腎臟缺血性再灌流傷害調控單核球衍生的樹突細胞之發育 Renal ischemia/reperfusion injury modulates development of monocytes-derived dendritic cells

中文摘要

受損的組織及細菌的產物會啓動樹突細胞的成熟與遷移。樹突細胞被公認是最佳的抗原呈現細胞，樹突細胞吞噬抗原物質與MHC 抗原結合，將抗原訊息表達並遷移至淋巴組織啓動免疫反應。於不同的成熟條件下樹突細胞分別刺激 T-helper 1 (Th1) 或 Th2 反應。外科手術傷害、運動及身體精神壓力均會影響樹突細胞表型與功能，此時樹突細胞啓動了不同程度之刺激與抑制的免疫反應，而這些反應決定了病程之進展及預後。臨床上，腎臟之缺血/再灌流傷害常導致腎臟移植排斥與缺血性腎衰竭，而腎臟缺血/再灌流傷害如何影響樹突細胞之免疫反應機制仍未清楚，尤其是腎移植後期排斥現象，雖與手術之缺血/再灌流傷害關係密切，且樹突細胞參與了重要角色，然而其間確切之相關性仍未被釐清。本研究之目的是爲了解腎臟缺血/再灌流傷害是否會改變樹突細胞之發育過程，其中包括了分化、表型、細胞激素產生、T 細胞增殖等功能。樹突細胞的來源分別來自週邊血及骨髓的單核球。

Sprague-Dawley 大鼠接受缺血/再灌流手術或假手術後，每日收集血液與尿液評估腎功能 (肌酸酐廓清率 CCr) 共 14 天。於再灌流後第 2 天及第 14 天，觀察來自週邊血單核球及骨髓單核球的樹突細胞之分化能力、表面抗原 (CD11c、CD80、CD86、MHC-II, IA) 及功能性指標 (如 MLR；IL-12 分泌；T 細胞分泌的 IFN-gamma、IL-4)，並觀察傷害後腎臟組織的樹突細胞。

手術後之腎功能 (CCr) 明顯下降，至第 5 天回復正常，源自週邊血單核球衍生的樹突細胞之分化能力明顯地於缺血/再灌流第 2 天增加，且伴隨著樹突細胞表現 MHC-II、分泌 IL-12 受被刺激的 T 細胞 IFN-gamma 產量均上升。相對於周邊血單核球，源自骨髓的單核球所分化的樹突細胞能力則於手術後第 2 天明顯被抑制。無論樹突細胞來自週邊血或骨髓的單核球於腎臟傷害後第 14 天，其分化能力、表型及功能均與假手術組無統計差異。於缺血/再灌流後第 2 天，可明顯觀察到樹突細胞浸潤於腎臟外側髓質區域。

腎臟缺血/再灌流傷害引起之腎衰竭急性期間，來自週邊血單核球的樹突細胞表現出分化、表型、細胞激素及功能被活化的現象，這可能是反應著成熟樹突細胞於應付免疫挑戰之準備狀態。相對地，來自骨髓之單核細胞的樹突細胞於缺血/再灌流傷害後，反而表現出分化能力被抑制的現象，而其功能並沒有影響，而在此同時樹突細胞會浸潤腎臟外側髓質區。由此可知，於腎臟缺血/再灌流傷害誘發的腎衰竭之急性期的週邊血單核球分化爲樹突細胞的能力上升，而骨髓單核細胞分化成樹突細胞能力下降以及樹突細胞浸潤於受損之組織爲缺血性腎衰竭之重要之病理機制，此提供未來腎衰竭及移植器官排斥於治療上的一個理論基礎。

英文摘要

Damaged tissues and microbial products trigger the maturation and migration of dendritic cells (DCs) (important antigen-presenting cells [APCs]) to secondary lymphoid organs where the presented antigen stimulates T cell activation. DCs stimulate T-helper 1 (Th1) or Th2 responses depending on the maturation conditions. These conditions may profoundly influence immunological outcome and the role that DCs play in this outcome. DCs play a central role in both stimulating and suppressing immune responses and are impacted by surgical injury, exercise, and other physiological stressors. Ischemia/reperfusion (I/R) injury has been associated with ischemic acute renal failure (ARF), which is a major cause of native kidney and allograft dysfunction. DCs appear to play a central role in the initiation of an adaptive immune response to tissue injury stemming from I/R. The DC response to renal I/R injury has not been defined or clearly distinguished from the DC response to injury due to the renal transplant surgery itself. Also unclear is whether the DC response has a role in the injury phase or in the regeneration process after I/R. This objective aims to determine whether renal I/R injury alters the differentiation, maturation, and activation of myeloid DCs from peripheral blood monocytes (PBMo) and bone marrow monocytes (BMMo). The focus of this study was on DCs derived from monocytes and the impact of renal I/R on these cells only. Sprague-Dawley rats were subjected to I/R injury or sham-operated. Creatinine clearance (CCr) was monitored daily during the 14 days of reperfusion that followed the ischemic insult. At 2 and 14 days of reperfusion, the following properties of DCs were assessed: the amount of generated DCs from PBMo and BMMo, surface markers [CD11c, CD80, CD86, and MHC-II (IA)], functional status including magnitude of mixed lymphocyte reaction (MLR), production of IL-12 p70 by DCs, production of IFN-gamma and IL-4 by DC-stimulated T cells and the presence of DCs in the kidney.

CCr was greatly reduced in the injured rats 0-4 days after ischemia. Two days after I/R injury to kidney, the numbers of DCs differentiated from PBMo, IL-12 production by DCs, expression of MHC-II (IA), and IFN-gamma production by DC-stimulated T cells were significantly increased in the I/R injured group (compared to the sham-operated group). In contrast to PBMo derived DCs, the generation rate of BMMo-derived DCs was decreased in the I/R injured group at 2 d following I/R. After 14 d of reperfusion, there was no between-group differences in the numbers DCs derived from either PBMo or BMMo, MLR, expression of CD80, CD86, and MHC-II (IA), and production of IL-12, IFN-gamma and IL-4. The immunohistochemistry showed infiltrating DCs in the outer medulla of the I/R injured kidney at 2 d but not 14 d of reperfusion.

The upshift in differentiation of DCs derived from PBMos with a corresponding increase in IL-12 production of mature monocyte-derived DCs and higher production of IFN-gamma by DC-stimulated T cells may reflect a preparatory step in the pathway leading to mature immunogenic DCs and increase in Th1 cell numbers in the acute phase of renal I/R injury. I/R stress reduces the number of DCs differentiated from BMMos but not the functional activity of these DCs. This decrease may reflect a stress-induced downshift in the capacity of BMMos to differentiate into DCs and a parallel upshift in the capacity of DCs to infiltrate the kidney. In summary, the upshift in PBMo differentiation to DCs, downshift in BMMo differentiation to DCs, and infiltration of the kidney at 2 d of reperfusion may be part of an important pathophysiologic acute stress response to ischemic renal failure. Our findings may provide a rational basis for investigating immunosuppressive agents, targeting suppression of DC differentiation, for preventing transplant organ failure at the early stage following I/R injury.