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Hypoxia induces intercellular adhesion molecule-1 on cultured human tubular cells

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Hypoxia induces intercellular adhesion molecule-1 on cultured human tubular cells. The adverse effects of acute renal ischemia are partly mediated through an infiltration of inflammatory cells into the tubulointerstitium. The expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) by resident renal cells (endothelial cells and tubular cells) may facilitate this process. We investigated whether hypoxia stimulates the expression of ICAM-1 by cultured human proximal tubular cells (HPTC). Hypoxic culture conditions ($PO_2 < 4$ kPa) stimulated the expression of ICAM-1 by HPTC in a time-dependent manner ($P < 0.0001$) as demonstrated by quantitative flow cytometry analysis. Quantitative PCR demonstrated an increase in ICAM-1 transcription. Re-oxygenation of tubular cells did not increase ICAM-1 expression further. TNFα concentration in culture supernatants increased with hypoxia, but blocking experiments demonstrated that TNFα was not implicated in hypoxia-induced expression of ICAM-1. Furthermore, the cytokines IL-6 and IL-1β were not involved, but the effect of hypoxia was blocked by PDTC, an antioxidant that may inhibit the activation of the transcription factor NF-κB. These data demonstrate that hypoxia is a stimulus that induces the synthesis and expression of the adhesion molecule ICAM-1, presumably via the activation of NF-κB.

Ischemic damage to both native kidneys and kidneys after renal transplantation is a common clinical problem. Following an episode of renal ischemia, there is a marked infiltration of leukocytes into the tubulointerstitium, which contributes to ischemia-reperfusion injury [1–4]. Renal proximal tubular cells are known to express adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), in a variety of pathological conditions including transplant rejection and glomerulonephritis [1, 4, 5]. It is thought that the expression of these adhesion molecules on the cell surface is required to mediate the adhesion of leukocytes to tubular cells. In an experimental model of renal ischemia and reperfusion in the rat, administration of an anti-ICAM-1 antibody has been shown to preserve renal function [2]. Furthermore, a protective effect of antisense oligonucleotides for ICAM-1 has been demonstrated in reperfusion injury of the kidney [6]. Also, in clinical renal transplantation the administration of anti-ICAM-1 antibodies protects against renal dysfunction and reduces the occurrence of rejection [7]. The benefit seen with the use of anti-ICAM-1 in renal transplantation could, in part, result from a protective effect of this antibody on hypoxia-reperfusion injury [2, 4]. *In vitro*, the expression of ICAM-1 and VCAM-1 by tubular cells in culture is stimulated by cytokines [interleukin-1β, tumor necrosis factor-α (TNF-α) and interferon-γ] [1, 4, 8–12]. This is dependent on protein kinase C since cytokine-stimulated adhesion molecule expression can be inhibited by inhibitors of protein kinase C and be mimicked by the use of protein kinase C agonists [13, 14]. Tubular epithelial cells are also able to produce a variety cytokines, including TNF-α [15] and IL-6 [16–18], which could in turn modulate the synthesis and expression of adhesion molecules on the tubular epithelial cell surface in an autocrine manner.

The effects of hypoxia on the expression of adhesion molecules have been studied *in vitro* on cultured endothelial cells. Exposure of endothelial cells to hypoxia followed by re-oxygenation induces an increase in the adhesiveness of endothelial cells for neutrophils and related leukocytic cell lines [19–21] as well as for lymphocytes [22]. This increased adhesiveness is associated with the stimulation of transcription and of expression of the adhesion molecules ICAM-1 [19, 20] and E-selectin, formerly named endothelial leukocyte adhesion molecule-1 (ELAM-1) [19]. The release of interleukin-1α [19] and platelet activating factor (PAF) [21] has been shown to mediate the effects of hypoxia on the synthesis and expression of adhesion molecules by cultured endothelial cells. In other studies, hypoxia has been shown to activate the transcription and synthesis of inflammatory mediators such as IL-8 and MCP-1 [23] through direct activation of the transcription factor NF-κB by phosphorylation of its inhibitor IκB [23, 24].

