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Anti-IL-2 receptor antibody decreases cytokine-induced apoptosis of human renal tubular epithelial cells (TEC)

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Abstract

Background. Transplant rejection is mediated by T-cell activation which is modulated by interleukin-2 (IL-2) binding to IL-2R (CD25). Monoclonal anti-IL-2 receptor antibody is used in renal transplantation to reduce rejection. Interestingly, proximal tubular epithelial cells (TEC) express CD25, similar to T cells. We have demonstrated that IL-2 induces murine TEC apoptosis through down-regulation of the caspase-8 inhibitor protein c-FLIP. Anti-CD25 antibody may be useful clinically to limit renal injury, but this has not been tested in human TEC.

Methods. Human PT-2 TEC were isolated and cloned from the urine of transplant patients. Apoptosis was determined by FACS with Annexin-V FITC. Protein expression was studied using western blot, and mRNA levels by quantitative real-time (PR-PCR).

Results. We demonstrated that the morphology of a human kidney cell line (PT-2) cloned from urine was consistent with proximal TEC and expresses alkaline phosphatase, cytokeratin, vimentin, CD13, CD26, and low levels of E-cadherin. Basal IL-2 receptor (CD25) was up-regulated by IL-2/IFN-γ stimulation, and cytokine exposure induced apoptosis in a dose-dependent manner. Apoptosis with IL-2/IFN-γ was associated with increased caspase-8 activity and decreased endogenous caspase-8 inhibitor c-FLIP mRNA and protein expression. IL-2/IFN-γ-induced
apoptosis could be blocked by pre-treatment of PT-2 with anti IL-2R antibody (basiliximab) but not control IgG antibody.

**Conclusions.** These data demonstrate for the first time in human TEC that IL-2 and IFN-γ can induce TEC apoptosis which can be blocked by CD25 blockade antibody. These data suggest that anti-CD25 mAb might similarly attenuate inflammation-induced TEC injury in vivo. Kidney-expressed CD25 may represent a clinically important new target for attenuating early inflammatory injury in donor kidneys and preserving renal function during anti-rejection therapy.

**Keywords:** apoptosis, human tubular cell; IL-2 receptor; interleukin-2; kidney

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**Introduction**

IL-2 is a well-characterized T-cell cytokine with central roles in inflammation and immune signaling. Its actions are generally mediated by T cells, B cells and natural killer cells which possess high-affinity IL-2 receptors, typically resulting in escalating functional activity of these target cells.

IL-2 receptor consists of at least three distinct subunits, the α chain (IL-2Rα, 55k, Tac or CD25), the β chain (IL-2Rβ, p75 or CD122), and the γ chain (IL-2Rγ, γc or p64). The affinity of IL-2R depends on which subunits are expressed at the cell surface: β and γ chains together form the intermediate-affinity receptor, whereas the high-affinity receptor requires the additional presence of the α subunit [1–3].

Basiliximab (Simulect®, Novartis) is a chimeric murine/human monoclonal antibody that is selectively directed against the IL-2 receptor α chain, also known as CD25, which is expressed on T lymphocytes in response to antigenic activation. Basiliximab has been shown to be effective in reducing the rate of acute rejection in kidney transplantation and also improving both the rate of graft and patient survival [4–6]. Currently, 30–40% of deceased-donor and living-donor renal transplant patients receive an IL-2R antibody as induction therapy to decrease rejection [7].

In specific patient subsets, the benefit of IL-2R Abs in reducing acute rejection is increased with greater HLA mismatch [8]. In contrast to polyclonal or monoclonal lymphocyte-depleting antibody preparation, anti CD25 therapy is not usually associated with side effects associated with T-cell activation or cytokine release [9]. Since calcineurin inhibitors are all potentially nephrotoxic, anti-IL-2R has also been used in an attempt to decrease CNI exposure early post-transplantation. IL-2R antagonist can also minimize the requirement for steroids in some patients [10]. Interestingly, there was an early benefit of IL-2R antibody on renal function (GFR and urine output) in registration trials of anti CD25 therapy that was not explained on the basis of rejection per se [5].

