Inflammatory mediators in human renal dysplasia

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Abstract

Background. Cytokines regulate many processes in the immune system and have recently been implicated in normal organogenesis. We previously demonstrated that the archetypal inflammatory cytokine tumour necrosis factor-α (TNF-α) is expressed in the murine metanephros, and exogenous TNF-α inhibits nephrogenesis and increases macrophage numbers in vitro (Cale et al., Int J Dev Biol 1998; 42: 663–674). The phenotype seen, with an arrest of ureteric bud branching and failure of mesenchymal to epithelial conversion, is similar to human renal dysplasia.

Methods and results. In normal human fetal kidneys we demonstrated the presence of macrophages and T cells and also documented TNF receptor expression on ureteric bud derivatives. In contrast to normal tissues, TNF-α protein was detected in dysplastic kidneys. This factor was also detected in the urine of fetuses with obstructive uropathy and TNF receptors were expressed in dysplastic tubules. Furthermore, we noted a fetal distribution of macrophages and T cells in dysplastic tissues and persistent expression of the adhesion molecules neural cell adhesion molecule and intercellular adhesion molecule.

Conclusions. We suggest that abnormal expression of cytokines early in renal development dysregulates normal patterns of adhesion molecule expression and inflammatory cells, and may contribute to the pathogenesis of renal dysplasia.

Key words: ICAM-1; macrophage; NCAM; renal dysplasia; TNF-α

Introduction

Nephrogenesis commences at week 5 of gestation in the human when the ureteric bud branches off the Wolfian duct into an area of intermediate mesoderm, the metanephric mesenchyme. The first event in mesenchymal conversion to epithelium is aggregation and condensation of mesenchymal cells around ureteric bud tips. These condensates form primitive vesicles and then comma and S shaped bodies before ultimately forming the glomeruli and collecting ducts. The first glomeruli develop at approximately 9 weeks. In parallel with, and reciprocally dependent on mesenchymal development, the ureteric bud branches sequentially to form the collecting ducts and the urothelium of the renal pelvis and ureter [1].

Disruption in the expression of a number of genes in transgenic mice, including bcl-2, wnt-4 and bone morphogenic protein 7, results in a phenotype resembling human congenital renal dysplasia [2]. In this condition there appears to be a developmental arrest with severely abnormal renal architecture. This includes the presence of dysplastic tubules surrounded by collars of undifferentiated tissue of presumed mesenchymal origin and abnormally developed glomeruli. Cystic changes and the presence of cartilage in these tissues is also characteristic [1,2]. These pathological changes may occur in isolation or as part of a syndrome. There may also be associated obstruction of the urinary tract secondary to abnormal development of the pelvi-ureteric system. For example, the atretic ureter seen in multicystic dysplastic kidneys or in association with posterior urethral valves [1].

We have previously demonstrated that the classical inflammatory cytokine tumour necrosis factor-α (TNF-α) is expressed in the murine metanephros and, in increased concentrations, inhibits normal development in organ culture resulting in a phenotype that resembles renal dysplasia [3]. Furthermore, macrophages, which are regulated by and may themselves produce cytokines, are present in the developing rodent renal rudiment from its inception [3,4] and TNF-α produces a disproportionate increase in their numbers in vitro [3].

We hypothesized that cytokines, produced by macrophages or intrinsic metanephric cells, may disrupt the normal process of nephrogenesis and contribute to the pathogenesis of renal dysplasia via their interactions with inflammatory cells, adhesion and other molecules. In the present study we aimed to delineate normal patterns in the human metanephric kidney of macrophages, T cells, the cytokine TNF-α and its receptors, and neural cell and intercellular adhesion molecules (NCAM and ICAM-1). These results were compared...
with expression patterns in fetal and postnatal dysplastic kidneys.

Methods

All chemicals were obtained from the Sigma Chemical Company unless otherwise stated.