Renal proximal tubular cells are especially sensitive to exposure to hypoxia [3, 25, 26]. As stated above, these cells may secrete various cytokines, including potent inducers of the expression of ICAM-1 such as TNF-α, the secretion of which could be stimulated by hypoxia. Therefore, the current studies were designed to examine the effects of hypoxia on the expression of ICAM-1 by tubular cells and to define the mechanism involved. The studies demonstrate that hypoxia induces the synthesis and expression of the adhesion molecule ICAM-1 by cultured human tubular cells, independent of the secretion of the cytokines TNF-α, IL-6, and IL-1β. Pyrrolidine dithiocarbamate (PDTC), an antioxidant that inhibits the activation of transcription by NF-κB, reduced the effect of hypoxia on ICAM-1 expression.

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Methods

Materials

Cell culture media were obtained from Gibco (Irvine, UK). Defined medium additives, collagenase type II, bovine collagen type I were from Sigma (St. Louis, MO, USA). The recombinant human cytokines tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were purchased from R & D Systems (Abingdon, UK).

Cell culture

Human tubular cells were isolated as previously described [27] by a modification of the method of Detrisac et al [28]. The outer cortex was dissected from the normal pole of kidneys removed for treatment of carcinoma or from donor kidneys deemed unsuitable for transplantation and cut into 1 mm^3 pieces. These were suspended in type II collagenase, 1 mg/ml at 37°C for 30 minutes. The digest was passed through a series of sieves of diminishing mesh sizes (500, 250, 90 μm) and the glomeruli removed on the top of the 90 μm mesh. The tubular fragments that passed through the sieves were seeded into 75 cm^2 flasks that had been previously coated with bovine collagen type I and adsorbed fetal calf serum proteins.

The cells were grown in DMEM:F12 with the addition of 25 mM HEPES buffer, insulin (5 μg/ml), transferrin (5 μg/ml), selenium (5 ng/ml), tri-iodo-thyronine (4 pg/ml), hydrocortisone (36 ng/ml), benzyl penicillin (100 IU/ml) and streptomycin (50 μg/ml). At the third passage and thereafter, 5% heat-inactivated fetal calf serum was added to the above medium since it was found that the serum was required to achieve cell confluence. Experiments were performed with cells from the second to the fifth passages.

Cell characterization

Cytospins of cells were prepared for immunostaining with mouse monoclonal antibodies to cytokeratin (clone MNF116; Dako, Glostrup, Denmark), human epithelial membrane antigen (clone E29; Dako), and factor VIII related antigen (clone F8/86; Dako). Standard histochemistry was used to detect nonspecific esterase [29, 30]. Cultured human endothelial cells (gift of Dr Bryan Williams, University of Leicester, UK) were used as a positive control for factor VIII related antigen staining.

Cyclic 3′5′ adenosine monophosphate (cAMP) production by the cells was measured in response to a 15 minute stimulation by parathyroid hormone (10^{-6} M) or arginine vasopressin (10^{-6} M) in the presence of the phosphodiesterase inhibitor IBMX (isobutyl-3-methyl xanthine 5 mM). cAMP was measured by radioimmunoassay (Amersham, UK) after extraction with 0.01 N HCl as previously described [31].

Exposure of cells to hypoxia/re-oxygenation

Hypoxic culture conditions were produced by placing the cells in a mini-incubator perfused with a 95% N_2/5% CO_2 gas mixture saturated with water. Temperature was maintained at 37°C. The cells were exposed to hypoxia for a maximum of 24 hours, during which time oxygen tension in the culture medium was 3.1 ± 0.5 kPa (vs. 17.5 ± 1.4 kPa in 95% air/5% CO_2 control conditions). Re-oxygenation was performed by placing the cells in an incubator gassed with 95% air/5% CO_2.

Cell viability in hypoxic and control conditions was assessed by trypan blue exclusion and the measurement of lactate dehydrogenase activity in the supernatants.

