏性蛋白質免疫調節活性研究

Studies on the immunomodulatory activities of dioscorin, the major storage protein.

中文摘要

本研究探討山藥儲藏性蛋白質 dioscorin 的體內及體外免疫調節活性。以 RAW 264.7 細胞株評估 dioscorin 對於一氧化氮的產生、吞噬反應、氧化噴發以及分泌細胞激素 (IL-1β, IL-6, TNF-α) 的影響；並以 BALB/c 小鼠的脾細胞與腹腔吞噬細胞進行體外實驗，探討 dioscorin 對於腹腔吞噬細胞的吞噬活性與腹腔吞噬細胞以及 BALB/c、C57BL/6 兩種品系小鼠脾細胞在 dioscorin 刺激後產生細胞激素的影響。將 dioscorin 與人類單核球共同培養，分析單核球分泌細胞激素 (IL-1β, IL-6, TNF-α) 的濃度。體內實驗將 dioscorin 管灌餵食 BALB/c 小鼠，探討餵食期間小鼠周邊血中淋巴球分布及血中多形性顆粒球與單核球的吞噬能力，腸道分泌性抗體 IgA 的濃度於每週收集一次糞便以 ELISA 法分析濃度，最後犧牲小鼠分析脾臟中淋巴球分布、自然殺手細胞活性、與 dioscorin 餵食對 Th-1 相關細胞激素（IL-2、IFN-γ ）與 Th-2 相關細胞激素（IL-4、IL-6、IL-10 型）分泌的影響。實驗結果顯示，dioscorin 能藉由促進 RAW 264.7 細胞株表現 iNOS 而產生一氧化氮 (2.5-3.5 μM)，促進 RAW 264.7 的吞噬能力達 1.5-2.0 倍。對於 RAW 264.7 細胞及人類單核球分泌細胞激素方面發現有顯著性促進 IL-1β, IL-6, TNF-α分泌。Dioscorin 促進小鼠腹腔吞噬細胞的吞噬能力達 1.1 倍，促進腹腔巨噬細胞 IL-6 分泌 (6.0-9.0 ng/mL)；在小鼠脾細胞的體外刺激，發現 dioscorin 能促進脾細胞增生，對於 PHA 所誘導的細胞增生具有協同促進，爲 PHA 的 1.4 倍。Dioscorin 培養 BALB/c 脾細胞 48 小時後，Th-2 相關細胞激素(IL-6 與 IL-10)具有濃度依順性上升，Th-1 相關細胞激素（IL-2）則爲濃度依順性下降；而 Dioscorin 對於另一品系 C57BL/6 小鼠脾細胞培養 48 小時後，Th-1 相關細胞激素（IL-2）反而具有濃度依順性上升，顯示 dioscorin 對於促進小鼠脾細胞分泌細胞激素具有雙向性。將 dioscorin 與人類單核球共同培養，發現 dioscorin 能促進 IL-1β, IL-6, TNF-α的分泌。在體內實驗結果顯示，餵食 dioscorin 能使增加小鼠周邊血中 B 細胞與自然殺手細胞數目與脾臟淋巴細胞當中的自然殺手細胞數目；促進小鼠先天性免疫活性，包括吞噬及自然殺手細胞活性；餵食 dioscorin 小鼠脾細胞對於 PHA 誘導的細胞增生能力比控制組小鼠脾細胞高；對於 Th-2 型細胞激素，包括：IL-4 與 IL-10 分泌促進較明顯，另外 IFN-γ 的濃度在餵食組小鼠明顯較高；小腸壁上的 Peyer’s patces 的數目在餵食 dioscorin 後明顯增加，腸道分泌性抗體 IgA 濃度隨著餵食的時間增加而上升。由上述實驗結果顯示，dioscorin 具有體內、體外的免疫調節活性。

英文摘要

The major storage protein of yam (Dioscorea alata cv. Tainong No.1) tuber, dioscorin, was used to investigate the immunomodulatory activities by a series of in vitro and in vivo assay systems. Initially, the in vitro assays included nitric oxide production, stimulated phagocytosis, and cytokine production (TNF-α, IL-1β, and IL-6) of RAW 264.7 and peritoneal macrophages from BALB/c mice after being treated with different concentrations of dioscorin in the presence of polymyxin B. The spleen cells of BALB/c and C57BL/6 mice treated with dioscorin were used to perform the effects of dioscorin on cytokine production. The effect of dioscorin on cytokines production from human monocytes was also investigated. The in vivo immunomodulatory effects of dioscorin on BALB/c mice were studied by orally administration of dioscorin under the dose of 2.5 mg/kg/day and 20 mg/kg/day for 21days. The lymphocyte subpopulation in peripheral blood and stimulated phagocytosis of polymorphonuclear cells and monocytes were analyzed by flow cytometry at day0, 7, 14, and 21. The feces of mice were collected once a week for measuring the concentration of sIgA in gastrointestinal tract. After being sacrificed at day 21, spleens were removed and used to prepare splenic cells and to analyze the stimulated proliferation, cytotoxicity of natural killer cells, and cytokine production. The results of in vitro assays showed that dioscorin could increase the NO production through iNOS activation and cytokines (including IL-1β, IL-6, and TNF-α) production of RAW 264.7 cells and enhanced the phagocytosis and oxidative burst. Dioscorin also stimulated the proliferation of BALB/c mice spleen cells and synergistically enhanced the proliferative activity compared to the PHA alone. T helper type 2 cytokines, IL-6 and IL-10, were stimulated and the T helper type 1 cytokine, IL-2, was inhibited in dioscorin cultured with BALB/c mice splenic cells. However, IL-2 production was increased dose-dependently of dioscorin cultured with C57BL/6 mice splenic cells. Cytokines production on mice splenic cells showed that dioscorin might regulate the immune responses through the T helper type 2 responses and exhibit dual effects on T helper type 1 responses. Dioscorin increased the cytokines (including IL-1β, IL-6, and TNF-α) production of human monocytes. The results of in vivo experiments showed that dioscorin might enhance the innate immunity of BALB/c mice, including stimulated phagocytic activity and natural killer cell activity, and the profile of cytokine secretion toward T helper type 2 responses. The number of Peyer’s patches and secreted IgA concentration were increased in mice fed with dioscorin. From the results of above-mentioned, we concluded that dioscorin exhibited immunomodulaory activity both in vitro and in vivo.