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Anti-dsDNA antibody induces soluble fibronectin secretion by proximal renal tubular epithelial cells and downstream increase of TGF-β1 and collagen synthesis

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A B S T R A C T

The level of anti-dsDNA antibodies correlates with disease activity in lupus nephritis, but their role in pathogenic mechanisms remains to be defined. We investigated the effect of anti-dsDNA antibodies isolated from lupus nephritis patients on fibronectin synthesis and downstream fibrogenesis in proximal renal tubular epithelial cells (PTEC). Kidney biopsies were obtained from patients with active severe proliferative lupus nephritis. In vitro studies with cultured PTEC were performed to investigate the effect of human polyclonal IgG anti-dsDNA antibodies and mycophenolic acid (MPA). The role of IL-6, IL-8, MCP-1, TNF-α, TGF-β1, and MAPK and PKC signaling pathways on soluble and cell-associated fibronectin synthesis was investigated using neutralizing antibodies or specific inhibitors. The effect of exogenous endotoxin-free soluble fibronectin on downstream fibrotic processes was also examined. Fibronectin expression was markedly increased in the tubulo-interstitium of lupus nephritis renal biopsies and it co-localized with IgG deposition. Anti-dsDNA antibodies significantly increased both secreted and cell-associated fibronectin, through prior activation of ERK, p38 MAPK, JNK, PKC-α and PKC-βII. There was downstream induction of IL-6, IL-8, MCP-1, TNF-α and TGF-β1. MPA inhibited the induction of inflammatory and fibrotic processes by anti-dsDNA antibody. Exogenous soluble fibronectin induced TGF-β1 secretion and type I collagen synthesis in PTEC in a dose-dependent manner. Our data demonstrate that anti-dsDNA antibody contributes to tubulo-interstitial fibrosis in lupus nephritis through its action on PTEC. Anti-dsDNA antibody induces both cell-associated and soluble fibronectin secretion in PTEC, the former adds to extracellular matrix deposition while the latter amplifies the fibrotic process through induction of TGF-β1 and collagen type I. The pro-fibrotic effects of anti-dsDNA antibody are ameliorated by MPA.

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1. Introduction

Lupus nephritis is a common and severe organ manifestation of systemic lupus erythematosus affecting up to 60% of patients. It is characterized by anti-dsDNA antibody production and immune-mediated injury to the kidney parenchyma [1], progressing from inflammation to fibrosis. In addition to glomerular abnormalities, approximately 70% of lupus nephritis patients show immune deposition along the renal tubular basement membrane which correlates with tubulo-interstitial infiltration of inflammatory cells, tubular atrophy and interstitial fibrosis [2,3]. The tubulo-interstitium accounts for 85–90% of the kidney volume, and proximal tubular epithelial cells (PTEC) are the predominant cell type, contributing to both initiation and amplification of tubulo-interstitial inflammation and fibrosis. The degree of tubulo-interstitial damage is more powerful than glomerular lesions in predicting poor renal prognosis [4,5].

Irrespective of the original kidney disease, fibrosis is a final common pathway leading to end-stage renal failure. It is characterized by the accumulation of matrix proteins, destruction and replacement of normal kidney parenchyma by fibrous tissue.

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Fibronectin is a major component of the extracellular matrix. In the normal kidney, fibronectin is predominantly localized to the mesangial matrix and glomerular basement membrane (GBM) where it plays an important role in mediating the attachment of resident glomerular cells to the GBM, and stabilizing the structure of the glomerular tuft [6,7]. Fibronectin expression in the Bowman's capsule and tubular basement membrane is increased during kidney injury. Under pathological conditions, fibronectin is the principal matrix protein present in glomerulosclerotic lesions and is the first matrix component that is deposited within the tubulo-interstitium [8].

We have previously demonstrated that human anti-dsDNA antibodies could bind to PTEC, and such binding was followed by changes in cell morphology and function [3]. In view of the active role of PTEC in tubulo-interstitial inflammation and fibrosis in renal diseases [9–12], we further investigated the effect of anti-dsDNA antibodies on PTEC fibronectin synthesis and the ensuing pro-fibrotic processes. Since mycophenolic acid (MPA) is a commonly used current standard-of-care therapy for lupus nephritis [13–15], we also investigated the effect of MPA. In this regard, there is evidence to suggest that in addition to its inhibitory action on lymphocytes, MPA can exert direct effects on non-immune cells, and inhibit cell proliferation, epithelial-to-mesenchymal transition and IL-6 secretion [16–19]. Our results show that human anti-dsDNA antibodies induce both soluble and cell-associated fibronectin in PTEC through the activation of ERK, p38 MAPK, PKC-α and PKC-βII. The pro-fibrotic processes are further amplified through the induction of cytokines and growth factors and the downstream effects of soluble fibronectin on TGF-β1 and collagen type I synthesis. Our data also show an inhibitory effect of MPA on inflammatory and fibrotic processes induced in PTEC by anti-dsDNA antibodies.