Flow cytometry analysis

For flow cytometry analysis, cells were cultured in six well plates (6 × 10 cm^2). At the end of the experimental period, cells were rinsed three times with HBSS at 4°C, then detached with trypsin-EDTA for five minutes at 37°C. Trypsinization was stopped by the addition of PBS containing 1% NaN_3 and 10% FCS. Thereafter, cells were pelleted, centrifugated and incubated for 10 minutes at 4°C in PBS containing 1% NaN_3 and 1% goat serum. After two washes with PBS/1% NaN_3, cells were incubated with the primary antibody for 30 minutes at 4°C. Antibodies used were a murine monoclonal anti-ICAM-1 (BBIG-I1; R&D Systems clone) at a concentration of 1 μg/50 μl/25,000 cells, a murine monoclonal anti-VCAM-1 (clone BBE 3; R&D Systems) at a concentration of 1 μg/50 μl/25,000 cells and, as a control, a murine monoclonal anti-human von Willebrand factor (clone F8/86; Dako) at a concentration of 0.3 μg/50 μl/25,000 cells. After three washes with PBS/1% NaN_3, cells were incubated for 30 minutes at 4°C with a FITC-labeled goat monoclonal anti-murine IgG, then washed twice and fixed with PBS containing 1% NaN_3 and 1% paraformaldehyde.

Flow cytometry analysis was performed with a FACScan, (Becton-Dickinson, Mountain View, CA, USA). To standardize cell-associated immunofluorescence between different experiments, the instrument was calibrated with quantitative fluorescein microbead standards [32, 33] (Flow Cytometry Standards Corp, Research Triangle Park, NC, USA). A standard curve was constructed by plotting FITC molecules per bead versus log of mean fluorescence intensity. The presence of ICAM-1 on the cells was expressed as the number of fluorescent molecules (fm) per cell.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Tubular cells cultured in six well plates were rinsed three times with PBS at 4°C, and then harvested with a sterile rubber policeman. Polyadenylated RNA was then prepared using a Micro-FastTrack kit (Invitrogen, San Diego, CA, USA). Single strand cDNA was then synthetized by reverse transcription using a method modified from Sambrook, Fritsch and Maniatis [34]. Briefly the RNA sample was incubated for one hour at 42°C with 1 μg of polyA+ RNA, 20 μg/ml oligodeoxythymidine and 200 units M-MLV RNase H-reverse transcriptase (Gibco BRL, Gaithersburg, MD, USA). Amplifications were performed on a 50-μl reaction mixture containing 5 μl of cDNA-RNA hybrids, 200 μM dATP, dCTP, dGTP, 150 μM dTTP, 50 μM biotinylated-11-dUTP, 20 mM Tris-HCl pH 8.55, 2.5 mM $MgCl_2$, 16 mM $(NH_4)_2SO_4$, 150 μg/ml bovine serum albumin (BSA), 1 mM of each oligonucleotide primer and 2.5 units of Thermus aquaticus (BioTaq^{TM}) polymerase (Bioprobe Systems). The reaction was subjected to defined rounds of temperature cycling with a thermocycler. A typical temperature cycle was 30 seconds at 95°C (denature), five seconds at 56°C (anneal) and one minute at 72°C (elongate) [35].

The oligonucleotide primers for the PCR are shown in Table 1 [35, 36]. Oligodeoxynucleotides were synthesized according to a published sequence for ICAM-1 [37]. Human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) primers were provided by Clontech Laboratories, Inc. (Palo Alto, CA, USA).

The exponential range of amplification was determined for

Table 1. Oligonucleotides of 5′ primers and 3′ primers of target genes

mRNA species	Sense	Sequence (5′-3′)	Nucleotides	Size of PCR product-bp
ICAM-1	(+)	GTCCCCCTCAAAAGTCATCC	105–124	943
	(−)	AACCCCATTCAGCGTCACCT	1028–1047	
G3PDH	(+)	TGAAGGTCGGAGTCAACGGATTTT	71–96	983
	(−)	CATGTGGGCCATGAGGTCCACCAC	1030–1053	

each primer set. The number of cycles was adjusted so samples were analyzed in the linear phase of amplification. As previously reported [35], the number of cycles was 18 for G3PDH, and 22 for ICAM-1.

Quantitation of cDNA-PCR amplified products

Ten microliters of each PCR reaction mixture was electrophoresed in agarose gel, blotted onto a nylon membrane (Hybond N+; Amersham), and then coupled to streptavidin-horseradish peroxidase complex (Amersham). The relative density of the signals from each lane of PCR products were determined by scanning densitometry [35].

