

Interleukin-13 Increases Podocyte Apoptosis in Cultured Human Podocytes

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Purpose: Podocytes are important architectures that maintain the crucial roles of glomerular filtration barrier functions. Despite this structural importance, however, the mechanisms of the changes in podocytes that can be an important pathogenesis of minimal change nephrotic syndrome (MCNS) are not clear yet. The aim of this study was to investigate whether apoptosis is induced by interleukin (IL)-13 in cultured human podocytes.

Methods: Human podocytes were treated with different IL-13 doses and apoptotic cells were analyzed using terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL assay) and fluorescence-activated cell sorting (FACS).

Results: The IL-13 increased the number of TUNEL-positive cells in a dose-dependent manner at 6 and 18 hours ($P < 0.05$ and $P < 0.05$, respectively). The apoptosis rate was appeared to be increased slightly in the IL-13-stimulated podocytes (8.63%, 13.02%, and 14.46%; 3, 10 and 30 ng/mL, respectively) than in the control cells (7.66%) at 12 hours by FACS assay.

Conclusion: Our study revealed that IL-13 expression may increase podocyte apoptosis. Blocking the IL-13 signal pathway can potentially play an important role in regulating the apoptosis of podocytes.

Key words: Interleukin (IL)-13, Podocytes, Apoptosis, Minimal change nephrotic syndrome (MCNS)

Introduction

Minimal change nephrotic syndrome (MCNS) accounts for 84.5% of idiopathic nephrotic syndrome (INS) in children, and 10 to 25% of INS in adults^{1,2}. About 80–90% of children with childhood INS are steroid-sensitive nephrotic syndrome (SSRS) and around 60% of MCNS children show SSRS^{3,4}. Even though initial response rate to steroids is 90% to 95%, 20–60% of SSRS children relapse and about 60–90% of them will have five or more relapses with increased morbidity^{4,5}. Resistance to therapy, also called as steroid-resistant nephrotic syndrome (SRNS), occurs in 50% of focal segmental glomerulosclerosis (FSGS) and 10% of MCNS often progress to renal failure requiring dialysis and transplantation despite various immunosuppressive treatments^{6–9}.

MCNS is composed of two important pathologic features: 1) absence of glomerular immune complex deposition and 2) foot process effacement (podocytes seem to be fused together a flattened morphology)⁸. Foot process

effacement is closely related the changes in the selective barrier in the slit diaphragm, which is composed of a number of proteins: nephrin, P-cadherin, CD2-associated protein, zona occludens (ZO)-1, Fat cadherin, podocin, and Neph1^{10,11}.

Interleukin (IL)-13 is a kind of T cell-derived cytokine¹². It is also reported to be an important cytokine in MCNS and we previously reported that IL-13 may increase podocyte permeability through the modulation of ZO-1^{13,14}. However, there have been no reports about podocyte apoptosis related to IL-13. The aim of this study was to investigate whether apoptosis is induced by IL-13 in cultured human podocytes.

Materials and methods

1. Cell culture of human podocytes

Human conditionally immortalized podocytes (AB8/23) was cloned from human glomerular cultures. Dr. Moin A. Saleem (University of Bristol, Bristol, UK) characterized and generously provided them. Then human podocytes were maintained in RPMI 1640 (WelGENE Inc., Daegu, South Korea) supplemented with 10% heat-inactivated fetal bovine serum (FBS), Insulin-Transferrin-Selenium-Pyruvate Supplement (ITSP; WelGENE Inc.), and antibiotics. Once every 2 days, fresh media was supplied.

Cells were cultivated at 33°C (permissive conditions) in a culture medium supplemented with human recombinant ITSP to induce expression of temperature-sensitive large T antigens and to stimulate human podocyte proliferation. To induce differentiation, podocytes were maintained at 37°C (non-permissive conditions) for at least 2 weeks, and for subcultures, 0.05% trypsin was used to detach cells from the culture dishes.

