Br. J. Pharmacol. (1991), **103**, 1663–1668

Excretion of metabolites of prostacyclin and thromboxane by rats with nephrotoxic nephritis: effects of interleukin-1

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1 To obtain direct evidence of abnormal eicosanoid biosynthesis in rats injected with anti-glomerular-basement-membrane antibodies (a-GBM), products derived from thromboxane A_2 (TXA_2) and prostacyclin (PGI_2) were measured in 24 h urine collections before and after a-GBM.

2 Administration of a-GBM (9.5 mg) caused albuminuria, decreased creatinine clearance, increased numbers of intra-glomerular neutrophils and increased excretion of TXB_2, 2,3-dinor-TXB_2 (products of TXA_2) and 6-oxo-$PGF_{1\alpha}$ and 2,3-dinor-6-oxo-$PGF_{1\alpha}$ (products of PGI_2) at 24 h.

3 Interleukin-1 (IL-1_β; 5 μg) alone caused an increase in PGI_2 metabolite excretion but had no effect on TXA_2 metabolites. It had no effect on creatinine clearance but increased numbers of glomerular neutrophils by approximately 4–5 fold compared to a-GBM.

4 Pretreatment of rats with IL-1_β before a-GBM synergistically increased albumin excretion but only additively increased eicosanoid excretion. Numbers of intra-glomerular neutrophils and creatinine clearance were unchanged compared to IL-1_β alone.

5 The cyclo-oxygenase inhibitor, ibuprofen (10 mg kg^{-1} i.p., twice daily for 4 days) inhibited both serum TXB_2 production and urinary prostaglandin excretion. It also caused an almost complete attenuation of albumin excretion. Creatinine clearance and glomerular neutrophils remained unchanged after a-GBM/IL-1_β.

6 We conclude that the 50% inhibition of thromboxane production induced by ibuprofen does not modify the fall in creatinine clearance of accumulation of neutrophils in the glomerulus caused by the a-GBM. This degree of inhibition of eicosanoid production was associated with a striking decrease in proteinuria, but this may reflect a haemodynamic rather than a disease modifying action.

Keywords: Prostacyclin; thromboxane A_2; interleukin-1; nephritis; anti-glomerular-basement-membrane antibodies

Introduction

Intravenous administration of heterologous antibody to glomerular basement membrane (nephrotoxic globulin, a-GBM) causes neutrophil-dependent glomerular injury characterized by albuminuria (Unanue & Dixon, 1967). Several biochemical mediators have been implicated in the pathophysiology of the ensuing nephrotoxic nephritis which, however, remains incompletely understood. Indirect evidence based on measurements of eicosanoid production by isolated glomeruli *ex vivo*, and on the effects of various inhibitors of the arachidonic acid cascade on renal function in nephrotoxic nephritis (NTN) has pointed to a role for increased eicosanoid formation. During the first 2 to 3 h after administration of a-GBM, the glomerular filtration rate has been shown to fall (Lianos *et al.*, 1983), and this may be mediated via increased production of the vasoconstrictor thromboxane A_2 (TXA_2); in the later stages of the disease (by 24 h) the glomerular filtration rate has returned to normal, perhaps because of increased synthesis of vasodilator prostaglandins (PGE_2 and/or PGI_2) (Stork & Dunn, 1985). Direct evidence of altered eicosanoid formation in NTN *in vivo* is lacking.

Intercurrent bacterial infections can cause relapse of glomerulonephritis in man (reviewed by Heptinstall, 1983). Bacterial endotoxin stimulates production of several cytokines, including interleukin-1 (IL-1_β; Werber *et al.*, 1987). We recently found that parenteral injections of small doses of human recombinant IL-1_β substantially increased albuminuria caused by a-GBM (Tomosugi *et al.*, 1989). IL-1_β has been shown to be capable of stimulating prostaglandin synthesis *in vitro* (Mizel *et al.*, 1981; Rossi *et al.*, 1985; Conti *et al.*, 1986; Levine & Xiao, 1986) so it is possible that altered eicosanoid biosynthesis mediates or otherwise influences the effect of these cytokines on the course of NTN.