Interestingly, expression of IL-2R is not restricted to lymphoid cells only, but has also been reported on human fibroblasts, endothelial cells and intestinal epithelial cells. A previous report has also shown that human proximal tubular cells can express high-affinity IL-2 receptor which regulated the production of complement component C3 during renal inflammation [11]. Our group has previously reported that IL-2 can increase apoptosis of mouse kidney TEC along with caspase-8 activation [12]. Therefore, as IL-2 receptor is expressed in renal tissue, anti CD25 antibody has the potential to affect parenchymal cells as well as immune cells clinically. However, whether IL-2 has a direct effect on human renal TEC or if anti CD25 antibody could block IL-2 effect on human TEC has not been tested. The aim of this study was to confirm the expression of IL-2R on human TEC isolated from a transplant patient and to determine whether IL-2 could induce apoptosis in these human TEC through a caspase 8/c-FLIP-regulated pathway and whether blocking IL-2 receptor could attenuate renal cell injury directly in vitro.

**Materials and methods**

**Characterization of PT-2 cells**

PT-2 TEC were isolated and cloned from centrifuged urine, obtained with formal consent from a transplant recipient undergoing acute rejection. Work with this line has been approved by appropriate institutional review (PA, Dalhousie University). PT-2 cells have rapid growth to confluence with contact inhibition in culture medium [Dulbecco’s modified Eagle’s medium (DMEM): Hams F12] (50:50) (Invitrogen-Gibco, Carlsbad, CA, USA), supplemented with 5% bovine calf serum, hormone mix [5 μg/mL insulin, 1.25 ng/mL prostaglandin E1 (PGE1), 34 pg/mL triiodothyronine, 5 μg/mL transferrin, 1.73 ng/mL sodium selenite and 18 ng/mL of hydrocortisone]) and 25 ng/mL epidermal growth factor (EGF). PT-2 cells are γGTP- and appear to have characteristics of proximal TEC. Media were replaced in confluent monolayer cultures before experiments with serum-free K1 medium.

PT-2 cells were seeded to each well of 24-well plates overnight (Starstedt, Newton, NC, USA). On the next day, the cells were removed with 0.5% trypsin-EDTA (Invitrogen GibCO) and washed with 1× PBS twice. Following the washes, the cells were incubated in 10% goat serum for 5 min on ice. Many proximal cell lines that have been widely employed for *in vitro* studies vary in their epithelial characteristics and markers as well as patterns of cell adhesion molecule expression. PT-2 cells retain a phenotype indicative of well-differentiated tubular epithelial cells with proximal cell morphology by light microscopy and form confluent monolayers with contact inhibition as well as biochemical characteristics. To characterize, 1 μL of anti-E-cadherin-FITC, anti-CD13-PE, anti-CD26-PE or isotype control antibody (BioLegend, San Diego, CA, USA) was added, and the PT-2 cells were incubated for an additional 30 min on ice. In addition, aminopeptidase N/CD13 and dipeptidyl peptidase IV/CD26 are widespread membrane-bound peptidases found on cultured renal cells, and E-cadherin expression is considered a marker of differentiated epithelial phenotype. Cells were washed twice with 1× PBS before FACS analysis. To further characterize the PT-2 cell line, expression of alkaline phosphatase, cytokeratin and vimentin was assessed by using a previously described technique [13]. PT-2 cells, grown on glass culture slides (BD Falcon, Bedford, MA, USA), were fixed with citrate–acetone–formaldehyde fixative solution and then stained for alkaline phosphatase with a solution of naphthol AS phosphatase and fast blue BB, followed by counterstaining with neutral red solution according to Sigma Procedure No. 86 (Alkaline phosphatase kit, Sigma-Aldrich, St. Louis, MO, USA). For histochemical stains of cytokeratin and vimentin, PT-2 cells were fixed in ice-cold acetone, followed by rinsing in TBS and stained by immunoperoxidase with an Elite Veetastain ABC kit (Vector Laboratories). Cells were stained for cytokeratin with rabbit anti-cytokeratin mAb (Abcam Inc, Cambridge, MA, USA) or for vimentin with goat anti-vimentin mAb (Abcam Inc, Cambridge, MA, USA).
Slides were examined using light microscopy. Negative controls were performed by omitting the primary Abs.