Collection of samples

The local Ethical Committees approved all sample collection. Early human fetuses were provided by the Medical Research Council-funded Human Embryo Bank at the Institute of Child Health, London, UK. Metanephric kidneys from six normal fetuses aged from 5 to 14 weeks were used in the present study using collection methods previously described [3] and gestational age was determined by standard criteria from external morphology. Fetal kidneys for RNA and protein extraction were dissected as soon as possible after embryo collection and snap frozen. All other kidneys were examined and classified by a histopathologist, and all dysplastic kidneys were multicystic. Fetal normal (n = 10) (17–38 weeks gestation) and dysplastic (n = 7) (19–37 weeks gestation) samples were obtained at post mortem. Of the dysplastic samples obtained, two fetuses were documented as having urethral obstruction, all had hydropptic lungs and two fetuses had hydrocephalus. Kidneys from children who had died from sudden infant death syndrome and with no other demonstrable abnormality were used as postnatal normal samples (n = 7). Postnatal dysplastic samples (n = 8) were collected at elective surgical removal from children aged from 4 to 24 months. Two of the eight kidneys were documented as obstructed, and none had any significant extrarenal manifestation [3]. Samples of fetal urine were residual volumes collected for diagnostic purposes from fetuses with obstructed urinary tracts [6]. Samples of the bladder or renal pelvis of 13 cases with gestational ages from 13 to 30 weeks were collected by ultrasound-guided transabdominal aspiration [6]. Uretines were stored at −70°C prior to analysis.

Nested reverse transcription–polymerase chain reaction (RT–PCR) for TNF-α

RNA was extracted from fetal kidneys using TRI-REAGENT (Molecular Research Center, Cincinnati, Ohio) according to the manufacturer’s protocol. RNA (300 ng) was treated with DNase I to degrade contaminating DNA. Reverse transcription was then performed as described elsewhere [7]. cDNA was subjected to two rounds of PCR using nested primers. The 25-μl PCR reaction consisted of cDNA (10 μl of the RT product used for the first reaction, 5 μl of the first reaction product used for the second reaction) 250 μM of dATP, dCTP, dGTP and dTTP, 1 × NH₄ reaction buffer (Promega, London, UK), 1.5 mM MgCl₂, 100 ng of each primer and 1.25 U Taq polymerase (Biopro, UK). Thirty cycles of denaturation at 95°C, annealing at 49°C (first reaction) or 54°C (second reaction) and extension at 72°C were performed for each reaction. Sequences of the first primer pair (5’–3’, with nucleotide numbers corresponding to the genomic sequence) were ATGAGCAGTGAAGCATG (nucleotides 796–813) and TACACGGGCAATGATCCC (nucleotides 2565–2589), and the second ATCCGGGACGTGGAGCTG (nucleotides 814–830) and AAAGTAGACCTGCAGAC (nucleotides 2556–2564) [8]. This gave expected product sizes of 699 and 666. Primers for β-Actin were obtained from Clontech (Palo Alto, CA, USA).

Enzyme linked immunosorbent assay (ELISA) for TNF-α

A sandwich ELISA for TNF-α in fetal urine was performed, as described elsewhere [3], using 4 μg/ml of TNF-α capture antibody and 200 ng/ml detection antibody (R and D Systems, Abingdon, UK).
Results

The distribution of cells and patterns of protein expression were studied during normal human nephrogenesis (‘fetal’) and in the postnatal period. This was then compared with expression patterns seen in sections from antenatal and postnatal dysplastic kidneys. Staining patterns were described as being in different anatomical compartments. In normal tissues ‘ureteric bud’ and ‘mesenchyme’ included only structures within the nephrogenic zone, ‘glomeruli’ included the glomerular endothelium, and ‘interstitium’ described the loose stroma deep to the nephrogenic zone and outside all tubular, glomerular, vascular and nervous structures. In dysplastic tissue, ‘peri-tubular tissue’ described the collar of cells immediately surrounding dysplastic tubules and all tissue lying outside this area which was not nerves or vessels was described as ‘interstitial’. ‘Vessels’ included lymphatics, veins, arteries and capillaries. Cells were described as ‘outside vessels’ if they appeared to be separate from both the blood vessel lumen and wall, and ‘within the vasculature’ if they were associated with the blood vessel lumen or wall. Data is summarized in Table 1.