Standard curves for ICAM-1 and G3PDH were constructed by analysis of serial dilutions of purified cDNA. cDNA was quantified over the linear portion of the standard curves. Outside this range, cDNA was quantified on diluted samples. G3PDH, a ubiquitous and highly expressed gene, was used as internal standard, and the ICAM-1 signals were normalized to G3PDH values. The data were presented as relative values (ICAM-1/G3PDH) and plotted against time. The results from three independent amplifications on two separate studies are presented.

Cytokine measurements

Cytokine concentrations in the culture medium (IL-1β, TNF-α and IL-6) were measured using commercially available ELISAs (R&D Systems).

Blocking experiments

The biological effects of TNF-α were blocked using a neutralising antibody (clone BDA 13; R&D Systems) at a concentration of 10 μg/ml, that is, 10 times the concentration used to neutralize 100% of the biological activity of 0.25 ng/ml recombinant human TNF-α. In separate experiments it was confirmed that this antibody did not modify any flow cytometry parameters (data not shown).

PDTC, an antioxidant, was used as an inhibitor of the transcription factor NF-κB [38]. This compound blocks the dissociation of IκBα from the NFκB protein, inhibiting the effect of various agonists such as phorbol esters, IL-1 and TNF-α [38]. At the concentration used (100 μM), it is not toxic to cell cultures.

Analysis of results

Results are expressed as mean ± SD and have been analyzed with analysis of variance, paired or unpaired *t*-test or Scheffé's test, as appropriate, with a significance level of $P < 0.05$.

Results

Cell characterization

The human kidney cortical cells formed monolayers that had the typical cobblestone appearance of epithelial cell monolayers. The cells formed domes on tissue culture plates. They stained positively for epithelial membrane antigen and cytokeratin but negatively for Factor VIII related antigen. The cells stained positively for nonspecific esterase, which distinguishes them from glomerular epithelial cells [28]. Exposure of the cells to parathyroid hormone in the presence of IBMX resulted in an approximately 6.8-fold increase in cAMP concentration compared to cells exposed to IBMX only. A 2.7-fold increase in cAMP was seen upon exposure to AVP. These results would indicate that the cells are predominantly of proximal tubular origin [27, 39, 40].

Effects of hypoxia and re-oxygenation on cell viability

After 24 hours of exposure to hypoxic culture conditions, cell viability was not affected: trypan blue exclusion was greater than 98%, and lactate dehydrogenase activity in the supernatants was comparable in hypoxic cells and cells cultured in normal conditions (101 ± 39 U/ml vs. 86 ± 39 U/ml, P = NS, results of 3 experiments performed in triplicate). When hypoxic cells were re-oxygenated, trypan blue exclusion was maintained, lactate dehydrogenase activity in the supernatants being not different from control or hypoxic cells (92 ± 42 U/ml).

Induction of ICAM-1 upon exposure of tubular cells to hypoxia

Under control conditions it was found that cortical tubular cells strongly expressed ICAM-1 (1.33 ± 0.19 10^6 fm per cell), confirming previously published data [8, 9, 11–13, 41]. This is 50-fold greater expression than is seen in human mesangial cells from the same kidney (data not shown). Exposure of cortical tubular cells to hypoxic culture conditions induced a time-dependent increase in the expression of ICAM-1 (Fig. 1), with 1.60 ± 0.01 10^6 fm per cell after four hours ($P < 0.01$), 1.68 ± 0.15 10^6 fm per cell after 12 hours ($P < 0.01$), and 1.88 ± 0.17 10^6 fm per cell after 24 hours ($P < 0.0001$). When the cells were re-oxygenated in a 95% air/5% CO_2 atmosphere, no further increase in ICAM-1 expression was demonstrated, and indeed, ICAM-1 expression returned to control levels (Fig. 2).

While exposure of cortical tubular cells to hypoxia induced membrane expression of ICAM-1, the expression of VCAM-1 was not modified (control 3.08 ± 1.74 10^5 fm per cell, 24 hr of hypoxia 2.92 ± 1.20 10^5 fm per cell).