2. IL-13 treatment conditions

To imitate MCNS-like conditions, cells were incubated with various concentrations of IL-13 (PeproTech Inc., Rocky Hill, NJ, USA) during the indicated time periods (6, 12 and 18 hours). IL-13 was administered with various concentrations (3, 10, 30, and 100 ng/mL) into 0.5% RPMI at 37°C.

3. Measurement of apoptosis

1) Terminal deoxynucleotidyl transferase dUTP nickend labeling (TUNEL assay)

TUNEL assay was done using In situ Cell Death Detection Kit (Roche Molecular Biochemicals, Mannheim, Germany). Podocytes that were grown on type I collagen-coated glass coverslips incubated for 24 hours were fixed in 4% paraformaldehyde for 1 hour, followed by permeabilization with 0.1% Triton X-100 for 10 minutes at room temperature. After coverslips were mounted in mountant, the samples were immediately evaluated using a fluorescence microscope. The TUNEL index (apoptotic podocytes) was determined by counting the positively and negatively stained cells in each of 10 fields of vision. Cell numbers were converted to percent apoptotic cells for statistical analysis.

2) Fluorescence-activated cell sorting (FACS)

We conducted a flow cytometric analysis to elucidate whether IL-13 has apoptotic effects on podocytes. The apoptotic rate was calculated by a single-color flow cytometric analysis depending upon the IL-13 concentrations (control, 3, 10, and 30 ng/mL) and apoptotic hours (6 and 12 hours). Equal numbers of podocytes were cultivated on 6 cm tissue culture plates in medium. After IL-13 modulation, cell culture medium was collected and saved. Cells were washed once with 1.5 ml of phosphate-buffered saline (PBS). PBS used for washing was combined with the saved culture medium. Podocyte cells were analyzed with the Mofro Astrios flow cytometer (FACSCalibur-S System, Becton Dickinson Biosciences, San Jose, CA, USA). The number of apoptotic cells was calculated by multiplying the percentages of apoptosis and necrosis as determined by FACS.

4. Statistical analysis

Results are described as mean \pm standard deviation, as appropriate under different conditions. Statistical significance was evaluated by the non-parametric Kruskal-Wallis analysis or Student's t-test. *P*-values <0.05 were considered significant.

Results

1. Apoptotic rate on TUNEL assay

Apoptosis is a programmed cell death which results the series of events including alterations in the plasma membrane, activation of enzymes¹⁵. DNA fragmentation can be detected in terminal stages of apoptosis by labeling with fluorescent tagged nucleotides through a technique called as TUNEL¹⁶. Apoptotic podocytes were identified as TUNEL positive cells while the normal cells lacked nuclear staining. IL-13 increased the number of TUNEL-positive cells in a dose-dependent manner at 6 and 18 hours ($P < 0.05$ and $P < 0.05$, respectively) (Fig. 1, 2).

2. FACS assay for apoptosis

The apoptotic effects of IL-13 on podocytes were analyzed by FACS. There was no significant difference in the rate of apoptosis in controls and podocytes stimulated by different concentrations (3, 10 and 30 ng/mL) at 6 hours (9.73% vs. 13.89%, 12.52%, 10.83%, respectively, Fig. 3); at 12 hours, however, the rate of apoptosis was slightly increased in IL-13 stimulated podocytes (8.63%, 13.02%, 14.46%) than control cells (7.66%, Fig. 4).

Discussion

Podocytes are important structures which maintain the crucial roles of glomerular filtration barrier functions^{9,10}. In 2007, Lai et al. suggested that IL-13 overexpression could

lead to podocyte injury with downregulation of proteins such as nephrin, podocin, and dystroglycan and a concurrent upregulation of B7-1 in MCNS induced rat¹⁷. After that, our previous studies reported that IL-13 was involved in the changes of ZO-1 proteins in podocytes which could