2. Material and methods

2.1. Chemicals and reagents

All chemicals were of the highest purity and were purchased from Sigma Aldrich (Tin Hang Technology, Hong Kong) unless otherwise stated. Tissue culture flasks were purchased from Falcon (Becton–Dickenson, Gene Company Limited, Hong Kong) and tissue culture medium (DMEM/F12) and supplements purchased from Invitrogen (Life Technologies Corporation, Hong Kong). BD OptEIA ELISA kits for IL-6, IL-8, MCP-1, TNF-α and TGF-β1 were purchased from BD Biosciences Pharmingen (Bio-Gene Technology Limited, Hong Kong). QuantiMatrix human fibronectin ELISA kits were purchased from Chemicon International (Onwon Trading Limited, Hong Kong). Inhibitors to ERK (PD98059) and JNK (SP600125) were purchased from Cell Signaling Technology (Genetimes Technology International Holding Limited, Hong Kong), and inhibitors to p38 MAPK (SB203580) and PKC (Gö6976) were purchased from Calbiochem, Merck (Onwon Trading Limited, Hong Kong). MPA was provided by Roche (Palo Alto, California).

2.2. Immunohistochemical analysis of tubulo-interstitial fibronectin expression and IgG deposition

Renal biopsy specimens were obtained from 10 patients with active diffuse proliferative lupus nephritis (ISN/RPS Class IV G/S, A or A/C). Normal kidney specimens from 6 patients who underwent nephrectomy were used as controls. Samples were washed three times with PBS in between all steps. To detect fibronectin expression, cryosections (8 μm) were incubated with mouse anti-human fibronectin antibody (dilution 1:50) for 1 h at 37 °C in a humidified chamber followed by the Texas red-conjugated secondary antibody (dilution 1:400). IgG deposition was assessed by incubation with FITC-conjugated goat anti-human IgG (dilution 1:100). Epifluorescence was viewed using an Axioskop 2 plus fluorescent microscope. Six separate images along non-overlapping sections of the tubulo-interstitium were captured per biopsy sample and fluorescence scored blindly on a scale of 0–3, where 0 = no staining, 1 = weak staining, 2 = moderate staining, and 3 = strong staining [20].

2.3. Isolation of human anti-dsDNA antibodies

Polyclonal IgG anti-dsDNA antibodies were isolated from the sera of 10 patients with biopsy-proven active proliferative lupus nephritis after informed consent, using sequential affinity chromatography as previously described [3,21]. The purity of anti-dsDNA antibodies was confirmed by 10% SDS-PAGE, and the absence of immune complexes in anti-dsDNA antibody preparations was confirmed with polyethylene glycol precipitation [21]. All anti-dsDNA antibody preparations were treated with DNase to remove bound DNA prior to use in experiments. Anti-dsDNA activity in antibody preparations was determined using a quantitative commercial ELISA according to the manufacturer's instructions (BioRad, Hong Kong).

2.4. Culture of proximal renal tubular epithelial cells

Experiments were conducted in both primary and immortalized human PTEC. Primary PTEC were purchased from Lonza (Gene Company Limited, Hong Kong) and cultured in Renal Cell Growth Medium supplemented with SingleQuots and 0.5% FCS according to the manufacturer's instructions. Primary cells of the 3rd–5th passage were used in our studies. HK-2 cells are human PTEC immortalized by transduction with the human papilloma virus 16 E6/E7 genes [22] and have been shown to behave similarly to primary human PTEC [3,22,23]. HK-2 cells were cultured in DMEM/F12 medium supplemented with 5% FCS. Ninety percent confluent primary PTEC or HK-2 cells were growth arrested in DMEM/F12 medium for 72 h prior to experimentation. Primary PTEC were used to assess the effect of anti-dsDNA antibodies on fibronectin synthesis and phosphorylation of signaling pathways, and HK-2 cells were used to assess the effect of MPA on anti-dsDNA antibody- and cytokine-mediated changes in fibronectin synthesis. Primary PTEC and HK-2 cells will be referred to as PTEC in this study.

PTEC were incubated with serum free medium (SFM), control human IgG, or anti-dsDNA antibodies (final IgG concentration 10 μg/ml) for periods up to 72 h to assess their effect on fibronectin synthesis. This IgG concentration was chosen based on maximum cellular binding and its effect on cellular functions [3]. In separate studies, PTEC were incubated with inhibitors to ERK (PD98059, 50 μM), p38 MAPK (SB203580, 20 μM), JNK (SP600125, 20 μM), and PKC (Gö6976, 20 μM), or neutralizing antibodies to IL-6, IL-8, MCP-1, TNF-α and TGF-β1 (100 ng/ml for all) for 1 h prior to incubation with control human IgG or anti-dsDNA antibodies to assess the involvement of signaling pathways and cytokines/chemokines in fibronectin synthesis. The concentration of inhibitors used in these studies were optimum concentrations that mediated maximum inhibition of each signaling pathway without causing any cytotoxic effects [24]. To assess the effect of MPA on fibronectin synthesis, PTEC were incubated with MPA (5 μg/ml) for 1 h at 37 °C prior to the addition of control human IgG, anti-dsDNA antibodies, or exogenous IL-6, IL-8, MCP-1, TNF-α and TGF-β1 (10 ng/ml for all cytokines), and incubated for periods up to 72 h. This MPA concentration corresponded to the blood trough level attained in patients receiving a daily dose of 2–3 g mycophenolate mofetil [25,26]. This concentration of MPA was not cytotoxic and had no effect on cell viability (data not shown). To determine the

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