The induction of ICAM-1 expression on exposure of cortical tubular cells to hypoxia was less than that seen when the cells were exposed to 10 ng/ml TNF-α for 24 hours (3.36 ± 0.72 10^6 fm per cell, $P < 0.0001$). This TNF-α-induced ICAM-1 expression was comparable to that reported in previous studies [9, 11–13, 41].

In parallel with the increase in membrane expression of ICAM-1, there was a time-dependent induction of ICAM-1 mRNA in hypoxic tubular cells, compared to normoxic cells (Fig. 3). The ICAM-1/GAPDH mRNA ratio increased up to 12 hours of hypoxia and decreased thereafter. The ICAM-1/GAPDH

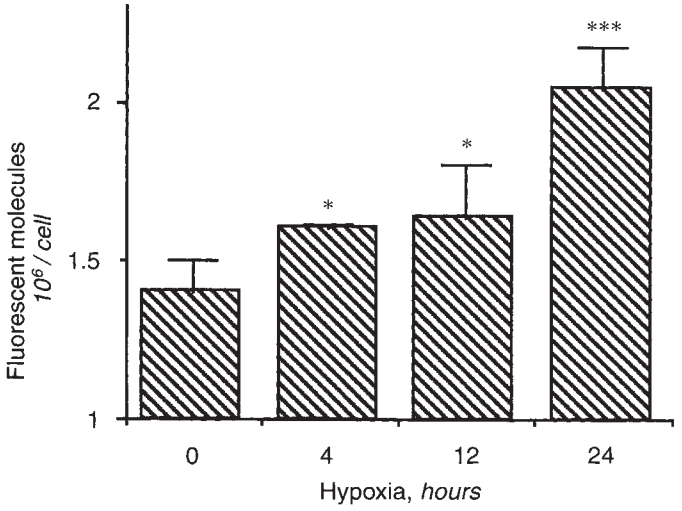

Fig. 1. *Time-dependent quantitative membrane expression of ICAM-1 by cortical tubular cells exposed to hypoxic culture conditions.* Results are presented as the mean ± SD of 3 experiments, each performed in triplicate. $^{*}P < 0.01$, $^{***}P < 0.0001$.

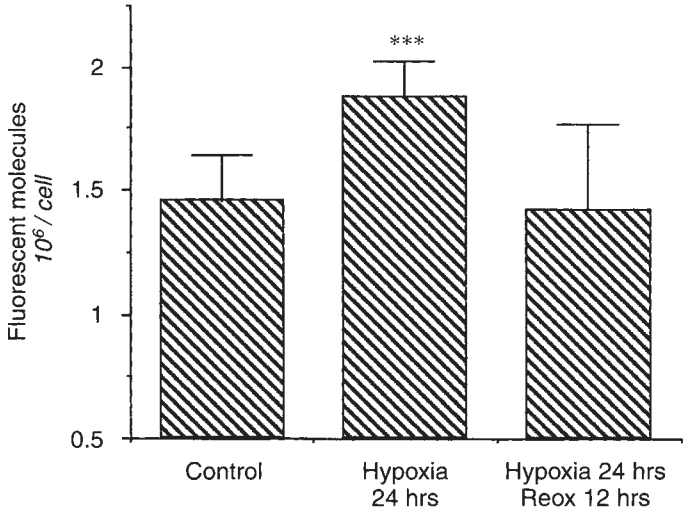

Fig. 2. *Membrane expression of ICAM-1 by cortical tubular cells exposed to hypoxic culture conditions and re-oxygenated.* Results are presented as mean ± SD of 2 experiments, each performed in triplicate. $^{***}P < 0.0001$.