The objects of our present study were to seek direct evidence of abnormal eicosanoid formation in NTN and to investigate the effects of IL-1_β on eicosanoid biosynthesis in this condition. Thromboxanes and prostaglandins are readily formed in response to trauma during tissue isolation of blood sampling (Dollery *et al.*, 1984; Granström *et al.*, 1985). The least invasive way of investigating eicosanoid biosynthesis is by measurement of eicosanoid derived products in urine (Roberts *et al.*, 1981). We therefore measured hydrolysis products and metabolites of TXA_2 and PGI_2 in urine, to provide measures of biosynthesis of these eicosanoids *in vivo*. Cyclo-oxygenase-inhibition has been shown to be beneficial in reducing proteinuria in patients with nephrotic syndrome (Donker *et al.*, 1978; Velosa & Torres, 1986; Vriesendorp *et al.*, 1986). To study the role of prostaglandins in NTN, we have also studied the effect of ibuprofen, a cyclo-oxygenase inhibitor. This drug has previously been shown to be effective in protecting against haemodynamic alterations induced in glomerulonephritis in the rat by Takahashi *et al.* (1990), and a similar dosing regimen was used as in this study.

Methods

Animals

Male Sprague-Dawley rats were purchased from Olac, U.K. They were given free access to food and water until they were 180–200 g, when they were selected at random for the following experiments.

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Protocol

Induction of nephrotoxic nephritis Rats were housed individually in metabolism cages which facilitated the collection of their urine. They were allowed to acclimatize overnight before starting the experiments. Water was still supplied *ad libitum* but food was withdrawn for the whole period of the urine collections.

Urine collection commenced at 9 h 00 min on day 0. Next day the urine was removed and a second 24 h collection started. The animals were lightly anaesthetized with ether, and injected via the dorsal tail vein with 1 ml of vehicle (0.9% saline), a-GBM (9.5 mg), IL-1_β (Biogen, Geneva; 5 μg $\equiv 5 \times 10^7$ units) or a-GBM with IL-1_β ($n = 6$ for each of the four groups). a-GBM was prepared as described previously (El Nahas *et al.*, 1985). On day 2, the animals were again anaesthetized, the peritoneum opened, and the animals exsanguinated via the abdominal aorta. The left kidney was removed and preserved in 10% neutral-buffered formalin for histological analysis.

Cyclo-oxygenase inhibition In a separate experiment, 2 groups of 6 rats were injected intra-peritoneally twice-daily with 1 ml of vehicle (0.9% saline) or ibuprofen (Sigma Chemical Co, Poole, Dorset; 10 mg kg^{-1}) a dose previously determined to be effective at inhibiting serum TXB_2 production by >95%, for the whole period of the experiment. After four days, nephritis was induced with a-GBM + IL-1_β, as above.

Assay procedures

Urinary prostaglandins were assayed by the Barrow *et al.* (1989) modification of the method of Chiabrando *et al.* (1987). Briefly, urine volume was measured and deuterated internal standard (5 ng) of each prostaglandin to be measured added. Phosphate buffer (0.1 M, pH 8.4, 10 ml) was added and the samples left to equilibrate for at least one week at −20°C. They were then defrosted, centrifuged, and extracted on Sephadex-4B immuno-affinity columns containing immobilized antibodies raised against thromboxane B_2 and 6-oxo-$PGF_{1\alpha}$. These cross-reacted with the respective 2,3-dinor-metabolites. The columns were washed with Milli-Q grade water (Millipore Corp.) and eluted with acetone:water (95:5) v/v, 3 × 0.5 ml. This was blown to dryness and the samples derivatised and analysed on a Finnigan-MAT 4500 gas-chromatograph/mass spectrometer, using electron-capture detection. Inter- and intra-assay variations were each <10%, and recovery through the extraction was >60%.