**Flow cytometric analysis (FACS)**

Cells (2.5 × 10^6) were plated overnight in each 24-well plates and induced the following 24 h with recombinant human IL-2 (R&D systems Inc, Minneapolis, MN, USA), then another following 48 h with recombinant human IFN-γ or not (BD PharMingen, Mississauga, ON, Canada). Recombinant human TNF-α (BD PharMingen, Mississauga, ON, Canada) was used as positive control.

PT-2 cells were cultured for up to 72 h, and cells were released from culture plates by a brief incubation with trypsin-EDTA solution (Sigma-Aldrich, St. Louis, MO, USA). For analysis of surface protein expression, cells were incubated with basiliximab (Simulect®, a chimeric murine/human IL-2 receptor α chain monoclonal antibody, anti-CD25 receptor antibody, a kind gift from Novartis, St. Louis, MO, USA) or with mouse IgG (Sigma Aldrich) as negative control, followed by a secondary staining of phycoerythrin (PE) goat anti-human IgG Fe gamma fragment specific from ebioscience (San Diego, CA, USA). The cells were also incubated with anti-CD25 conjugated with fluorescein isothiocyanate (FITC) (BD PharMingen, Mississauga, ON, Canada) in phosphate-buffered saline (PBS) containing 10% normal goat serum at 4°C for 30 min, then washed with PBS twice.

For apoptosis assay by FACS, the cells were incubated with Annexin-V conjugated with FITC in 1× binding buffer (BD PharMingen) for 15 min, following which, apoptotic cells were identified by Annexin-V-FITC staining.

The density of fluorescein binding on the cell was measured by a flow cytometer and analyzed by CellQuest software (BD Bioscience, Mississauga, ON, Canada). Thin line indicated the background staining.

**mRNA analysis by quantitative real-time PCR**

The level of total c-FLIP mRNA in PT-2 cells was analyzed by real-time PCR (RT-PCR). Total RNA was extracted from TEC cultures using TRI reagent (Sigma Aldrich) following the provided protocol. cDNA pools were synthesized with the First-Strand Synthesis System according to the manufacturer's protocol (Stratagene). Primers were designed using Primer Express, primer designing software from Applied Biosystems. For human c-FLIP-L (112 bp), the following primers were used: 5'-GCCTGTATGCCCGAGCACCG-3' (antisense) and 5'-GCAGGGG-GAGCCCTTGGATGAG-3' (sense). For human beta actin mRNA (250 bp): 5'-CATGTCAGTTGATCATCCAGGC-3' (sense) and 5'-CTCCTATGGTCAGCCAGAT-3' (antisense). The gene sequences were obtained from the www.ncbi.nlm.nih.gov database. Real-time quantitative PCR was performed on standardized quantities of cDNA using the Brilliant SYBR Green QPCR Master Mix kit, and the MX4000 system (Stratagene). The beta actin mRNA amplification was used as the endogenous control. The normalized delta threshold cycle value and relative expression levels (2^-ΔΔCT) were calculated according to the manufacturer’s protocol.

