Glomerular adenine nucleotidases

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CHAPTER 8

ATTENUATION OF ANTI-THY1 GLOMERULONEPHRITIS IN THE RAT
Anti-inflammatory activity of platelet inhibiting pharmaca

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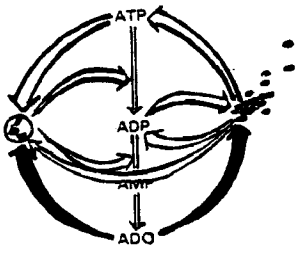

SUMMARY

Both ecto-ADPase and prostacyclin (PGI_2) are inhibitors of platelets and neutrophils. Therefore, we studied their role in acute glomerulonephritis, characterized by platelet and neutrophil influx. We tested the analog of PGI_2 (Iloprost) and 2chloro-adenosine (2Cl-ADO), an analog of adenosine, the end-product of nucleotidase activities, in rats with anti-Thy1 nephritis. After 24 hrs, platelet aggregation and intraglomerular influx of O_2^- producing neutrophils was studied immunohistochemically in kidneys of these animals. It is shown that, while both 2Cl-ADO and Iloprost treatment caused significant reduction of intraglomerular platelet aggregation, only after 2Cl-ADO treatment reduction of intraglomerular O_2^- producing activity occurred. Both agents were able to abolish proteinuria in an additional group of nephritic rats studied for 11 days. It is concluded that PGI_2 and glomerular ectonucleotidases are potentially able to attenuate this form of acute glomerulonephritis by direct anti-aggregatory actions and possibly even more so via generation of adenosine.

INTRODUCTION

Endothelial prostacyclin (PGI_2) production has been considered as an important platelet inhibiting mechanism (1-3). PGI_2 probably also has an anti-inflammatory role (4-6). In addition, blood vessel walls possess ADPase activity (7-9) with potent antithrombotic activity as demonstrated in the kidney microvasculature in vitro (10,11) and in vivo (12,13).
Like PGI_2, this ecto-nucleotidase may also display anti-inflammatory activity since ADP and ATP potentiate neutrophil responses (14-16). Therefore, hydrolysis of both adenine nucleotides by nucleotidases (17,18) may be of significance, in particular since adenosine (ADO), a potent inhibitor of inflammatory cells (15,19), can be generated in conjunction with 5'-nucleotidase.
Although in vitro studies have indicated that both ADO and PGI_2 are potent inhibitors of platelet aggregation and inflammatory cell responses, the activities of nucleotidases and PGI_2 have never been sudied in vivo during acute inflammatory processes.
The purpose of the present study was to investigate the effects of PGI_2 and ADO during anti-Thy1 nephritis, a model of acute glomerulonephritis (GN) characterized by intraglomerular platelet accumulation and neutrophil influx (12). In order to inhibit degradation of PGI_2 and ADO, which both have a short half-life in vivo, the stable analogs Iloprost and 2-chloro-adenosine (2Cl-ADO) were applied in the present study.
Results show that these analogs are potent inhibitors of intraglomerular platelet aggregation in vivo. In addition, 2Cl-ADO significantly inhibits O_2^- production of inflammatory cells in situ, whereas both 2Cl-ADO and Iloprost are able to prevent proteinuria in nephritic rats.
The inhibitory activity of both analogs upon the inflammatory process in vivo shows the protective potential of PGI_2 production and ADO generation in glomeruli, although the latter principle seems a more effective anti-inflammatory mechanism as compared to PGI_2. Furthermore, the present data support the notion that activated platelets play an important

role in the development of proteinuria during anti-Thy1 GN, once again stressing the necessity of an intact platelet inhibiting mechanism in rat glomeruli.

METHODS

ANIMALS

Female inbred PVG rats (200-220 g) fed on standard chow (Hope Farms Woerden, The Netherlands) and 3 months of age were used. Peritoneal exsudate cells were elicited in Lewis rats (300 g), since more cells could be harvested using this strain.

PREPARATION OF NEUTROPHIL SUSPENSIONS

Peritoneal exsudate cells (PEC) were obtained 16 hr following an i.p. injection of 7 ml 10% proteose pepton (Difco) with 0.8% heart infusion broth (Difco) as decribed previously (12). Suspensions were prepared according to standard procedures (12) and volumes were adjusted to a cell concentration of 7.0×10^5 cells per ml Hanks buffered salt solution (HBSS).