Serum TXB_2 was measured by radio-immunoassay (Fuller *et al.*, 1984). Albuminuria was measured by gel rocket-immuno-electrophoresis (Laurell, 1972). The gels were run at 2 V cm^{-1} overnight, washed, and stained with Coomassie Blue. Creatinine clearance was determined from measurement of serum and urine creatinine concentrations.

Morphology

Specimens for light microscopy were fixed in neutral-buffered formalin, embedded in wax, and sections cut at 3 μm. These were stained for neutrophils with chloroacetate esterase. Severity of infiltration was assessed as numbers of positive-staining cells in 50 sequential glomeruli per section. Slides were randomized and coded before counting.

Statistical analysis

Data from the initial induction experiments were analysed by analysis of variance, and from the ibuprofen study with an unpaired t test. Comparisons were made between rats on different treatment limbs on day 2 of the protocol. Data are expressed as mean ± s.e.mean and differences considered significant when $P < 0.05$.

Results

The results of the renal morphology and functional studies are summarised in Table 1. a-GBM alone decreased creatinine clearance by 68% at 24 h. IL-1_β had no effect, but in combination caused some attenuation of the a-GBM-induced fall, though this was neither statistically different from either the saline control group, or the a-GBM alone treated group. Neutrophil infiltration into the glomerulus occurred following a-GBM ($P < 0.05$). Both IL-1_β and a-GBM/IL-1_β caused a significantly greater influx ($P < 0.001$), with no difference between these two treatments. Rats given a-GBM alone displayed a small but significant ($P < 0.05$) albuminuria (Figure 1), consistent with earlier findings. Whilst IL-1_β alone had no effect, when given in conjunction with a-GBM it resulted in a very large and highly significant ($P < 0.001$) synergistic

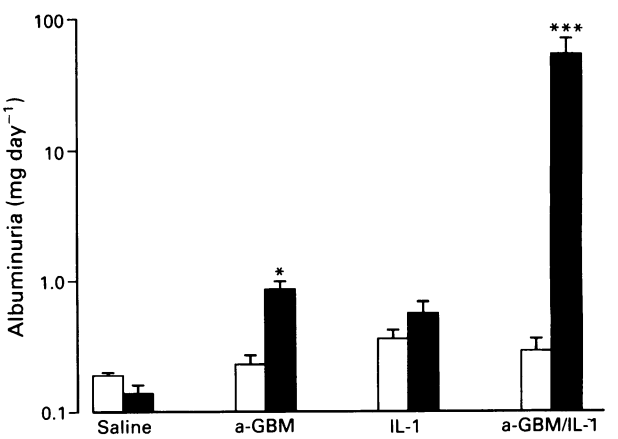

Figure 1 Urinary excretion of albumin following saline, anti-glomerular-basement-membrane antibodies (a-GBM, 9.5 mg), interleukin-1 (IL-1_β, 5 μg) or a-GBM/IL-1_β (9.5 mg; 5 μg). Results are mean (vertical bars show s.e.mean) for 24 h pre- (open columns) and post-treatment (solid columns), $n = 6$ for all groups. There were no differences in excretion rate between any of the groups on the pretreatment day. Excretion rate is shown on a $\log_{10}$ scale. * $P < 0.05$; *** $P < 0.001$ vs pretreatment day.

Table 1 Effect of anti-glomerular-basement-membrane antibodies (a-GBM) and/or interleukin-1_β (IL-1_β) on creatinine clearance and numbers of infiltrating glomerular neutrophils

	Saline	*a-GBM*	IL-1_β	*a-GBM/IL*
CCr (ml min^{-1})	1.55 ± 0.37	0.49 ± 0.09**	1.78 ± 0.48	0.91 ± 0.41
Neutrophils	1.67 ± 0.61	6.5 ± 1.43*	34.5 ± 10.5***	23.5 ± 1.98***

All measurements were made 24 h post-treatment. CCr = creatinine clearance; Neutrophils = infiltrating glomerular neutrophils/50 glomeruli. a-GBM (9.5 mg) and IL-1_β (5 μg) were given intravenously in 1.0 ml of 0.9% saline. Data are mean ± s.e.mean, $n = 6$ for all groups. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$ vs. saline control.