**Western blot**

Total protein expression of PT-2 cell cultures was examined as described previously [14]. Briefly, whole cell lysates were collected in lysis buffer [10 mmol/L Hepes (pH 7.9), 10 mmol/L KCl, 0.1 mmol/L EDTA, 0.1 mmol/L ethyleneglycol tetraacetate (EGTA), 0.1% NP 40, 1 mmol/L dithiofretiole (DTT) and complete protease inhibitor cocktail] (Roche, Mannheim, Germany). These were mixed with 2× sodium dodecyl sulfate (SDS) sample buffer [20 mmol/L Tris–HCl (pH 6.8), 5% (wt/vol) SDS, 10% (vol/vol) mercaptoethanol, 2 mmol/L EDTA, and 0.02% bromophenol blue] and boiled for 5 min. The protein content of each cell lysate was determined by Bio-Rad assay (Bio-Rad Laboratories, Hercules, CA, USA). Of total protein from each sample, 100 μg was fractionated by 10% SDS–polyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose membranes (Bio-Rad), blocked with 5% Carnation fat-free milk in Tris-buffered saline Tween-20 (TBS-T) for 1 h, and then probed with rabbit anti-procaspase 8 antibody (R&D systems Inc, Minneapolis, MN, USA) or anti-c-FLIP polyclonal antibody (Stressgen Biotech, Victoria, BC, Canada) in TBS containing 2.5% of milk at 4°C overnight. The specific protein binds recognized by the antibodies on the membrane were visualized by enhanced chemiluminescence (ECL) assay (Amersham Pharmacia Biotech, Buckinghamshire, England). Blots were re-probed using anti-beta-actin IgG (Sigma-Aldrich) for confirmation of loaded protein in each sample.

**Statistical analysis**

Comparisons between groups were performed using analysis of variance (ANOVA) or t-test as appropriate with Statview 5.0 (SAS, Cary, NC, USA). To compare levels between groups, we used analysis of variance. P-values of <0.05 were considered significant.

**Results**

**Expression of IL-2 receptor (CD25) in human PT-2 TEC**

To study the role of IL-2 receptor in renal TEC, we used a unique human (PT-2) cell line that derived from patient urine. Many proximal cell lines (HK-2, LLC-PK1, NRK-52E and others) that have been widely employed for in vitro studies vary in their epithelial characteristics as well as patterns of cell adhesion molecule expression. Although PT-2 cells have rapid growth to confluence in K1 medium, are γGTP+, appear to have a typical proximal tubular cell morphology with uniformly elongated and spindle-shaped or cobblestone-like growth, and exhibit contact inhibition in monolayers (Figure 1A), further characterization was performed. Alkaline phosphatase activity was demonstrated in PT-2 cells, as shown in Figure 1B with areas of intense blue staining within pink cytoplasm background. In addition, the cells stained positively (brown color) for cytokeratin (Figure 1C) and vimentin (Figure 1D), both being characteristic of epithelial cells. Staining pattern was equivalent to that observed with CS3.1 used as further controls, which are cloned mouse proximal TEC [15]. PT-2 cells express low levels of E-cadherin (Figure 1E) and aminopeptidase N (CD13, Figure 1F), as well as high levels of dipetidyl peptidase (CD26, Figure 1G).

Previous studies have demonstrated the expression of functional surface receptors for IL-2 in human renal proximal tubular epithelial cells. We have shown that TEC apoptosis in response to exogenous IL-2 and confirmed the expression of IL-2R including α, β and γ chains in mouse TEC response to IL-2 and IFN-γ [12]. To test this in human cells, we first tested if PT-2 cells express high-affinity IL-2 receptors. PT-2 cells were pre-exposed to IL-2 (40 ng/mL) for 24 h and then cultured for another 48 h with IFN-γ (20 ng/mL). As shown in Figure 2A and B, CD25 is upregulated by combination of IL-2 and IFN-γ stimulation (MFI = 13.17 ± 0.4%, P < 0.01) when compared with cells without cytokine stimulation (4.53 ± 0.6%, P = 0.01) when compared with cells without cytokine stimulation (4.53 ± 0.48%). A lower degree of upregulation was observed in PT-2 cells with either IL-2 (6.32 ± 0.61%) or IFN-γ (5.9 ± 0.63%) alone. To further confirm the surface expression of high-affinity IL-2R, cytokine-treated PT-2 cells were labelled using an antibody specific for high-affinity IL-2 receptor (basiliximab, Simulect®), followed by a secondary IgG-PE. Similar results were noted, with IL-2 receptor increasing in PT-2 cells from MFI of 5.33 ± 0.53% in untreated cultures to 13.35 ± 3.53% (P < 0.01) in IL-2/IFN-γ-treated cultures (Figure 3A and B).
IL-2- and IFN-γ-induced human TEC apoptosis

