膠原蛋白免疫性 — 膠原蛋白及膠原蛋白抗體之特質

Collagen Immunogenicity - Characterization of Collagen and Collagen Antibodies

中文摘要

膠原蛋白(collagen)是結締組織中含量最豐富的蛋白質，也是細胞間質(extracellular matrix)中主要的組成份子之一。由於其特殊的三股螺旋結構(triple-helix)，使得膠原蛋白不僅提供一個組織構成的骨架，也提供細胞一個十分良好的生長環境。由於膠原蛋白的免疫反應極低(low immunogenicity)，目前已有許多以膠原蛋白爲基礎的生物醫學材料(biomedical materials)，廣泛地被運用在各種組織的修復治療之中，如心臟瓣膜(heart valves)、燙傷敷料(wound dressing)等。而膠原蛋白的免疫特性在過去的研究中已有相當程度的了解；但是要能夠偵測在傷口癒合過程中(wound healing process)膠原蛋白的生成及轉變，則必須更精確地掌握膠原蛋白與其抗體的免疫特性。在本論文研究中，我們比較數種不同來源之不同型膠原蛋白與抗體間之相互作用，同時也嘗試以酵素處理的方式，改變膠原蛋白的免疫反應，並以簡單的免疫測試方法，來探討膠原蛋白其抗原-抗體之間的反應特性；進而培養軟骨細胞，再以相同的方法偵測軟骨細胞所生合成的膠原蛋白，在繼代培養之下的改變。

在實驗上，我們萃取了紐西蘭大白兔、豬、大白鼠、牛的第一型膠原蛋白，以及兔、豬的第二型膠原蛋白，經由 SDS-PAGE 電泳法，分析胃蛋白 (pepsin)處理前後，蛋白質分子量的改變；並且以本實驗室所製作的豬抗兔第一型和第二型膠原蛋白多株抗體(polyclonal antibody)，以及所購得之羊抗牛第二型膠原蛋白多株抗體，以及鼠抗雞第二型膠原蛋白單株抗體(monoclonal antibody)，來比較其間免疫反應的差異。利用酵素連結免疫分析法(enzyme-linking immunoassay, ELISA)以及西方點墨分析法(western blot)，我們得到了以下幾點結果：(1) 豬抗兔第一型膠原蛋白多株抗體對於兔第二型膠原蛋白擁有相對 45%的交叉反應(cross-reaction)，而第二型膠原蛋白多株抗體則對兔之第一型膠原蛋白擁有相對 3%的交叉反應；此外，羊抗牛第二型膠原蛋白對兔之第一型膠原蛋白擁有相對 16%的交叉反應。(2) 在酵素處理之後，豬抗兔之第一型膠原蛋白多株抗體對於兔子第一型膠原蛋白的專一性並無明顯改變，但是對於大白鼠第一型膠原蛋白的交叉反應則在酵素處理後明顯地降低。而豬抗兔及羊抗牛之第二型膠原蛋白多株抗體對豬之第二型膠原蛋白也在酵素處理之後明顯下降。(3) 相對於豬之第二型膠原蛋白，鼠抗雞第二型膠原蛋白中段序列之單株抗體則對酵素處理之後的兔子第二型膠原蛋白產生了五倍的反應。

在軟骨細胞的繼代培養過程中，多株抗體明顯地測得第二型膠原蛋白逐漸減少，而第一型膠原蛋白卻相對地逐漸增多的現象；然而由於軟骨細胞的初級繼代培養

僅能維持七至九代的生命，我們便嘗試分析不朽化之軟骨細胞 (immortalized chondrocyte)。初期我們紀錄了此不朽化軟骨細胞的生長速度，並與初級培養的軟骨細胞比較其形態上的不同。我們發現：(1) 隨著培養代數的增加，初級培養的軟骨細胞其形狀會愈來愈大，但是不朽化軟骨細胞的大小則一直沒有改變。(2) 不朽化之軟骨細胞之倍增速率平均爲 19~21 小時，第 13 及 27 代之變化不大。而經過長期的培養，初級培養的軟骨細胞以及不朽化軟骨細胞都有丘狀體(dune)形成的現象。目前不朽化軟骨細胞已培養至四十五代，若能順利培養至五十代，則延長細胞壽命的目標便完成。至於此一不朽化之軟骨細胞是否可能癌化，仍待更進一步的測試。

總括本論文的研究，我們得到以下的結論：(1) 單一多株抗體對不同物種的膠原蛋白之間，交叉反應的程度不同，顯示出不同物種的膠原蛋白，其抗原決定位置上的結構不盡相同。同時也證實本實驗室自行製作的抗體能夠區分不同物種的膠原蛋白。(2) 膠原蛋白在經過酵素處理之後，免疫反應的降低，顯示出膠原蛋白引起免疫反應的位置，已被酵素破壞，因而將胃蛋白 處理之某些膠原蛋白應用於生物醫學材料之製作，可能會降低其免疫性。(3) 多株抗體能區分軟骨細胞所生成的膠原蛋白，可應用於免疫組織染色上的運用。(4) 軟骨細胞的不朽化，也許可作爲長期供應軟骨細胞來源，但不朽化軟骨細胞之特性仍待更進一步的研究，並觀察是否可作爲臨床之應用。

英文摘要

Collagen is the most abundant protein component of the extracellular matrix (ECM). It provides a scaffold for cell attachment and migration, and also possesses specific, intrinsic mechanic properties. Because the low immunogenicity, collagen-based products are compatible to those synthetic polymers for clinical use, including heart valves and wound dressing. To monitor changes in collagen types during wound healing process, collagens and anti-collagen antibodies were purified and characterized. The objective of this thesis is to study whether collagen immunogenicity can be altered by pepsin treatment based on ELISA and Western blot analyses. The antibodies were subsequently used to explore changes in collagen synthesis patterns by primary cultured and immortalized chondrocytes.

For the purposes, type I collagens were extracted and purified from rabbit, swine, rat and bovine tendon; the type II collagens were extracted and purified from rabbit and swine cartilage. The purity of type I collagen, and that of type II collagen were analyzed by SDS gel electrophoresis. The immunogenicity of collagens and pepsin-treated collagens were investigated by immunodetection (ELISA, Western Blot). The results suggested that there were little changes appeared before and after pepsin digestion except that the interaction of porcine anti-rabbit type I collagen

antibody with rat type I collagen was decreased greatly after pepsin digestion.
The porcine anti-rabbit type I and type II collagen antibodies were used to determine the collagen typing during primary chondrocyte culture. The results suggested that type I collagen synthesized by chondrocytes from passage 1 to passage 7 was increased gradually but the presence of type II collagen was diminished by passage 3. In order to keep the phenotypes of chondrocyte, the cells were immortalized since P0 by Deng et.al. The immortalized chondrocyte were cultured and the life span, doubling time and morphological changes were recorded. Both primary cultured and immortalized cultured chondrocytes formed dunes after long-term culture for 14 days. The immortalized chondrocytes has been subcultured to passage 45 and still continuing.
In conclusion, (1) Different species of type I collagens and type II collagens can be differentiated by porcine anti-rabbit collagen polyclonal antibodies. (2) Based on pepsin-digestion data, the immunogenicity of collagens from different species was different. This may be because of variation in respective type I collagen structure or amino acid sequence. (3) The type I and type II collagens from various passages of primary chondrocytes could be differentiated by porcine anti-rabbit type I and type II collagen antibodies. It was found that type II collagen decreased from passage 0 to 3 while type I collagen increased gradually from passage 0 to passage 7 of monolayer chondrocyte culture. (4) The immortalized chondrocytes has been cultured to passage 45 and still continuing.