TNFR1

Normal nephrogenesis

No TNFR1 immunoreactivity was detected in the nephrogenic zone at 9 or 14 weeks gestation (data not shown). TNFR1 was detected in developing glomeruli in a distribution consistent with endothelial expression (Figure 1A). Urothelium in the forming renal pelvis and calyces expressed TNFR1 (Figure 1C), as did larger fetal collecting ducts (Figure 1D). In normal postnatal samples TNFR1 staining was only detected at a lower level on glomerular endothelia and the walls of larger arteries (data not shown).

Dysplastic kidneys

Blood vessel walls expressed TNFR1 in both fetal and postnatal dysplastic kidneys. TNFR1 expression was noted in the rare fetal dysplastic glomeruli noted, but not in corresponding structures in postnatal samples. In antenatal dysplastic specimens, isolated TNFR1 immunoreactive cells were scattered in the interstitium, but these were not detected in postnatal samples (data not shown).

TNFR2

Normal nephrogenesis

As with TNFR1, TNFR2 was expressed on the endothelia of glomeruli and the urothelium at 9 and 14 weeks (data not shown). Individual cells within the mesangial area also expressed this molecule at these ages (data not shown). In parallel with TNFR1, expression of TNFR2 was down regulated postnatally with

Table 1 Summary of protein expression for the TNF-α axis and adhesion molecules

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‘Ureteric bud’ and ‘mesenchyme’ define tissues within the nephrogenic zone. ‘Glomeruli’ includes glomerular endothelium, ‘mature epithelium’ includes collecting ducts, urothelium of the renal pelvis and loops of Henle. ‘Interstitium’ defines the loose stromal tissue deep into or surrounding these tissues, and ‘vessels’ included staining in any layer of vessels outside glomeruli, including patterns consistent with endothelial staining. – no immunoreactivity; (+) weak immunoreactivity; + positive immunoreactivity.
Fig 1. Expression of TNF receptors. TNF receptors were localized using an antibody to their membrane bound portion (brown) and all sections were counterstained with methyl green. TNFR1 staining within glomeruli was detected at 13 weeks (arrow in A) in a distribution consistent with endothelial expression. Preabsorbing the antibody with an appropriate peptide markedly diminished staining (B). (C and D) Staining for TNFR1 was noted on the urothelium of the pelvis and forming calyces and on larger collecting ducts in the medulla. In fetal dysplastic tissue, staining (arrows) was seen on dysplastic tubules (E). (F) A dysplastic tubule in a postnatal kidney with individual cells strongly immunoreactive for TNFR2. Isolated cells (arrows) in the interstitium also stained positive for TNFR2 in fetal and postnatal dysplastic tissue (G). Bars represent 25 μm.
marked expression seen only in the arterial walls and minimal expression in glomerular endothelia (data not shown).

**Dysplastic kidneys**

TNFR2 was expressed more extensively than TNFR1 in dysplastic tissues. In fetal tissues dysplastic glomeruli, most endothelia and isolated cells in the interstitium expressed TNFR2 (data not shown). In addition, a punctate staining was noted in some dysplastic tubules (Figure 1E). A subset of cells in postnatal dysplastic tubules was strongly immunoreactive for TNFR2 (Figure 1F). Additional TNFR2 immunoreactive cells were noted in the postnatal dysplastic interstitium (Figure 1G) and endothelia of large vessels, and arterial walls in these samples expressed TNFR2 (data not shown).

**TNF-α**

**Normal nephrogenesis**

Using immunohistochemistry and two different antibodies to TNF-α, no protein was detected in normal fetal kidneys at 9, 10 or 14 weeks, or postnatally (Table 1 and data not shown). However, using the very sensitive technique of nested RT-PCR, TNF-α mRNA was detected at 7.5, 9 and 15 weeks gestation (Figure 2A).

**Dysplastic kidneys**

In fetal dysplastic tissues individual cells were noted in the interstitium that stained strongly for TNF-α (Figure 2B). Staining for CD68 in adjacent sections suggested that these cells were monocytes (Figure 2C). In addition, TNF-α was detected at low levels (5–19 pg/ml) in the fetal urine of seven of the 13 samples from fetuses with renal abnormalities examined (Figure 2D). No positive staining for TNF-α was detected in postnatal dysplastic samples.