BIOCHEMICAL ASSAY OF OXYGEN FREE RADICAL PRODUCTION

O_2^- production of PEC was assayed biochemically by measuring superoxide dismutase (SOD; Sigma Chemical Co, St.Louis) inhibitable cytochrome-c reduction according to standard methods (16). Cells were stimulated with 5 ng/ml phorbol myristate acetate (PMA) or with 0.5 mg/ml serum treated zymosan (STZ), prepared by adding 10 mg boiled zymosan (Sigma) per ml Lewis pool serum according to standard procedures (14). 2Cl-A (Sigma) and Iloprost (a gift from Schering AG, Berlin, Germany) were added to the incubation media in final concentrations of 0.2 and 2 μM.

HISTOCHEMISTRY

Tissue processing

Kidneys were removed under ether anesthesia and specimens were snap frozen in isopentane (-80 °C). Subsequently, cryostat sections (4 μM) were cut at -20°C and stained for intraglomerular antibody deposition, platelet aggregation and inflammatory cell influx. O_2^- production in situ and myeloperoxidase depositions were also demonstrated histochemically.

Intraglomerular antibody deposition

Sections were stained with FITC-conjugated rabbit-anti-mouse IgG (Nordic, Tilburg, The Netherlands) in a dilution of 1:100 in phosphate buffered saline, according to standard procedures and examined with a fluorescence microscope (E. Leitz Inc. Rockleigh N.J.).

Inflammatory cell influx

Monocytes were demonstrated by staining of kidney sections for non-specific esterase activity according to standard methods with α-naphthylacetate as the substrate (20).

Neutrophils were demonstrated using a monoclonal antibody against rat granulocytes (HIS48; 21). In view of the presence of mouse antibodies within glomeruli of nephritic rats (i.e. anti-Thy1 IgG), we labelled the mouse monoclonal antibody against granulocytes (HIS48) with biotin (Dakopatts, Glostrup, Denmark). Cells were subsequently demonstrated using the avidin-biotin-complex (Dakopatts), according to standard procedures (12) with 3,3'-diaminobenzidin (DAB; Sigma Chemical Co, St.Louis) in the final step.

Intraglomerular activity of neutrophils

Activity of neutrophils in glomeruli in situ was assessed by histochemical demonstration of

superoxide anion production and by immunohistochemical detection of myeloperoxidase (MPO) deposits along capillary walls.

Superoxide anion production by activated PMN was demonstrated in kidney cryostat sections using Mn^{++} and DAB as decribed previously (12). Inhibition of staining by addition of SOD to the incubation media (300 U/ml) confirms the demonstration of O_2^- production by this method.

MPO deposits were demonstrated using a rabbit polyclonal antibody against rat MPO. Antibodies were prepared by immunizing rabbits with purified MPO which was isolated from rats according to the method of Meuil (22). Staining for MPO upon aceton-fixed cryostat sections was performed using standard indirect fluorescence methods with anti-MPO serum in the first and FITC-conjugated goat-anti-rabbit IgG (Kirkegeerd and Perry Laboratories Inc., Maryland, U.S.A.) in the second step. Sections were examined with a fluorescence microscope (E. Leitz Inc. Rockleigh N.J.).

Platelet aggregation

Intraglomerular platelet aggregation was demonstrated using a mouse monoclonal antibody against rat platelets (PL-1;12). This antibody was also labeled with biotin for the reason mentioned above. Staining for platelets was performed as decribed above for neutrophils.

PROTEINURIA

Urine was collected by housing rats in metabolic cages. Protein excretion per 24 hr was subsequently measured using pyrogallol-red (Merck, Darmstadt, Germany) according to standard methods (23).

QUANTITATIVE ANALYSIS OF TISSUE SECTIONS AND STATISTICAL EVALUATION

Intensity of fluorescence staining for anti-Thy1 IgG in each single glomerular cross section (GCS) was measured using an exposure meter (Leitz Orthomat E, Automatic microscope camera). Using spot measurements, light exposure time (sec) upon 400 ASA film for each glomerular cross section was measured. At magnification 160x, the complete glomerular cross section can be covered by the spot and 50 glomerular cross sections are measured per kidney. Fluorescence intensity is inversely proportional to the exposure time. The mean staining intensity of control sections from saline injected rats is subtracted from the mean staining intensity of nephritic kidneys. Studies with kidney sections of 2, 3, 4, 6 and 8 μm thickness demonstrate a linear relationship between section thickness (T) i.e. intraglomerular IgG deposits and fluorescence intensity (I) measured using this method (Fig.1A, empirical equation: $I=2.2x10^{-3}T + 5.4x10^{-3}$; $r= 0.9923$). Furthermore, results of studies with GAM-FITC dilutions and measurements of FITC extinction during light exposure, demonstrate that differences in fluorescence intensity can be measured using this method (Fig.1B and C). Results are expressed in units (sec^{-1}).