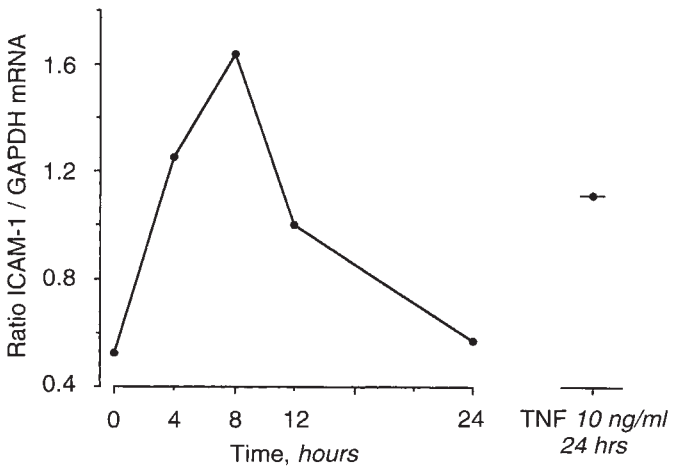

Fig. 3. *Time-course of ICAM-1 mRNA expression in cortical tubular cells exposed to hypoxia, as determined by RT-PCR analysis.* ICAM-1 mRNA is expressed as the ICAM-1/GAPDH mRNA ratio.

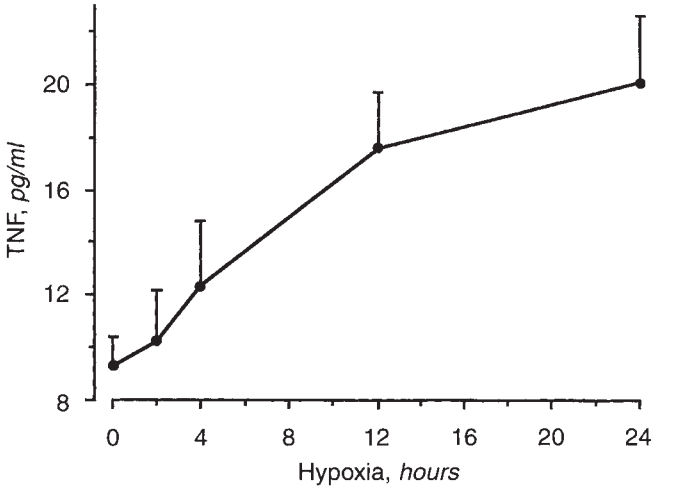

Fig. 4. *Concentration of TNF-α in the supernatants of tubular cells exposed to hypoxia.* Results are presented as mean ± SEM of 3 experiments, each performed in triplicate. Effect of time: $P < 0.002$ by ANOVA.

mRNA ratio after exposure of the cells to TNF-α for 24 hours is shown for comparison.

Role of cytokines in the induction of the expression of ICAM-1 by hypoxia

Exposure of tubular cells to hypoxia resulted in a small, but significant (by ANOVA) increase in TNF-α concentration in the culture medium with time (Fig. 4). To assess the contribution of raised TNF-α levels in the induction of ICAM-1 by hypoxia, an anti-TNF-α neutralizing antibody was used (Fig. 5). Although at the concentration used, the antibody was effective in preventing the induction of the expression of ICAM-1 by 10 ng/ml recombinant TNF-α at 24 hours, it had no effect on hypoxia-induced expression of ICAM-1. These experiments demonstrate that the secretion TNF-α by tubular cells in response to hypoxia did not mediate the effect of hypoxia on ICAM-1 expression.

IL-6 concentrations in the culture supernatants did not vary between normoxic conditions (3630 ± 870 pg/ml) and following exposure to hypoxia for four hours (2920 ± 480 pg/ml), 12 hours (2032 ± 289 pg/ml) or 24 hours (3121 ± 2002 pg/ml), suggesting that IL-6 was not involved in mediating hypoxia induced ICAM-1 expression. Furthermore, recombinant IL-6 at a concentration of 10 ng/ml for 24 hours had no effect on membrane ICAM-1 expression (data not shown).

IL-1β is known to be a powerful inducer of the expression of ICAM-1 in several cell types, and was able to induce ICAM-1 expression on these cultured cortical tubular cells (data not shown). However, immunoreactive IL-1β was not detected in the culture medium of the cortical tubular epithelial cells, either under hypoxic or normoxic culture conditions.