In our previous report, IL-2 as well as pro-inflammatory cytokines such as IFN-γ can induce apoptosis in murine TECs through a Fas/FasL-dependent mechanism [12]. To confirm the effect on apoptosis by cytokine stimulation in human cell line, PT-2 cells were pre-treated with IL-2 (20 or 40 ng/mL) for 24 h, followed by IFN-γ (0, 10 or 20 ng/mL) for a further 48 h in serum-free media. Cells were then analyzed for apoptosis by FACS using Annexin-V (Figure 4A and B). TNF-α treatment was also used as a positive control. Pre-incubation of PT-2 cells with IL-2 followed by IFN-γ resulted in greater apoptosis than observed with IL-2 or IFN-γ alone, in a dose-dependent manner. Furthermore, apoptosis using the combination of IL-2 (20 ng/mL) and IFN-γ (10 ng/mL) increased to 21.9 ± 3.69% (P < 0.01) compared with 11.00 ± 2.35% in non-treated TEC cultures. TEC apoptosis reached 40.9 ± 4.25% (P < 0.001) using higher concentrations of IL-2 (40 ng/mL) and IFN-γ (20 ng/mL) in cultures, compared with a modest increase with IFN-γ alone (15.80 ± 0.76% at 10 ng/mL, P < 0.05 and 20.75 ± 4.28% at 20 ng/mL, P < 0.001). These data demonstrate that IL-2 and IFN-γ can induce apoptosis in human TEC and that pre-exposure of TEC to IL-2 can augment the level of IFN-γ-induced apoptosis.

Fig. 1. PT-2 cells express tubular epithelial cell (TEC) morphology and markers. (A) Morphology of PT-2 cells grown in monolayer (×400). (B) Histochemical demonstration of alkaline phosphatase in PT-2 cells. Immune cytochemical demonstration of cytokeratin (C) and vimentin (D). The positive staining areas are depicted by arrows in the images. Flow cytometry demonstration of E-cadherin-FITC (E), CD13-PE (F), CD26-PE (G), (dark line) or isotype control antibody (grey line). FACS analysis was presented as percentage of increase in the intensity of fluorescence versus background staining.
IL-2- and IFN-γ-induced apoptosis in PT-2 cells is associated with caspase-8 activation

We have previously shown in mouse TEC that apoptosis in response to the pro-inflammatory cytokines IFN-γ and TNF-α occurs by a mechanism of Fas-mediated self-injury, which is related to the activation of caspase-8 pathway [12]. The Fas death signal pathway is mediated by procaspase-8 recruitment to the death-inducing signaling complex (DISC) with subsequent processing to mature caspase-8 which cleaves downstream caspases. Thus, we tested for loss of pro-caspase-8 using western blot analyses. Following incubation with IL-2 (40 ng/mL) or IFN-γ (20 ng/mL) in media without serum, procaspase-8 decreased with IFN-γ alone stimulation by 8 h, and maximally decreased with combined IFN-γ/IL-2 treatment. No change was seen by 4 h (Figure 5). These data suggest that cytokine-induced apoptosis of human TEC occurs through caspase-8 activation and that IL-2 augments IFN-γ-induced apoptosis.

