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Original contribution

Dendritic cells in renal biopsies of patients with acute tubulointerstitial nephritis☆,☆☆

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Summary Dendritic cells (DCs) play a critical role in the regulation of the adaptive immune response and can be separated into 2 major subsets: myeloid (mDC) and plasmacytoid (pDC) DCs. Acute tubulointerstitial nephritis (ATIN) is a common cause of injury to renal tubules and interstitium resulting from the interplay of tubular cells and inflammatory cells and their products. However, the involvement of DCs in ATIN is still unknown. In this study, the participation and localization of myeloid (CD1c) and plasmacytoid (CD303) DC subsets in the biopsies from patients with ATIN (n = 20), lupus nephritis (n = 17, positive control), or minimal change disease (n = 14, negative control) were investigated. DCs were identified morphologically within the tubulointerstitium in the renal biopsies by transmission electron microscopy interacting with surrounding tubules and inflammatory cells. Direct immunofluorescence showed that both CD1c+ DCs and CD303+ DCs exist in all the renal biopsies. As compared with minimal change disease, biopsies with ATIN had significantly increased CD1c+ DCs ($P < .001$) and CD303+ DCs ($P < .001$). The number of CD1c+ DCs in ATIN was significantly higher than that in lupus nephritis ($P < .02$), whereas the number of CD303+ DCs in ATIN was slightly but not significantly higher than that in lupus nephritis ($P = .2$). DCs in the biopsies with ATIN were restricted to the tubulointerstitium forming dense networks, and most of them maintained immature state. All these findings suggest that DC subsets may be differentially involved in the pathogenesis of ATIN. Their potential role in intrarenal regulation of immune responses in ATIN is proposed.

1. Introduction

Acute tubulointerstitial nephritis (ATIN) is a common cause of acute kidney injury and has become an important cause of acute renal failure. Renal biopsy of ATIN typically reveals interstitial edema, inflammation, and tubulitis with a predominance of T lymphocytes and other mononuclear cells, with variable numbers of eosinophils. Glomeruli are normal. Interstitial fibrosis develops later in the course of the illness. There is strong evidence that ATIN is immunologically mediated, and antigen-driven immunopathology is considered to be the key mechanism. The presence of helper-inducer and suppressor-cytotoxic T lymphocytes in the inflammatory infiltrate suggests that T-cell–mediated hypersensitivity reactions and cytotoxic T-cell injury are involved in the pathogenesis of ATIN [1]. In some cases, humoral mechanisms are involved with complement proteins, immunoglobulins, and anti–tubular basement membrane (TBM) antibodies [2]. However, the precise mechanism involved in the development of ATIN is still not known.

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Dendritic cells (DCs) are professional antigen-presenting cells that play an important role in the initiation of immune responses. Human DCs are classically defined as CD45-positive leukocytes that lack other leukocyte lineage (lin) markers, such as CD3 (T cell), CD19/20 (B cell), and CD56 (NK cell), and so on, and express high levels of major histocompatibility complex class II (HLA-DR) [3]. DCs also express costimulatory molecules including CD80 (B7.1) and CD86 (B7.2), which are up-regulated during DC activation [4]. Recently, 2 subsets of DCs have been recognized in human tissue: mDCs and pDCs [5]. Human mDCs express typical myeloid antigens CD11c, CD13, and CD33, and may be split into CD1c+ and CD141+ fractions. CD1c+ mDCs are the major population of human mDCs in blood, tissues, and lymphoid organs. They comprise approximately 90% of human mDCs. Human pDCs lack myeloid antigens CD11c, CD13, and CD33 but express CD303 and CD304 [3,6].

In the past 3 decades, DCs have been extensively studied in lymphoid and many nonlymphoid organs. However, because of difficulties in identifying and in isolating these cells from the human kidney, relatively little is known about their functional roles in human renal disease. In recent years, some characteristic markers were identified to distinguish individual DC subsets in human kidney. For example, CD1c is considered as a specific marker for mDCs in kidney. Besides mDCs, expression of CD1c has only been detected on a subpopulation of CD19+ small resting B lymphocytes in blood and on immature Langerhans cells [7,8]. CD303 is strictly confined to pDCs and is considered as a specific marker for pDCs [9]. Recent studies using these specific markers showed that DC subsets may participate in inflammatory renal diseases, such as lupus nephritis [10]. So far, it has not been shown whether and which subsets of DCs take part in inflammatory processes in ATIN. This study assesses the participation of DC subsets in inflammation due to ATIN in human kidney biopsies.

2. Materials and methods

2.1. Patients

A total of 20 frozen renal biopsies from patients with ATIN (from 2013 to 2014) were investigated. Fourteen frozen renal biopsies from patients with minimal change disease (MCD) and no other associated pathologies such as acute tubular injury served as negative/noninflammatory controls. Seventeen frozen renal biopsies from patients with lupus nephritis served as positive/inflammatory controls. The diagnosis was based on light microscopy, immunohistochemistry, and electron microscopy. Institutional review board approval was obtained from the Louisiana State University Health Science Center in Shreveport.

2.2. Histology and immunofluorescence

Sections of formalin-fixed, paraffin-embedded renal biopsies were stained with hematoxylin and eosin (H&E) and periodic acid–Schiff (PAS). For immunofluorescence staining, renal biopsies were placed in Michel solution, snap-frozen in cold isopentane, serially sectioned at 5 μm, and placed on coated glass slides. Slides were immediately fixed in cold acetone for 10 minutes and then air-dried. Each of the slides was incubated with the antibody of anti-CD1c (clone: AD5-8E7; Miltenyi Biotec, Auburn, CA) or anti-CD303 (Cat. no. orb14185; Biorbyt, Cambridge, United Kingdom). Several washing steps were performed. Appropriate, subclass-specific isotype controls were used. Double-labeling immunofluorescence was performed on selected biopsies of ATIN for anti-CD80 (Cat no. ABIN1701670, antibodies—online) and one of DC markers (anti-CD1c or anti-CD303).

2.3. Transmission electron microscopy

Renal biopsies with ATIN were fixed in 2.5% glutaraldehyde in 0.1 M (pH 7.0) phosphate buffer and postfixed in 1% osmium tetroxide in the same buffer. After dehydration in acetonic series, the fragments were embedded in epoxy resin. Ultrathin sections were cut on an LKB ultratome and mounted on copper grids. The sections were stained with uranyl acetate–lead citrate and examined using transmission electron microscopy (TEM; Hitachi 87650, Tokyo, Japan).

2.4. Quantification of DCs in renal cortex

The amount of DCs in renal cortex was determined by area measurement. Representative pictures of each biopsy were taken with an original magnification of ×400. The amount of renal DCs in the biopsies was determined by area measurement using the digital image analysis system ImageJ (Web site http://rsb.info.nih.gov/ij/). Three to 5 high-power fields with the most DCs were evaluated in each biopsy. Positively stained area was expressed as a fraction of the area of the high-power field examined. For double-labeling immunofluorescence, the amount of mature DCs (yellow area) was expressed as a fraction of total DCs (green area).

2.5. Statistical analysis

All numerical data were expressed as mean ± SEM and analyzed using 1-way analysis of variance in conjunction with Tukey post hoc test. $P < .05$ was considered to be statistically significant.

3. Results

3.1. Histologic characteristics of human kidney diseases

H&E staining of renal biopsies with ATIN revealed multifocal interstitial inflammatory infiltrates composed of predominantly mononuclear inflammatory cells and focally associated