**Recent macrophage and neutrophil infiltration**

An antibody to LI was used to detect macrophages and neutrophils that had recently migrated into tissues.

![Figure 2](image_url)

**Normal nephrogenesis**

At 5 weeks gestation, occasional L1 positive cells were detected within the intermediate mesoderm adjacent to the metanephros (data not shown). Positive cells were...
not detected within metanephroi at 5 or 9 weeks, but at 13 weeks occasional glomeruli contained immunoreactive cells and others were noted in the nephrogenic cortex and interstitium (data not shown). High power views showed single large nuclei in these cells suggesting they were of a monocytic lineage (data not shown). As glomerular development proceeded, L1 expressing cells were noted within these structures (data not shown). In postnatal kidneys the occasional L1-expressing cells detected all appeared to be contained within the renal vasculature or glomeruli (data not shown).

**Dysplastic kidneys**

L1 positive cells were readily detected in fetal dysplastic kidneys (Figure 3C–E). Large clusters of cells were detected in the interstitium surrounding dysplastic tubules (Figure 3C) and adjacent to the occasional glomerular structures found in dysplastic organs (Figure 3D). Clusters of immunoreactive cells were also noted in areas containing multiple small, condensed nuclei. The latter were consistent with apoptotic cells (Figure 3E). L1 positive cells were also detected in a similar distribution in sections from postnatal dysplastic kidneys. However, staining of serial sections with antibodies to CD68, L1 and neutrophil elastase (Figure 3F–H) demonstrated that L1 positive cells in postnatal samples were neutrophils rather than the macrophages seen in fetal samples (see below).

**Mature macrophages**

**Normal nephrogenesis**

CD68 positive cells were detected in a 5-week-old embryo adjacent to the metanephric mesenchyme. Between 9 and 22 weeks gestation CD68 positive macrophages were detected outside the vasculature in both the nephrogenic zone and medullary interstitium. As glomeruli developed, a significant proportion was noted to contain one or more macrophage. Postnatally, the frequency with which mature macrophages were detected was considerably less and these cells were only detected within glomeruli and the vasculature (data not shown).

**Dysplastic kidneys**

CD68 cells were detected in all fetal dysplastic kidneys examined. These were mainly scattered in the interstitial tissues, apparently outside the lumen and wall of blood vessels. Two patterns of mature macrophages were seen in postnatal dysplastic tissues. In tissues with less fibrosis and some preservation of structure, CD68 positive cells were detected scattered in interstitial areas with a distribution similar to the fetal samples. Very fibrous samples, however, contained no CD68 positive macrophages (data not shown).

**T cells**

**Normal nephrogenesis**

At 9 weeks gestation, rare CD3 positive cells were noted in the loose connective tissue surrounding the metanephros (data not shown). No cells were detected in the body of the organ at 9 or 14 weeks. At 17 weeks positive cells were noted in the nephrogenic zone, often adjacent to condensates (Figure 3A), within developing glomeruli (Figure 3B) and in deeper medullary blood vessels adjacent to tubules (data not shown). By 32 weeks gestation, and in normal postnatal samples, all CD3 positive cells seen appeared to be within the lumen of glomerular or other blood vessels (data not shown).

**Dysplastic kidneys**

CD3 positive T cells were detected in all fetal dysplastic tissues examined. Although many cells were confined to blood vessel lumens, an interstitial infiltrate was also noted. No T cells were detected outside the vasculature in the postnatal dysplastic kidneys analysed (data not shown).

**NCAM**

**Normal nephrogenesis**

At 9 weeks gestation, NCAM protein was expressed in the nephrogenic zone in condensed mesenchyme, with absent expression in ureteric bud derivatives and other epithelia (Figure 4A). Mesenchymal expression decreased with renal maturation (data not shown). From 14 weeks additional staining was seen in neurovascular bundles (data not shown). In postnatal kidneys, staining patterns were consistent with isolated expression in nerves (data not shown).

