中文題目：Anti-Thy1 腎炎中腎小球內 fractalkine mRNA 的表現和分佈

英文題目：Expression and localization of glomerular fractalkine mRNA *in anti-Thy1 antibody-induced glomerulonephritis*

計劃編號：NSC 89-2314-B-002-068

執行期限：88/08/01 ~ 89/07/31

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中文摘要

背景：最近在血管內皮細胞上所發現的 CX3C 化學激素— fractalkine，兼具黏附和趨化作用，主要標的細胞與 CC 化學激素一樣也是單核球和 T 淋巴球。Fractalkine 除了固定表現在細胞膜，也可以自膜上脫離，以醣化蛋白型式吸引單核球和 T 淋巴球。據我們所知，迄今還沒有研究報告 fractalkine 在 Anti-Thy1 腎炎中的表現，以及其與腎小球內巨噬細胞或 T 淋巴球聚集的時程（time course）關係。

方法：腎小球 total RNA 是以 guanidine thiocyanate-acid phenol-chloroform 方法抽取，再利用 M-MuLV 反轉錄酵素製成 RT，施行 PCR。北方墨點則按標準分子生物學方法操作。免疫組織化學染色及原位雜合則用來計算浸潤腎小球的單核球及淋巴球數目，以及證明 fractalkine mRNA 存在及分佈情形。

結果：腎炎誘發後兩小時即發現 fractalkine 基因表現增加並達到最高點，持續到第五天。原位雜合可偵測到腎小球內 fractalkine mRNA 訊息存在。其表現強度與浸潤腎小球之 ED-1 陽性單核球, CD-4 和 CD-8 陽性淋巴球的數目有密切相關。其分佈在腎炎早期爲內皮細胞，在晚期則爲腎小球間質支持細胞 (mesangial cells)。

結論：這些結果顯示在實驗性 anti-Thy1 腎小球腎炎誘發過程中，腎小球內 fractalkline 基因表現上升，mesangial cells 表現 fractalkine mRNA 則爲首次被報告。

英文摘要

Background Fractalkine is a chemotactic cytokine known to regulate macrophage and T cell accumulation at sites of inflammation. In rat crescentic glomerulonephritis, fractalkine expression is induced exclusively in glomerular endothelium, and plays a pivotal role in glomerular leukocyte infiltration. It is not known, however, whether other glomerular cells can produce fractalkine during different types of glomerular injury. To study this, we examined fractalkine expression in a rat model of glomerulonephritis characterized by intense mesangial cell proliferation, the anti-Thy1 nephritis, and explored cytokine regulation of its expression by *cultured mesangial cells.*

Methods Male Wistar rats with anti-Thy1 antibody-induced nephritis were killed at various time points to study glomerular fractalkine expression by Northern blotting, *in situ* hybridization, and immunohistochemistry. Fractalkine expression by cultured mesangial cells was also examined with Northern and Western blotting under both cytokine- and growth factor-stimulated conditions.

Results Northern blotting showed that glomerular fractalkine mRNA was up-regulated following induction of anti-Thy1 nephritis. *In situ* hybridization revealed that fractalkine mRNA signals were induced, mainly in the non-mesangial areas of the glomeruli, at 2 h and day 1 of nephritis. Cells expressing fractalkine mRNA at this stage were presumably *glomerular endothelial cells and a few glomerular epithelial cells. In contrast,* glomerular fractalkine mRNA and protein were expressed mainly by activated mesangial cells in the *proliferative phase of nephritis. There was a temporal association between* glomerular fractalkine mRNA levels and accumulation of leukocytes, in particular macrophages, in the glomerulus.

Conclusions This study demonstrates that glomerular mesangial cells produce fractalkine both *in vitro* and *in vivo. It is postulated that fractalkine produced by mesangial cells works in* concert with other chemoattractants, and plays a role in the glomerular recruitment of monocytes and T cells in acute mesangial proliferative disease.

計劃緣由

由靜脈單次注射 anti-thy1.1 抗體至大鼠體內會迅速誘發急性腎小球間質增生型腎炎產生。這種腎炎的急性期（腎炎誘導後 72 小時內）在病態生物學上的特點包括腎小球間質結構解體和巨噬細胞聚集腎小球間質內。接著大約從誘導後 72 小時起，逐漸出現腎小球間質支持細胞 (mesangial cell)增生和腎小球細胞外基質沈積現象。近來有許多証據顯示聚積腎小球內的巨噬細胞在人類和實驗性腎小球腎炎之致病機轉過程中扮演十分關鍵性的角色。腎小球內的活化巨噬細胞可經由分泌發炎激素而參與腎小球發炎反應，並調控腎小球功能。巨噬細胞侵潤腎小球的機轉相當複雜，目前認爲是經由黏附因子的固定作用及化學激素的趨化作用，促使血液中的單核球穿過內皮細胞間隙，到達發炎腎小球內轉變爲巨噬細胞。化學激素是一群由免疫和非免疫細胞製造、構造類似且具有趨化性的蛋白質所組成。依照它們前兩個 cysteine (C) 基之間的胺基酸數目，可再細分爲四類（CXC，CC，C，和 CX3C）。這些化學激素的表現在腎臟發炎時（無論是腎小球或腎小管發炎）可以很快地被誘發，吸引特定白血球族群進入發炎區域（這點和傳統趨化因子如補體 C5a 之非特定性吸引白血球是不一樣的）。一般而言，CXC 化學激素較吸引嗜中性白血球，CC 化學激素則較吸引單核球和 T 淋巴球。在許多實驗性腎炎模式，已有研究發現腎小球內 CC 化學激素如單核球化學趨化因子-1 （MCP-1）和巨噬細胞發炎因子-1（MIP-1）之表現增高發生在巨噬細胞侵潤腎小球之前。同時若利用抗體中和 MCP-1 活性，則腎小球內巨噬細胞侵潤會減少 40%，然而中和 MIP-1 活性卻無法降低巨噬細胞侵潤程度。由此看來，MCP-1 雖然是吸引單核球的重要化學激素，卻也不是唯一的因子。最近在血管內皮細胞上所發現的 CX3C 化學激素— fractalkine ，兼具黏附和趨化作用，主要標的細胞與 CC 化學激素一樣也是單核球和 T 淋巴球。 Fractalkine 除了固定表現在細胞膜，也可以自膜上脫離，以醣化蛋白型式吸引單核球和 T 淋巴球。據我們所知，迄今還沒有研究報告 fractalkine 在 Anti-Thy1 腎炎中的表現，以及其與腎小球內巨噬細胞或 T 淋巴球聚集的時程（time course）關係。