PT-2 cells express c-FLIP which inhibits apoptosis and is down-regulated by IL-2

Cellular FLICE like inhibitory protein (c-FLIP) is an intracellular anti-apoptotic homologue of procaspase-8. We have previously demonstrated the expression of c-FLIP in highly differentiated murine proximal TECs. We now show that human TEC express c-FLIP-L (Figure 6A) and that c-Flip-L mRNA is altered by cytokine exposure. Using the baseline expression of FLIP as a maximal reference (100%), c-FLIP-L decreases more with IFN-γ treatment (50.8 ± 11.1%, P < 0.05) than IL-2 treatment (94.0 ± 5.0%) exposure by 4 h, but maximally decreases with both IL-2 and IFN-γ exposure (5.4 ± 1.2%, P < 0.01). This observation was confirmed for c-FLIP protein using western
blot and an antibody which recognizes the major isoforms of c-FLIP in mammalian cells (Figure 6B). In PT-2 cells, c-FLIP-L protein (55 kDa) was primarily expressed, and not the shorter processed FLIP (43 kDa). These data suggest that loss of c-FLIP in cytokine-exposed PT-2 cells may permit enhanced caspase-8-mediated apoptosis in PT-2 cells.

**Pre-treatment of PT-2 with basiliximab blocked IL-2/IFN-γ-induced human PT-2 TEC apoptosis**

IL-2 as well as IFN-γ can induce apoptosis in human PT-2 cells. As IL-2 receptor increased in PT-2 cells with IL-2 and IFN-γ, we tested whether IL-2 receptor antibody might block its function and decrease cytokine-induced apoptosis. PT-2 cells were pre-treated with basiliximab (anti IL-2 receptor antibody, 20 μg/mL) or mouse IgG isotype control (20 μg/mL) for 1 h in serum-free media and then incubated with IL-2 (0 or 40 ng/mL) for 24 h prior to exposure to IFN-γ (0 or 20 ng/mL) for a further 48 h. As shown in Figures 7A and B, pre-treatment of PT-2 cells with basiliximab decreased apoptosis in response to IL-2 and IFN-γ (19.30 ± 2.60% vs. 37.46 ± 3.16%, P < 0.001). Control antibody had no effect on apoptosis. These data suggest that pre-treatment of human TEC with basiliximab can attenuate IL-2/IFN-γ-induced apoptosis in vitro and might also have a similar protective effect in vivo.

**Fig. 4.** IL-2/IFN-γ induce apoptosis in human PT-2 cells in a dose-dependent manner. Apoptosis was determined by FACS analysis with Annexin V-FITC staining, and presented as percentage increase in the intensity of fluorescence versus background staining. Human PT-2 cells were pre-incubated with low dose IL-2 (20 ng/mL) or high dose (40 ng/mL) for 24 h, then with IFN-γ (10 or 20 ng/mL) for 48 h. *P < 0.05, **P < 0.01, ***P < 0.001 vs. medium controls. #P < 0.001 vs. treatment with IL-2 (20 ng/mL) and IFN-γ (10 ng/mL). TNF-α was used as a positive control, but apoptosis was less than pre-incubation with IL-2 and exposure to IFN-γ. A representative experiment is presented in A. Data are presented as mean ± SD of duplicate samples (B) and are representative of three separate experiments.
expression of high-affinity IL-2 receptor in human TEC and characterized the regulatory effect of IFN-γ and IL-2 on the expression of IL-2 receptor in PT-2 cells (Figures 2 and 3).

TEC can interact through Fas and FasL, and contact of adjacent cells during inflammation can result in a form of self-injury that may contribute to kidney injury with ischemia and immune injury during rejection [27,28]. Fas–FasL interactions lead to processing of procaspase-8 to caspase-8, and this activated caspase-8, the most upstream caspase in the Fas apoptotic pathway, promotes caspase-3 activation, DNA fragmentation and cell death. Given the importance of this death pathway, cells have evolved mechanisms of protection, and this appears to be the case in kidney TEC. c-FLIP, also termed cellular FLICE (caspase-8)-inhibitory protein, was shown by our group to be expressed in TEC and is an inhibitor of this death receptor pathway through interference with procaspase-8 recruitment to the adaptor Fas-associated death domain protein (FADD). In humanrenal carcinoma cells, it has been shown that c-FLIP may alter resistance to chemotherapeutic-induced apoptosis, and c-FLIP maybe an important modulator of death receptor-mediated apoptosis in renal cancer cells [32,33]. c-FLIP-L is constitutively expressed in mouse TEC as well as renal cortex and may