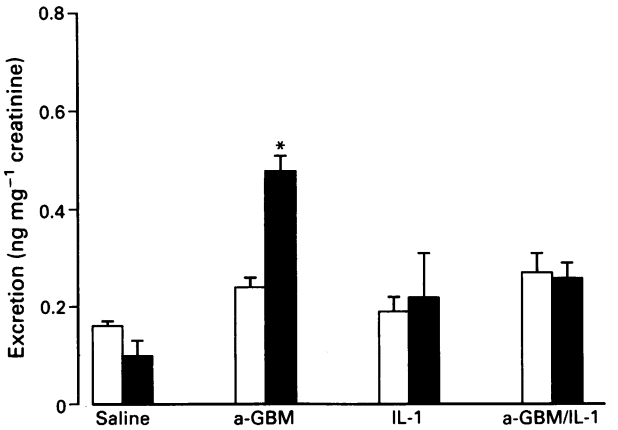

Figure 2 Urinary excretion of thromboxane B_2 (TXB_2) following saline, anti-glomerular-basement-membrane antibodies (a-GBM, 9.5 mg), interleukin-1 (IL-1_β, 5 μg) or a-GBM/IL-1_β (9.5 mg; 5 μg). Results are mean (vertical bars show s.e.mean) for 24 h pre- (open columns) and post-treatment (closed columns), $n = 6$ for all groups. There were no differences in excretion rate between any of the groups on the pretreatment day. * $P < 0.05$ vs. pretreatment day.

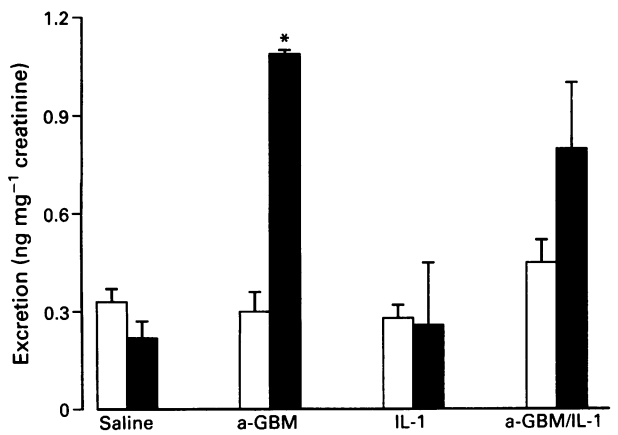

Figure 3 Urinary excretion of 2,3-dinor-thromboxane B_2 (2,3-dinor-TXB_2) following saline, anti-glomerular-basement-membrane antibodies (a-GBM, 9.5 mg), interleukin-1 (IL-1_β, 5 μg) or a-GBM/IL-1_β (9.5 mg; 5 μg). Results are mean (vertical bars show s.e.mean) for 24 h pre- (open columns) and post-treatment (solid columns), $n = 6$ for all groups. There were no differences in excretion rate between any of the groups on the pretreatment day. * $P < 0.05$ vs. pretreatment day.

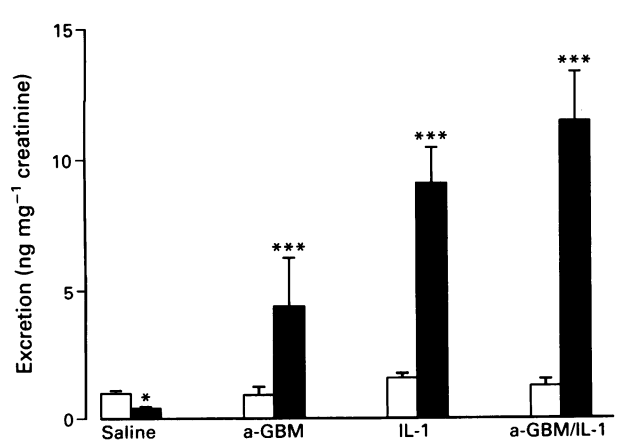

Figure 4 Urinary excretion of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) following saline, anti-glomerular-basement-membrane antibodies (a-GBM, 9.5 mg), interleukin-1 (IL-1_β, 5 μg) or a GBM/IL-1_β (9.5 mg; 5 μg). Results are mean (vertical bars show s.e.mean) for 24 h pre- (open columns) and post-treatment (solid columns), $n = 6$ for all groups. There were no differences in excretion rate between any of the groups on the pretreatment day. * $P < 0.05$; *** $P < 0.001$ vs. pretreatment day.