目的

因此本研究希望探討 (1) Anti-Thy1 腎炎中腎小球 fractalkine mRNA 訊息之動態變化，(2) Anti-Thy1 腎炎中腎小球 fractalkine mRNA 訊息之細胞定位，(3) Anti-Thy1 腎炎中腎小球 fractalkine mRNA 訊息與腎小球內巨噬細胞或 T 淋巴球數目之時程關係，(4) 抗發炎 藥物如前列腺素 E1 和 pentoxifylline 對 Anti-Thy1 腎炎中腎小球 fractalkine mRNA 訊息之影響 ，以了解 fractalkine 在 Anti-Thy1 腎炎中發炎細胞侵潤腎小球過程中扮演的病態生理角色。

結果

Northern blotting showed that glomerular fractalkine mRNA was up-regulated following induction of anti-Thy1 nephritis. *In situ* hybridization revealed that fractalkine mRNA signals were induced, mainly in the non-mesangial areas of the glomeruli, at 2 h and day 1 of nephritis. Cells expressing fractalkine mRNA at this stage were presumably glomerular endothelial cells and a few glomerular epithelial cells. In contrast, glomerular fractalkine mRNA and protein were expressed mainly by activated mesangial cells in the proliferative phase of nephritis. There was a temporal association between glomerular fractalkine mRNA levels and accumulation of leukocytes, in particular macrophages, in the glomerulus.

討論

The main findings of the present study are that MCs can synthesize fractalkine both *in vivo* and *in vitro*. Evidence in support of *in vivo* fractalkine expression by MCs was based on immunohistochemical double-staining, and combined *in situ* hybridization and immunohistochemistry which demonstrated glomerular fractalkine protein and mRNA expression by α-SMA-positive cells in the proliferative phase (days 3 to 5) of nephritis. In contrast, glomerular fractalkine mRNA in the mesangiolytic phase (2 h to day 1) of nephritis appeared to be expressed by other glomerular cells, in particular endothelial and some epithelial cells. Cultured endothelial cells can produce fractalkine in response to proinflammatory cytokines, such as TNF-α and IL-1β [1]. The present *in situ* hybridization findings revealed the presence of fractalkine mRNA distributing along glomerular capillary loops at 2 h and day 1 of nephritis, during which time glomerular TNF-α and IL-1β mRNAs were markedly elicited.

This finding is consistent with a recent study which demonstrated fractalkine protein expression by glomerular endothelium in a rat model of crescentic glomerulonephritis [2]. Thus, the sustained up-regulated glomerular fractalkine mRNA throughout the course of the nephritis is probably due to sequential expression by different intrinsic glomerular cells, with MCs prevailing in the proliferative phase. The present study, however, does not completely exclude the possibility that MCs express fractalkine mRNA in the early mesangiolytic phase of nephritis. Immune complexes have been shown to stimulate the expression of certain CC chemokines such as MCP-1 and RANTES by cultured MCs [3, 4]. Whether or not the *in situ* immune complex formation between the injected anti-Thy1 antibody and an antigen expressed on MCs might also induce fractalkine production by MCs in the early phase awaits further studies.

Infiltrating monocytes/macrophages and T cells are major sources of a number of CC and CXC chemokines [5]. However, they are probably not the source of fractalkine in the anti-Thy1 model for several reasons. First, the present study did not detect the presence of ED-1- or CD-8-positive cells expressing fractalkine mRNA in the nephritic tissues by combined *in situ* hybridization and immunohistochemistry. Second, no fractalkine mRNA could be detected in a macrophage cell line (J774.A1) or PBMCs isolated from nephritic rats by RT-PCR or Northern blotting. Finally, also by using RT-PCR, a previous study has shown that little or no fractalkine mRNA can be detected in peripheral blood cells, including monocytes, T cells, and NK cells [1].

This study has also shown that glomerular fractalkine mRNA levels correlate with accumulation of leukocytes, in particular macrophages, in the anti-Thy1 model. Compared to the CC or CXC chemokines, fractalkine is a larger molecular mass that can bind to extracellular matrix via its mucin-like domain [6]. This would lead to fractalkine sequestration and prolong its duration of action within the glomerular mesangium. Therefore, glomerular fractalkine expression may have a role in mediating not only migration but accumulation of circulating leukocytes within the glomerulus. While functional blocking studies against either fractalkine or its receptor are still needed in the anti-Thy1 model, a recent study has shown that administration of anti-fractalkine receptor antibody to rats with experimental crescentic glomerulonephritis dramatically attenuate glomerular macrophage and T cell infiltration, prevent crescent formation, and improve renal function [2].

計劃成果自評

本計劃在執行中雖有些許困難，但均能順利解決。本文已寫成文章並投搞。

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