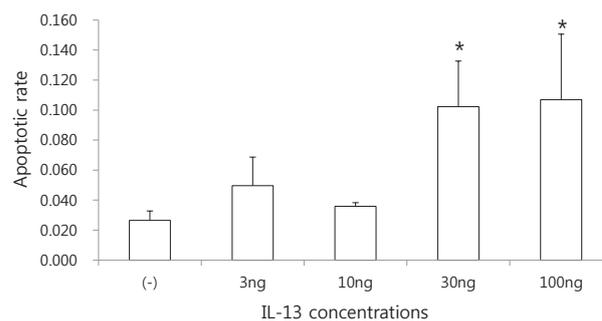


Fig. 1. TUNEL assay for apoptosis (6 hours). IL-13 increased the number of TUNEL-positive cell in a dose-dependent manner at 6 hours (* $P < 0.05$). IL-13, interleukin-13; TUNEL, terminal deoxynucleotidyl transferase dUTP nickend labeling.

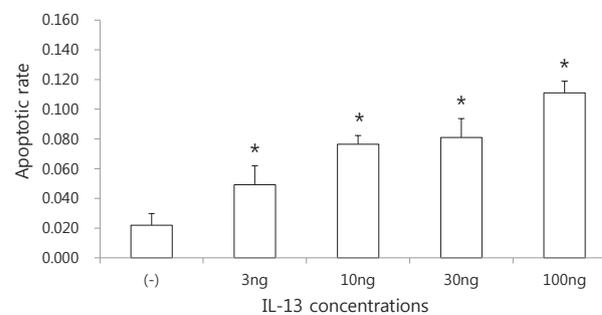


Fig. 2. TUNEL assay for apoptosis (18 hours). IL-13 increased the number of TUNEL-positive cell in a dose-dependent manner at 18 hours (* $P < 0.05$). IL-13, interleukin-13; TUNEL, terminal deoxynucleotidyl transferase dUTP nickend labeling.

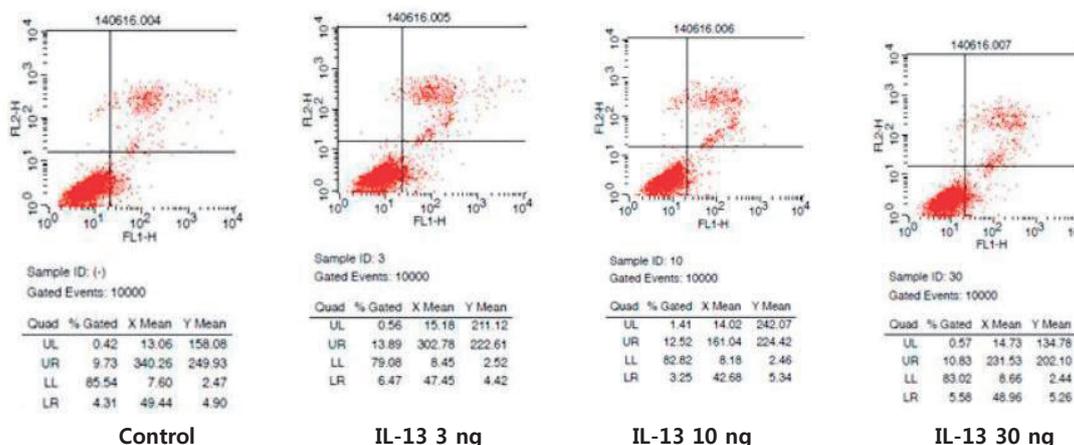


Fig. 3. FACS assay for apoptosis (6 hours). FACS, fluorescence-activated cell sorting; IL-13, interleukin-13.