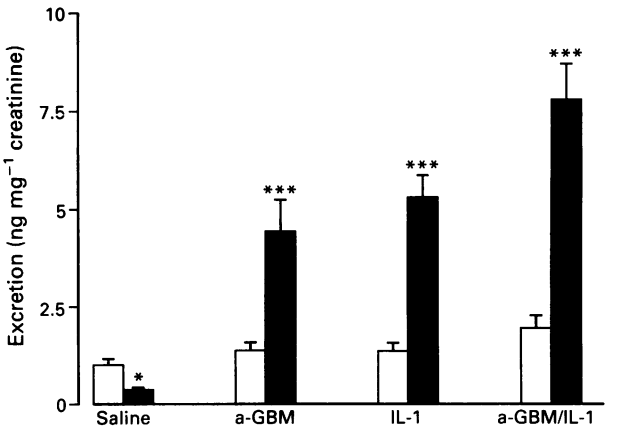

Figure 5 Urinary excretion of 2,3-dinor-6-oxo-prostaglandin $F_{1\alpha}$ (2,3-dinor-6-oxo-$PGF_{1\alpha}$) following saline, anti-glomerular-basement-membrane antibodies (a-GBM, 9.5 mg), interleukin-1 (IL-1_β, 5 μg) or a-GBM/IL-1_β (9.5 mg; 5 μg). Results are mean (vertical bars show s.e.mean) for 24 h pre- (open columns) and post-treatment (solid columns), $n = 6$ for all groups. There were no differences in excretion rate between any of the groups on the pretreatment day. * $P < 0.05$; *** $P < 0.001$ vs. pretreatment day.

increase in albuminuria, from 0.29 ± 0.07 to 54.6 ± 16.1 mg day^{-1}.

The results of the prostaglandin analysis are displayed in Figures 2–5. TXB_2 excretion was moderately elevated following a-GBM alone (Figure 2; 0.24 ± 0.02 vs. 0.48 ± 0.03 ng mg^{-1} creatinine; $P < 0.05$). Excretion of 2,3-dinor-TXB_2 was more markedly elevated following a-GBM alone (Figure 3; 0.3 ± 0.06 vs. 1.09 ± 0.01 ng mg^{-1} creatinine; $P < 0.05$). IL-1_β alone had no effect on excretion of either TXB_2 or 2,3-dinor-TXB_2. Following the combination of a-GBM/IL-1_β, 2,3-dinor-TXB_2 excretion was increased. Although this did not achieve statistical significance, it should be noted that excretion of 2,3-dinor-TXB_2 on pretreatment day was higher than for the other treatment groups. Excretion of 6-oxo-$PGF_{1\alpha}$ (Figure 4) and 2,3-dinor-6-oxo-$PGF_{1\alpha}$ (Figure 5) was substantially and highly significantly raised after a-GBM alone, IL-1_β alone, and to an even greater extent by the two given in combination. The effects of a-GBM and IL-1_β were approximately additive compared to either treatment alone.