Therefore, neither TNF-α, nor IL-6, nor IL-1β could be

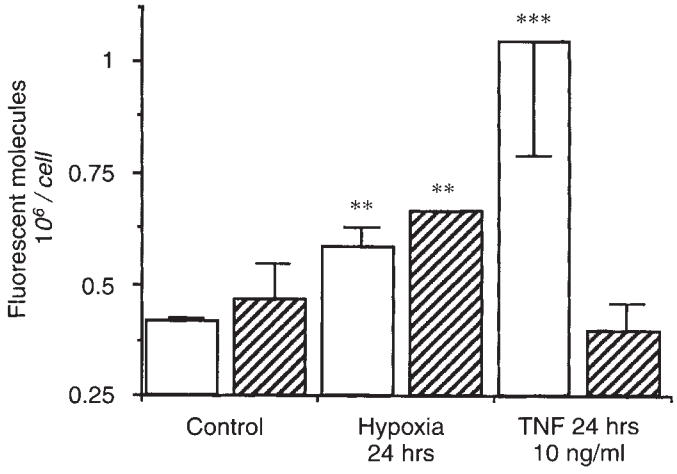

Fig. 5. *Membrane expression of ICAM-1 by tubular cells was measured after 24 hours of normoxic conditions, hypoxic conditions, or 10 ng/ml recombinant TNF-α, in the absence (□) or presence (▨) of an anti-TNF-α antibody (**P < 0.01, ***P < 0.001).*

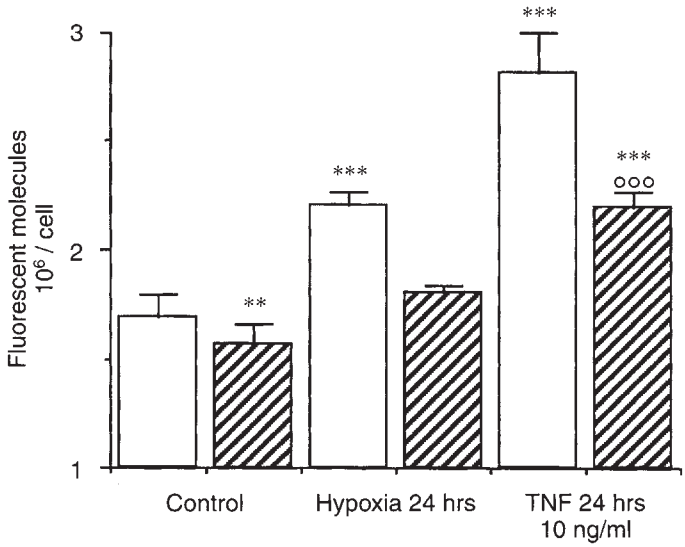

Fig. 6. *Membrane expression of ICAM-1 by tubular cells after 24 hours exposure to normoxic conditions, hypoxic conditions, or 10 ng/ml recombinant TNF-α, in the absence (□) or presence (▨) of 100 μM PDTC (***P < 0.0001 vs. control, °°°P < 0.001 vs. without PDTC).*

implicated in the induction of synthesis and expression of ICAM-1 seen on exposure of cortical tubular cells to hypoxia.

Effect of PDTC on hypoxia-induced expression of ICAM-1

Hypoxia has previously been shown to cause direct activation of NFκB in other cell culture systems [23, 24]. One hundred micromoles of PDTC was used to inhibit the activation of NFκB [38] in cortical tubular cells subjected to hypoxic or normoxic culture conditions. No variation in cell morphology was observed, nor in LDH activity in the supernatants, confirming that PDTC was not toxic to the cells, either hypoxic or normoxic (data not shown). The increase in ICAM-1 expression induced by hypoxia was nearly completely abolished when the cells were exposed to PDTC (Fig. 6). PDTC induced a small but significant decrease in the expression of ICAM-1 in normoxic conditions, and also had an inhibitory effect on the action of TNF-α. (TNF-α requires the activation of NFκB to induce the synthesis of ICAM-1.)

Discussion

An increase in the expression of ICAM-1 by renal tubular cells in response to hypoxia could contribute to the intense peri-tubular and interstitial leukocytic infiltration that is observed in experimental models of renal hypoxia [2, 3, 42]. A peritubular leukocyte infiltrate may directly modulate tubular epithelial cell function [43] and could also result in direct tubular cell damage and promote progressive interstitial damage [44]. Thus, the protective effect of anti-ICAM-1 antibodies in experimental renal hypoxia [2] could be explained in part by decreased adhesion of leukocytes to renal tubular cells, in addition to decreased adhesion of leukocytes to endothelial cells. It has also been suggested that the effects of ischemia on the function of renal allografts after transplantation may be mediated in part via the induction of ICAM-1 expression on tubular cells as a result of hypoxia [45]. Such a process might favor rejection; clinical observations would suggest that the degree of ischemia influences not only the incidence of acute renal failure in the early days following transplantation, but also the number of rejection episodes and the long-term survival of the kidney [45, 46].