**Dysplastic kidneys**

Marked expression of NCAM was noted in cells surrounding the majority of fetal dysplastic tubules (Figure 4C). Other staining in the interstitium was consistent with staining of longitudinal or cross sections of nerves (Table 1 and data not shown). This pattern was conserved in postnatal dysplastic tissue (Figure 4D).

**ICAM-1**

Throughout nephrogenesis and in postnatal life in both normal and dysplastic tissues, ICAM-1 expression was detected in a distribution consistent with the endothelia of the developing renal vasculature (data not shown). Expression on other structures is described below.

**Normal nephrogenesis**

At 9 weeks gestation, ICAM-1 was apically expressed in branches of the ureteric bud (Figure 5A). From 13
Fig. 3. Inflammatory cell distribution. (A and B) Stained with an antibody to CD3, the T cell receptor. (C–H) Stained for the L1 antigen expressed on migrating monocytes and neutrophils. (F) Stained for CD68 expressed on mature macrophages and stained for neutrophils using an antibody to neutrophil elastase (H). (A–E) Counterstained with methyl green and (F–H) with haematoxylin. T cells were noted in the nephrogenic zone (A) and within developing glomeruli (B). In fetal dysplastic tissue, monocytes were scattered in loose mesenchymal tissue adjacent to tubules (C) and dysplastic glomeruli (D). They were also noted in areas of cells with condensed nuclei (arrows) consistent with apoptosis (E). Postnatally, clusters of L1 positive cells were seen in dysplastic tissue (G). Many of these cells had a lobulated nucleus consistent with a neutrophil infiltrate, and staining of serial sections with antibodies to CD68 (F) and neutrophil elastase (H) demonstrated that these cells were neutrophils. (A, B and E) Bars represent 25 μm, (C and D) 80 μm and (F–H) 60 μm.
weeks gestation and in postnatal samples, no staining was seen in mesenchymal or epithelial structures.

**Dysplastic kidneys**

Individual cells within the interstitium of fetal dysplastic kidneys were immunoreactive for ICAM-1 (data not shown). ICAM-1 immunoreactivity was also detected on the apical surface of both fetal and postnatal dysplastic tubules (Figure 5C).

**Discussion**

In the present study we demonstrated the expression of TNF-α mRNA and TNF receptor proteins in the normal human metanephros, documented the presence of macrophages and T cells in these tissues and described the expression patterns of the adhesion molecules NCAM and ICAM-1 during nephrogenesis. When data were compared to that generated from human dysplastic kidneys, where there is an apparent arrest of normal renal development, we noted abnormal expression of TNF receptors, the presence of cells expressing TNF-α protein in fetal dysplastic tissue and TNF-α in the urine of fetuses with obstructive uropathy, and associated renal dysplasia. In addition we demonstrated distinctive epithelial and peritubular expression of ICAM-1 and NCAM respectively and a persisting interstitial inflammatory cell infiltrate in dysplastic tissues.