Discussion

Kidney transplantation remains the optimal therapeutic option for patients with end-stage kidney disease. Acute and chronic rejection leading to graft loss is dependent on adaptive immunity, innate immunity and graft-specific factors. Long-term graft survival may benefit by a greater understanding of the mechanisms of parenchymal cell injury and, in particular, tubular epithelial cells. More studies are now focusing on endogenous kidney factors and TECs as they appear to have a key role in regulating intragraft immune responses which may contribute to kidney injury by multiple mechanisms [16–20].

IL-2 can be detected in renal allografts post-transplantation as well as in various forms of autoimmune nephritis, although not as prominently as pro-inflammatory cytokines such as IFN-γ [21–24]. Acute transplant rejection is predominantly mediated by T-cell activation which is modulated by IL-2 binding to IL-2 receptor (CD25). Therefore, monoclonal anti-CD25 receptor antibody (basiliximab) has been extensively applied to renal transplantation to reduce immune rejection and may also attenuate lymphocyte-mediated ischemia–reperfusion injury [25].

Mouse and human renal proximal TEC have been shown to express IL-2 receptor α (CD25), β and γ chains on their surfaces. IL-2 receptor has also been detected in kidney tissue of crescentic glomerulonephritis patients by immunohistochemistry. Expression has been seen in cortical interstitial cells, renal tubular epithelial cells, and cells within glomerular crescents [26]. Such reports have demonstrated the wide expression of IL-2R on various tissues and non-T cells, but do not speculate on the potential role of IL-2R on kidney parenchymal cells. In addition, previous work has clearly demonstrated that the expression of IL-2R can be up-regulated with IL-2 and/or IFN-γ in human TEC culture [11]. In our study, we confirmed the
function to protect parenchymal cells from inflammation-associated apoptosis [12]. The expression of c-FLIP may be similarly important in human kidney.

Our previous data have shown that both IL-2 and IFN-γ can induce apoptosis in murine TECs, and activate caspase-8 [12]. IFN-γ may augment caspase-8 activation by increasing surface Fas expression to facilitate greater interaction with FasL on adjacent murine TECs. IL-2, in contrast, has no effect on Fas expression by TEC and might participate in TEC apoptosis through effects on c-FLIP Fas [12]. In our present study, IL-2 and IFN-γ together appear to be required to induce maximal apoptosis in human TEC (Figure 4). While we have not detailed the mechanism of increased apoptosis induction in human TEC following IL-2 and IFN-γ exposure, it is related to down-regulation of c-FLIP which may lead to subsequent caspase-8 activation (Figures 5 and 6). Importantly, in our study, pre-treatment of TEC with anti-IL-2 receptor monoclonal antibodies reduced cytokine-induced apoptosis which may have relevance to basiliximab treatment in human kidney transplantation (Figure 7). In addition to reduced T-cell activation, blocking IL-2R may have an additional benefit in TEC survival.