The current study provides direct evidence that exposure of cultured cortical tubular cells to hypoxia results in an increase in membrane expression of the adhesion molecule ICAM-1 and an increase in ICAM-1 mRNA. On the contrary, the expression of VCAM-1, another adhesion molecule, was not influenced by hypoxia.

Hypoxia is known to induce the transcription of the adhesion molecules ICAM-1 and E-selectin in cultured endothelial cells. This appears to be dependent on the activation by hypoxia of autocrine cytokine loops, particularly IL-1α [19]. We hypothesized that a similar mechanism of autocrine cytokine stimulation might mediate the effects of hypoxia on tubular epithelial cells. TNF-α has previously been implicated in the regulation of ICAM-1 expression by these cells [14] and TNF-α is also known to be a potent inducer of the synthesis of other cytokines. The current studies confirmed that TNF-α was able to induce the synthesis of ICAM-1 by human tubular cells in culture. However, although hypoxia induced a small, but significant increase in the secretion of TNF-α by the tubular epithelial cells into the culture medium (Fig. 4), when the effects of TNF-α were blocked with a monoclonal antibody the effect of hypoxia on ICAM-1 expression was not modified. The same concentration of monoclonal antibody was able to totally block the effect exogenously added recombinant TNF-α on ICAM-1 expression. Therefore, the stimulation of the expression of ICAM-1 by tubular epithelial cells in culture by hypoxia does not depend on the autocrinal secretion of TNF-α.

Cultured renal tubular cells also synthesize IL-6 in large amounts [16–18], and as IL-6 may modulate the expression of ICAM-1 in other culture systems [47], the role of IL-6 as a mediator of the effects of hypoxia was also examined. In the current experiments high concentrations of IL-6 were found in the supernatants of cultured renal tubular cells, confirming previous observations [16–18]. However, the levels of IL-6 were not influenced by exposure of the cells to hypoxia. Furthermore,

pharmacological doses of recombinant IL-6 had no effect on the expression of ICAM-1 by tubular cells, effectively ruling out a contribution of this cytokine in mediating the effects of hypoxia.

Since IL-1β is a powerful inducer of the synthesis of adhesion molecules, the effect of hypoxia on the synthesis of IL-1β was also assessed. However, no IL-1β was detected in the culture medium of either control cells or of cells cultured under hypoxic conditions.

Recent studies have shown that hypoxia itself may directly activate gene transcription. Exposure of cultured endothelial cells to hypoxia induces direct activation of the nuclear factor NF-κB through phosphorylation of its inhibitor IκB [23, 24]. In turn the activation of NF-κB stimulates the transcription and secretion of the cytokines IL-8 and MCP-1 [23]. NF-κB sites are also present in the promoter of the ICAM-1 gene [48]. Therefore, it is possible that hypoxia could directly activate the transcription of ICAM-1, through activation of NF-κB. In support of this hypothesis, when PDTC was added to the supernatants of cultured tubular cells, the effect of hypoxia on ICAM-1 expression was abrogated. PDTC may have directly blocked the activation of the transcription factor NFκB, as suggested by previous studies [38], or may have acted through its antioxidant properties, resulting in lower activation of NFκB. This suggests that hypoxia could directly activate the transcription of ICAM-1 through activation of NFκB.

In conclusion, hypoxia can stimulate the expression of ICAM-1 on cortical tubular epithelial cells. The modulation of autocrinal production of cytokines TNFα, IL-1β and IL-6 does not play a role in the stimulation of the synthesis and expression of ICAM-1 by tubular epithelial cells in response to hypoxia. The activation of NFκB is, however, probably required. These observations may explain in part the peritubular infiltration of leukocytes that is seen following an ischemic insult to the kidney. Modulation of this process may have therapeutic implications for abrogating post-ischemic renal dysfunction.

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