**TNF-α axis in normal renal development and dysplasia**

TNFR1 and 2 proteins were expressed in the normal human metanephros, and their distributions were consistent with endothelial expression [7]. Strict temporal regulation of receptor expression was noted with down regulation in normal postnatal samples. In contrast to normal tissues, epithelial expression of TNFR2 was noted in fetal and postnatal dysplastic kidneys. This differential up-regulation of TNFR2 compared to TNFR1 is in keeping with the known rapid inducibility of TNF-α protein in fetal dysplastic tissue and TNF-α in the urine of fetuses with obstructive uropathy, and associated renal dysplasia. In addition, mRNA for TNF-α was detected, demonstrating that the developing kidney has the capacity to produce TNF-α protein as has been documented in the murine metanephros [3]. As considerable regulation of TNF-α occurs at the post-transcriptional level [11], we cannot conclude from this data whether low levels of TNF-α protein are present in the normal metanephros. TNF-α protein expression was detected in fetal dysplastic tissues. In addition, TNF-α protein was demonstrated in the urine of fetuses with obstructed urinary tracts and renal dysplasia. Although normal fetal urine
controls are not available as fetal urine is not sampled
from normal kidneys, these two results support our
hypothesis that fetal dysplastic kidneys are exposed to
TNF-α. Not only does TNF-α inhibit nephrogenesis
in vitro [3] but TNF-α mRNA is elevated in the
tubular cells of obstructed mature rat kidneys [12]. Furthermore, animal studies have demonstrated
increased levels of TGFβ in the urine of obstructed
kidneys [13], and TGFβ, like TNF-α, inhibits renal
development in animal models [14]. Renal obstruction
has been shown in animal studies to contribute to the
pathogenesis of renal dysplasia [15] and may co-exist
with renal dysplasia in humans [1,2]. We suggest, there-
fore, that the TNF-α detected in the tissue and urine
from fetal dysplastic kidneys may contribute to its
pathogenesis. Our observation of TNFR2 expression
on the developing urothelium is also of interest in this
context. TNF-α in urine may act via these receptors
and affect the growth and development of the ureter
and pelvi-ureteric junction. This molecule therefore pro-
vides a potential functional basis for the association
between urinary tract obstruction and renal dysplasia
such as is seen in multicystic dysplastic kidneys with
atretic ureters [1,2].

**Inflammatory cells in normal renal development and
dysplasia**

Increased amounts of apoptosis occur in human renal
dysplasia [16]. TNF-α increases apoptosis in cultured
metanephrroi in vitro [3]. It is also chemotactic for
macrophages [17] and macrophage numbers increase
in murine metanephric cultures in the presence of
TNF-α [3]. These cells phagocytose apoptotic cells in
the developing murine kidney [3] and it has been
suggested that they may trigger cell death and therefore
be an integral component of the normal development
of other organs [18]. The distribution of inflammatory
cells in the developing human kidney has not previously
been studied. The patterns of tissue infiltration of the
fetal kidney with macrophages reported here is consist-
ent with that seen in mice [3,4] and the timing of the
renal macrophage and T cell infiltrate with what
is known of the normal haematopoietic process in
humans [19]. We suggest that low levels of TNF-α in
the normal metanephiros act as a chemotactic signal
for these cells. The normal down regulation of TNFRs
on endothelia may result in decreased influx of these
cells and hence the lower numbers and different distri-
bution of inflammatory cells with increasing renal
maturity. Any dysregulation of TNF-α, as demon-
strated here, or other cytokine axes in renal dysplasia
may affect this pattern and result in the persistence of
interstitial inflammatory cells noted in fetal renal
dysplasia. The type of inflammatory cell infiltrate differed
between fetal and postnatal dysplastic samples: L1
positive cells were predominantly monocytic in fetal
tissue, while neutrophils were seen postnatally and
T cells were seen in fetal but not postnatal samples. Different cytokines are chemoattractive for different inflammatory cells [20] and this data suggests that the cytokines involved in fetal dysplasia may differ from those in postnatal life. One possible trigger for this change is ascending urinary tract infection, which is unlikely to occur in utero but may be a factor in infants and children. The functions of macrophages and other inflammatory cells in normal and abnormal renal development remain to be elucidated, although they may include phagocytosis of dying cells and the production of cytokines which may then contribute to the pathological process in these organs.

Adhesion molecules in normal and abnormal renal development

Cytokines regulate the expression of adhesion molecules in fetal and adult organ systems. For example, NCAM is upregulated by TNF-α [17] and TGFβ [21], while expression of ICAM-1 is altered by TNF-α and TGFβ [22]. These cytokines inhibit nephrogenesis in mice models [3,23] and we hypothesized that cytokine actions in nephrogenesis may include regulation of adhesion molecule expression. We are not aware of other studies reporting normal fetal expression of either ICAM-1 or NCAM in the human metanephros. However, the pattern of NCAM protein we detected, with early mesenchymal expression and down regulation on maturation and epithelial conversion parallels that reported in rodents [24]. The pericentric expression of NCAM around dysplastic tubules resembles its fetal distribution, when it is expressed in mesenchyme surrounding ureteric bud branches. Persisting expression of this pro-adhesive molecule may inhibit the further differentiation and morphogenesis of these cells. Work inhibiting the actions of NCAM in vivo or in vitro has not demonstrated that its presence is necessary for normal nephrogenesis [24,25]. However, of more relevance to our data would be studies where NCAM has been over-expressed in the kidney.