**Fig. 7.** IL-2/IFN-γ-induced apoptosis is blocked by pre-treatment with basiliximab in IL-2R-bearing human PT-2 cells. Apoptosis of PT-2 cells was determined by FACS analysis with Annexin V-FITC staining, and presented as percentage of increase in the intensity of fluorescence versus background staining. PT-2 cells were pre-incubated with IL-2 40 ng/mL in the presence of IgG control 20 μg/mL or IL-2 receptor antibody (basiliximab, Simulect, Novartis) 20 μg/mL for 24 h, and then treated in the absence or presence of IFN-γ 20 ng/mL for 48 h. *P < 0.05, ***P < 0.001 vs. with IgG control. A representative experiment is presented in A. Data are presented as mean ± SD of duplicate samples (B) and are representative of three separate experiments.
The primary focus of allograft rejection is invasion of proximal tubular epithelial cells within the renal cortex. In the present study, we used a novel tubular epithelial cell line with spontaneous in vitro growth capacity and with morphological and biochemical features consistent with a proximal TEC origin. However, many proximal cell lines (HK-2, LLC-PK1, NRK-52E and others) that have been widely employed for in vitro studies are known to vary substantially in their epithelial characteristics, surface markers, function and patterns of cell adhesion molecule expression [13,34]. This remains a limitation in the use of cell lines in vitro and extrapolation to proximal TEC function in vivo. However, given that previous reports have confirmed the expression of CD25 in isolated proximal TEC, our data are in support of a benefit of CD25 blockade in proximal TEC during inflammation. Furthermore, as alloimmune responses are directed against all tubular epithelial cells, strategies to block immune injury of TEC in general would be expected to have a benefit following transplantation.

In summary, these data demonstrate for the first time the capacity of IL-2 to regulate cytokine-induced apoptosis in human TEC in vitro. While mechanisms remain unknown, down-regulation of c-FLIP leading to increased caspase-8 activation may contribute to kidney injury. Blocking IL-2 receptors on human renal TEC in vitro decreased apoptosis, which may be relevant to clinical use if anti-CD25 mAb can similarly attenuate inflammation-induced TEC injury in vivo. Further studies to block IL-2 receptor expression in donor kidney graft prior to transplantation or in patients with acute rejection should be considered for further investigation. In addition, kidney-expressed CD25 may be a useful and clinical novel adjunct therapeutic target to prevent tubular injury and preserve renal function during acute rejection therapy.

Supplementary data

Supplementary data is available online at http://ndt.oxfordjournals.org.

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Conflict of interest statement. None declared.

References

Mindin: a novel marker for podocyte injury in diabetic nephropathy

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Abstract

Background. GeneChip Expression Analysis was employed to survey the glomerular gene expression profile in a type 2 diabetes (T2D) model of KK/Ta mice fed with a high-calorie diet (HC), and we focused on the role of mindin (also called spondin 2), whose expression is upregulated by HC.

Methods. Isolated glomeruli from three 20-week-old KK/Ta mice fed with HC or a standard diet (SD) were dissected. Total RNA was extracted and labelled for hybridization using the Affymetrix GeneChip Mouse Genome 430 2.0 Array. The gene expression profile was compared between the HC and SD groups using GeneSpring 7.3.1 software. Mindin expression was examined using real-time PCR, western blot analysis and immunohistochemical staining in the glomeruli, cultured podocytes and urine samples of both mice and humans.

Results. Podocyte foot process effacement was observed in mice fed with HC. The mindin protein expression levels in mice were localized in the podocytes, and their levels in the glomeruli were increased in the HC group compared with the SD group. The levels of urinary mindin in the HC group at 16 weeks of age were also significantly higher than those in the SD group although albumin/creatinine ratio (ACR) did not differ between the groups. Furthermore, the levels in patients with T2D were higher than those in healthy individuals and increased gradually with increases in ACR.

Conclusions. Mindin could be related to podocyte injury and appears to be an early biomarker of the progression of diabetic nephropathy.

Keywords: biomarker; high-calorie diet; KK/Ta mouse; spondin 2

Introduction

Diabetic nephropathy (DN) is a major cause of end-stage kidney disease (ESKD) in the USA, Japan and most of Europe [1]. Almost 30% of diabetic patients develop DN despite strict blood glucose and/or blood pressure control [2]. Since DN occurs in familial clusters [3–5] and not all patients with poor metabolic control develop nephropathy, genetic factors may contribute to susceptibility to this disease. Genetic analysis of the whole kidney is difficult for studying diabetic glomerulopathy because glomeruli occupy only a small part of the kidney. Recent studies have shown that podocyte injury plays a role in the pathogenesis of various glomerular diseases, including DN [6–9]. However, the precise molecular mechanisms underlying DN remain unclear.

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