Effect of cyclo-oxygenase inhibition

Pretreatment of animals with ibuprofen reduced serum TXB_2 to less than 5% of the value for non-ibuprofen treated animals post a-GBM/IL-1_β. The reductions in prostanoid excretion were less than the reduction in serum TXB_2 (Table 2). This is probably partly a reflection of the timing of collection: blood for serum TXB_2 was taken near the presumed peak of ibuprofen inhibition, whilst the urine collections provided an integrated measure over 24 h. Following treatment with a-GBM/IL-1_β there was marked albuminuria (around 50 mg day^{-1}). Ibuprofen attenuated this to a level approaching that found in control non-nephritic animals (Figure 6). Ibuprofen had no detectable effect on creatinine clearance, either with or without a-GBM/IL-1_β. No glomerular thrombi were seen in any of the treatment groups. Glomerular neutrophils were not affected by ibuprofen pretreatment, though there was a trend towards increased numbers in the ibuprofen-treated group compared to control ($P < 0.08$).

Discussion

These experiments provide the first direct evidence that eicosanoid synthesis is increased in nephrotoxic nephritis *in vivo* in rats. Depending on the metabolite measured, there was a 2 to

Table 2 Effect of pretreatment with the cyclo-oxygenase inhibitor ibuprofen ($10\,mg\,kg^{-1}$) on serum thromboxane B_2 (TXB_2) and markers of renal damage and urinary prostaglandin excretion after anti-glomerular-basement-membrane antibodies and interleukin-1_β (a-GBM/IL-1_β)

	– Ibuprofen	*+ Ibuprofen*
Serum TXB_2 ($ng\,ml^{-1}$)	43.9 ± 17.2	2.05 ± 0.41***
Creatinine clearance	0.35 ± 0.02	0.35 ± 0.05
Albumin ($mg\,day^{-1}$)	29.51 ± 7.2	1.85 ± 0.41***
Glomerular neutrophils	9.83 ± 2.1	15.9 ± 2.8
Glomerular thrombi	<1	<1
TXB_2	0.32 ± 0.04	0.18 ± 0.02
2,3-dinor-TXB_2	0.93 ± 0.19	0.57 ± 0.21
6-oxo-$PGF_{1\alpha}$	9.74 ± 1.54	4.55 ± 1.59*
2,3-dinor-6-oxo-$PGF_{1\alpha}$	17.0 ± 3.25	9.08 ± 1.49*

Creatinine clearance = $ml\,min^{-1}$, glomerular neutrophils and thrombi = per 50 glomeruli, prostaglandin excretion = $ng\,mg^{-1}$ creatinine. a-GBM (9.5 mg) and IL-1_β (5 μg) were given intravenously in 1.0 ml of 0.9% saline. Ibuprofen was injected intraperitoneally, twice daily for 4 days before induction of nephritic injury. All measurements were made 24 h after a-GBM/IL-1_β. *** $P < 0.001$; * $P < 0.05$, both – ibuprofen vs. + ibuprofen.

4.7 fold increase in eicosanoid excretion with a-GBM alone, compared with the same animal under control conditions. This corroborates evidence based on measurements of eicosanoid production by isolated glomeruli from rats with NTN (Lianos *et al.*, 1983; Stork & Dunn, 1985). Excretion rates of 6-oxo-$PGF_{1\alpha}$ and 2,3-dinor-6-oxo-$PGF_{1\alpha}$ were increased to a similar extent by a-GBM, implying that PGI_2 biosynthesis is increased by a-GBM. Evidence from other groups has suggested that primary hydrolysis products of both eicosanoids are of renal origin, whereas the 2,3-dinor-metabolites are of extra-renal and probably systemic origin (Fitzgerald *et al.*, 1981; reviewed in Fitzgerald *et al.*, 1987). In man, we gave TXB_2 both by inhalation and by mouth (Taylor *et al.*, 1991) and demonstrated an increase in excretion of both TXB_2 (3–4 fold) and 2,3-dinor-TXB_2 (5–7 fold) suggesting that TXB_2 is not exclusively renally derived. Consistent with this evidence of extra-renal cyclo-oxygenase activation is histological evidence of damage in organs other than the kidney, notably the lung. Macroscopic pulmonary haemorrhages were noted in animals with NTN and never in vehicle-injected animals (data not shown). Capillary thrombi have been noted within the glomeruli of nephritic animals only when pretreated with bacterial lipopolysaccharide, IL-1_β or tumour necrosis factor (Tomosugi *et al.*, 1988), and platelets in these would be expected to be a rich source of thromboxane.