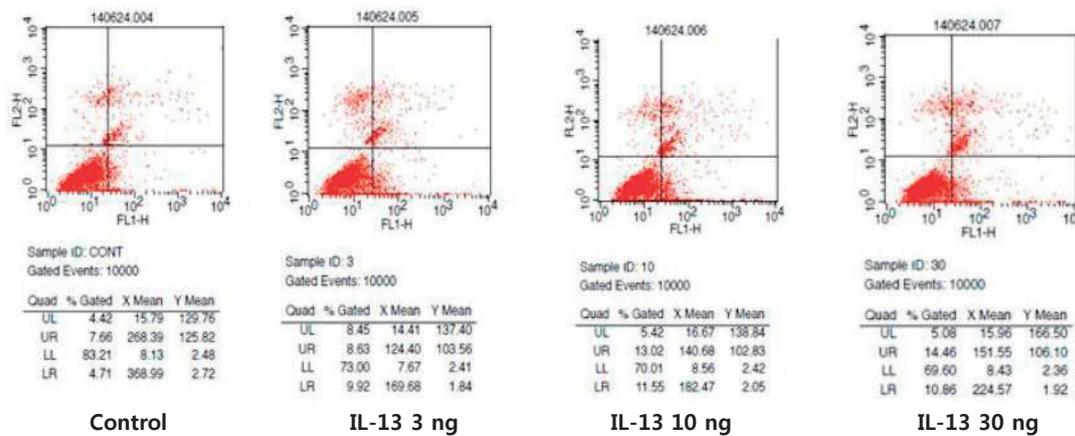


Fig. 4. FACS assay for apoptosis (12 hours). FACS, fluorescence-activated cell sorting; IL-13, interleukin-13.

be a pathogenesis of MCNS^{14,18}. Other recent studies have shown that increase in IL-13 production was to cause frequent relapse in SSNS^{13,19}. In this point of view, the aim of this study was to identify whether apoptosis in podocytes could be induced by IL-13.

There have been two suspected modes of podocyte apoptosis: 1) intrinsic apoptosis and 2) extrinsic apoptosis. Intrinsic apoptosis is related to a mitochondrion-centered control mechanism²⁰. On the other hand, extrinsic apoptosis is a caspase-dependent cell death related to activation of the caspase-9-caspase-3 cascade^{21,22}. Generally, caspase-3 is known as a key mediator as a leading enzyme in cell death²³. Pro-caspase-3 is usually processed by autoproteolytic cleavage and activated caspase-3 has been identified that are related to mitochondrial cytochrome c release and caspase-9 function^{24,25}. Manna et al. reported that IL-13 is a potent inhibitor of TNF-mediated activation and caspase-3 related apoptosis in 1998²⁶. In contrast, recent studies show IL-13 induces apoptotic pathways of cell death in head and neck cancer models^{27,28}.

IL-13 has been known as a causative factor of cell apoptosis in several previous studies. Borowski et al. reported that IL-13 can be a mediator on lung epithelial cells as an apoptotic effector, which leads to loss of functional airway tissue and finally results in asthma²⁹.

Several studies have already reported about the role of IL-13 as a pathogen in other cells and diseases other than podocytes. Heller et al. found that IL-13 can effect on intestinal epithelial barrier dysfunction and eventually cause ulcerative colitis³⁰. For this explanation, signal transduc-

tion qualities mediated by the respective receptors for IL-13 may result in distinct patterns of transcription regulation, ultimately explaining the unique role of IL-13 in asthma pathogenesis. JAK/STAT activation pathway can be an optional mechanism to explain these results³¹. This pathway is induced by the IL-13 receptor complex and then eventually causes cell apoptosis and destruction of cell junctions³¹. Exact mechanisms of these mediators, however, are unclear.

In this study, it is implicated that IL-13 can induce podocyte apoptosis. Our results and previous studies above indicate that IL-13 can be related to extrinsic apoptosis of podocyte, blocking this signal pathway.

Our study has several limitations: First, we were unable to reveal all exact mechanism of IL-13 in podocytes. Second, we did not include treatment attempt to prevent podocyte apoptosis into our experiments. Despite these limitations, our study is the first attempt to demonstrate the IL-13 as a key marker and potential treatment target to prevent changes related to cell death in human podocytes.

In conclusion, our preliminary results suggest that IL-13 may increase podocyte apoptosis. Such alterations can be relevant to the foot process changes in podocytes related to pathogenesis of proteinuria in the IL-13-induced MCNS model. Further studies about the methods of suppression of IL-13 are needed, and it will be noteworthy to explore whether IL-13 modulation can modify podocytes from MCNS.

Conflict of interest

The authors of the manuscript declare no conflict of interest.

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