ICAM-1 is widely expressed on endothelia [26], and the ICAM-1 expressed on endothelia in normal and abnormal renal tissues may contribute to the migration of macrophages into these tissues [26]. Renal epithelial expression of ICAM-1 has only been described in the presence of an ongoing inflammatory disease in mature kidneys [27]. We demonstrated that epithelial ICAM-1 expression occurs on the ureteric bud early in normal development, but this is rapidly down regulated and was not detected after 13 weeks in normal tissues. However, ICAM-1 expression was readily detected on epithelial-derived dysplastic fetal and postnatal tubules. No data is available on the functions of ICAM-1 during nephrogenesis. In adult human immune-mediated glomerulonephritis it has been suggested that increased expression of ICAM-1 enables the interaction of epithelial cells with macrophages, which then cause further tubular damage [27]. We did not however document the presence of macrophages in direct contact with dysplastic tubules. Persistent expression of ICAM-1 on dysplastic tubules may alter cell–cell adhesive properties and inhibit normal tubular morphogenesis.

Conclusions

We have previously demonstrated the deleterious effects on murine metanephric development of TNF-α, and have now documented abnormalities of expression of this cytokine and its receptors along with inflammatory cells and adhesion molecules in renal dysplasia. We suggest that these factors may interact and contribute to the pathogenesis of this disease.

Acknowledgements. We would like to thank Peter Cuckow, Jeeta Dhillon, Patrick Duffy, Peta Foxall, Fergal Quinn, Phillip Ransley, Tony Risdon, Charles Rodeck, Veronica Sams and Duncan Wilcox for their help in collecting samples used in this study. We are also grateful to Ester Papp who performed the TNF-α ELISA.

References

10. Vandenabeele P, Declercq W, Beyeart R, Fiers W. Cytokines regulate the expression of adhesion molecule expression. We are not aware of other studies reporting normal fetal expression of either ICAM-1 or NCAM in the human metanephros. However, the pattern of NCAM protein we detected, with early mesenchymal expression and down regulation on maturation and epithelial conversion parallels that reported in rodents [24]. The pericentric expression of NCAM around dysplastic tubules resembles its fetal distribution, when it is expressed in mesenchyme surrounding ureteric bud branches. Persisting expression of this pro-adhesive molecule may inhibit the further differentiation and morphogenesis of these cells. Work inhibiting the actions of NCAM in vivo or in vitro has not demonstrated that its presence is necessary for normal nephrogenesis [24,25]. However, of more relevance to our data would be studies where NCAM has been over-expressed in the kidney.

ICAM-1 is widely expressed on endothelia [26], and the ICAM-1 expressed on endothelia in normal and abnormal renal tissues may contribute to the migration of macrophages into these tissues [26]. Renal epithelial expression of ICAM-1 has only been described in the presence of an ongoing inflammatory disease in mature kidneys [27]. We demonstrated that epithelial ICAM-1 expression occurs on the ureteric bud early in normal development, but this is rapidly down regulated and was not detected after 13 weeks in normal tissues. However, ICAM-1 expression was readily detected on epithelial-derived dysplastic fetal and postnatal tubules. No data is available on the functions of ICAM-1 during nephrogenesis. In adult human immune-mediated glomerulonephritis it has been suggested that increased expression of ICAM-1 enables the interaction of epithelial cells with macrophages, which then cause further tubular damage [27]. We did not however document the presence of macrophages in direct contact with dysplastic tubules. Persistent expression of ICAM-1 on dysplastic tubules may alter cell–cell adhesive properties and inhibit normal tubular morphogenesis.

Conclusions

We have previously demonstrated the deleterious effects on murine metanephric development of TNF-α, and have now documented abnormalities of expression of this cytokine and its receptors along with inflammatory cells and adhesion molecules in renal dysplasia. We suggest that these factors may interact and contribute to the pathogenesis of this disease.

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