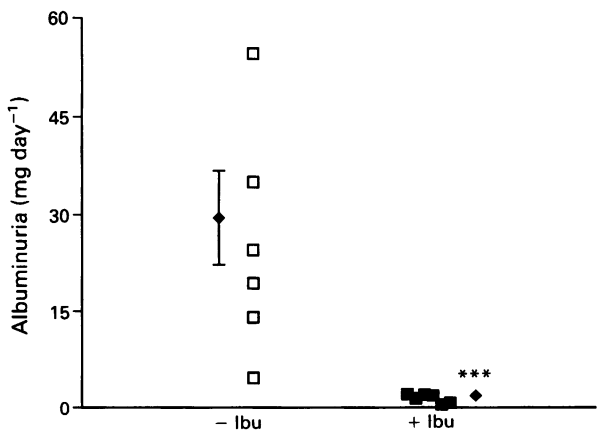

Figure 6 Urinary excretion of albumin following anti-glomerular-basement-membrane antibodies (a-GBM, 9.5 mg), interleukin-1 (IL-1_β, 5 μg): effect of pretreatment with ibuprofen ($10\,mg\,kg^{-1}$). Data represent values for individual animals either with (■; + Ibu) or without (□; – Ibu) pretreatment with ibuprofen. The mean excretion rate (vertical bars show s.e.mean if greater than symbol size) is also shown, offset from the individual data points. *** $P < 0.001$.

IL-1_β increased excretion of PGI_2- but not TXA_2-derived products, consistent with selective stimulation of PGI_2 synthesis. IL-1_β is well known to stimulate PGE_2 synthesis *in vitro* (Albrightson *et al.*, 1985; Beasley *et al.*, 1987; Kohan, 1989), but its effects on PGI_2 synthesis have been much less studied. Levine & Xiao (1985) demonstrated that IL-1_β stimulates 6-oxo-$PGF_{1\alpha}$ production by rat liver cells and Rossi *et al.* (1985) showed the same in vascular endothelial cells. We cannot identify the cellular site of the PGI_2 synthesis in the present experiments but this may well be vascular endothelial cells of the kidney and other organs, such as the lungs. The lack of effect of IL-1_β on TXA_2 biosynthesis was unexpected, since macrophages and polymorphonuclear leucocytes exhibit enhanced thromboxane synthesis after treatment with IL-1_β *in vitro* (Conti *et al.*, 1986). Increased TXA_2 production *in vivo* would have been a possible mechanism for the amplification by IL-1_β of the injurious effect of a-GBM, but our results do not support this hypothesis. On the contrary, the effect of IL-1_β on PGI_2 synthesis was additive with the effect of a-GBM itself, but IL-1_β attenuated the increase in TXB_2 and 2,3-dinor-TXB_2 excretion caused by a-GBM. A possible explanation is that increased PGI_2 synthesis caused by IL-1_β reduces platelet or white cell activation, resulting in reduced thromboxane formation. Despite an apparently favourable change in the balance of eicosanoid production, histological damage to the kidney and albuminuria were both increased by IL-1_β. A possible explanation is that the source of the increased prostacyclin production lay outside the kidney. It must be noted that histological damage to the glomeruli was assessed only by light microscopy and not by electron-microscopy of the membrane.

Nephrotoxic nephritis is a neutrophil-dependent injury and the results from this series of experiments show that injury is associated with increased numbers of neutrophils seen in the glomeruli. At 24 h post-induction of injury however, the numbers of infiltrating neutrophils is less than that seen at 2–4 h (Tomosugi *et al.*, 1989). It is not possible though to measure reliably prostaglandin excretion in rats over this period without cannulation of the bladder, a procedure which itself would increase prostaglandin production and therefore negate the 'non-invasive' advantage of urinary prostaglandin measurement over circulating plasma levels. It is interesting to note that in the group given IL-1 alone there was a significant increase in neutrophils seen in the glomerulus at 24 h but that there was no increase in albuminuria and no capillary thrombi seen. Both tumour necrosis factor (which has many overlapping, multiple specificities with IL-1; reviewed by Le & Vilcek, 1987) and bacterial lipopolysaccharide (which causes release of IL-1; Werber *et al.*, 1987) cause increased adhesiveness of neutrophils for endothelium *in vitro* (Gamble *et al.*, 1985). This increase has been noted before (Cashman *et al.*, 1989) and the lack of evidence of glomerular injury is presumed to be a reflection of the state of activation of these cells. Indeed, even under the electron microscope, where neutrophils were firmly adherent to underlying endothelium, there was no evidence of neutrophil granule release, and the endothelium appeared intact (S.J. Cashman, unpublished observation).

When animals were pretreated with the cyclo-oxygenase inhibitor ibuprofen, this decreased serum TXB_2 generation and excretion of urinary prostaglandin metabolites. Interestingly, it did not prove possible to inhibit urinary excretion of these products by more than 50%, despite a >95% inhibition of serum TXB_2. This is however in agreement with data from other groups working in man, and presumably is a reflection of the rate of recovery of cyclo-oxygenase activity following a given dose of drug (Fitzgerald *et al.*, 1983; Frazer & Ritter, 1987). Ibuprofen pretreatment reduced a-GBM/IL-1_β-induced albuminuria to levels comparable to those seen following a-GBM alone. This experiment does not therefore, tell us whether ibuprofen is reducing damage due to binding of

the a-GBM antibodies, or whether it is interfering with the synergism between this and IL-1_β.

The most likely explanation of the reduced proteinuria following ibuprofen is reduction of the intra-glomerular pressure. Two possible mechanisms have been proposed. The first is reduction of renin release and in consequence, a fall in angiotensin-II and diminished vasoconstrictor effect upon the efferent arteriole (Eriksson *et al.*, 1990). The second is reduced production of the vasodilator prostanoid, prostacyclin and resulting vasoconstriction of the afferent arteriole (Golbetz *et al.*, 1989). Our results tend to suggest that ibuprofen does not protect against injury *per se*, but inhibits net flux of albumin across equally damaged glomerular capillar walls. Consistent with this, work on angiotensin converting enzyme inhibitors showed that the ability of these drugs to inhibit proteinuria in patients with nephrotic syndrome is related to effects on filtration fraction (Heeg *et al.*, 1987). As the filtration fraction falls, proteinuria decreases, suggesting that the fall in proteinuria is related to reduction of intra-glomerular pressure. Similarly, ibuprofen is probably not protecting against the direct effects of the a-GBM, which is the primary cause of injury, but rather is attenuating the synergism between this and IL-1_β. Many of the effects of IL-1_β are known to be mediated through increased prostaglandin production and it could be that cyclo-oxygenase inhibition reduces interactions between neutrophils and endothelium, both potential sources of increased PGI_2 production.

In conclusion, we have confirmed *in vivo* previous *ex vivo* experiments suggesting that prostaglandin synthesis is elevated following injury due to anti-GBM antibodies, and also that IL-1_β is capable of causing increased prostacyclin synthesis. Inhibition of prostaglandin synthesis with the cyclo-oxygenase inhibitor ibuprofen reduced renal injury, as assessed by increased albumin excretion, but surprisingly had no effect on creatinine clearance. It has been proposed that increased thromboxane production contributes to the glomerular injury caused by a-GBM.

Although we have demonstrated increased production of thromboxane, the experiments with ibuprofen suggest that a 50% reduction in thromboxane production does not modify the glomerular injury (judged by creatinine clearance and glomerular neutrophil count), although it greatly reduces albuminuria. It is possible that a much greater degree of inhibition of thromboxane production might have modified the renal injury but it is difficult to achieve a very high degree of blockade of eicosanoid production *in vivo* with non-toxic doses of cyclo-oxygenase inhibitors.

This work was supported by the Medical Research Council.

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(Received November 27, 1990
Revised February 28, 1991
Accepted March 3, 1991)