To study inflammatory response upon nephritogenic antibody in rats of different experimental groups, inflammatory cell influx, which can be considered as the resultant of antibody deposition and chemotactic activity, was quantified. Therefore, the number of HIS48 and nonspecific esterase positive cells per 50 GCS were counted in each individual animal.

Intraglomerular neutrophil activity was assessed by counting the number of oxygen free radical producing cells in 50 glomerular cross sections (GCS). The total number of O_2^- positive cells per kidney section was related to the number of HIS48 positive cells per 50 GCS.

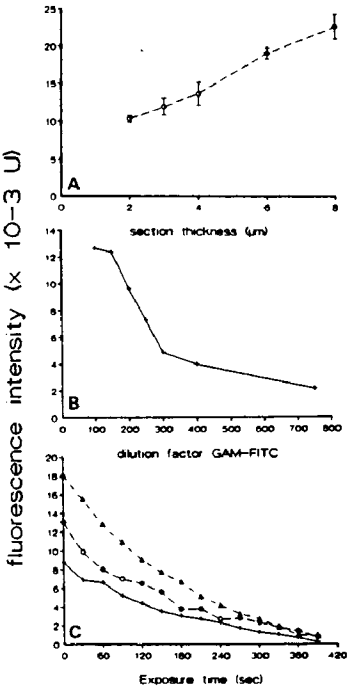

Fig.1: Glomerular fluorescence intensity, as measured using a light exposure meter, in kidney sections of rats with anti-Thy1 nephritis stained with GAM-FITC. Section thickness (A), GAM-FITC concentration (B) and FITC intensity (C) are varied to assess sensitivity of the method for measurement of fluorescence staining. Data of fig. A and B represent arithmetic means of 50 GCS per kidney section and results of fig. A are the mean (± SD) of three sections. Extinction of FITC intensity during light exposure (fig. C) is demonstrated in three different glomeruli as indicated by three separate curves. It is shown that variations in fluorescence intensity are readily detectable using this method.

Fluorescence intensity for MPO staining was quantified using the light exposure meter as described above for IgG deposits.

Intraglomerular platelet aggregation in vivo as detected by staining with PL-1 was quantitatively evaluated using standard computerized image analyzing techniques as described previously (12). The degree of intraglomerular platelet aggregation is expressed as mean percentage of glomerular area stained positive for platelets.

Results are expressed as arithmetic means (± SD) of six animals per group. Data were analyzed by Wilcoxon's test or analysis of variance (ANOVA) as indicated, and differences were considered significant at $p < 0.05$.

EXPERIMENTAL DESIGN

Six biochemical assays were performed to study effects of Iloprost or 2Cl-ADO upon radical production by PEC in vitro. Each assay was performed with a single batch of PEC, obtained from one animal.

For in vivo studies, rats received a single i.v. injection of monoclonal anti-Thy1 IgG (5 mg/kg b.w.; 20). Rats of group I (n=6) received subsequently 0.3 ml saline, whereas rats of group II and III received immediately afterwards a s.c. injection of respectively 2Cl-ADO (n=6) or Iloprost (n=6). Pilot studies demonstrated an optimal dose of 10 mg/kg b.w. for 2Cl-ADO, whereas Iloprost was administered in a dose of 1 mg/kg b.w. (24). To compare diseased versus non-diseased animals, we used saline treated rats (group IV; n=6) as control animals. 24 hr after injection of anti-Thy1 IgG or saline, kidneys were removed and

processed for histochemical studies as described above.

For longitudinal studies, additional rats received an injection of anti-Thy1 IgG and immediately afterwards the a s.c. injection of saline (group V; n=6), 2Cl-ADO (group VI; n=6) or Iloprost (group VII; n=6) in a dose of respectively 10 and 1 mg/kg b.w. Control rats received an i.v. injection of saline (group VIII; n=6). Urine samples from these rats were collected every other day, starting immediately after the injections (day 1) and finishing at day 11.

RESULTS

Immunohistological staining of kidney sections, 24 hr after injection of anti-Thy1 IgG or saline revealed abundant intraglomerular platelet aggregation in rats with anti-Thy1 IgG (fig.2) and no staining for platelets in glomeruli of rats receiving saline ($p < 0.005$, Wilcoxon). Rats treated with 2Cl-ADO or Iloprost after induction of anti-Thy1 GN showed significantly less intraglomerular platelet aggregation as compared to nephritic rats receiving no treatment ($p < 0.005$, Wilcoxon). Both 2Cl-ADO and Iloprost reduced aggregation in nephritic rats to a similar level (fig.2).

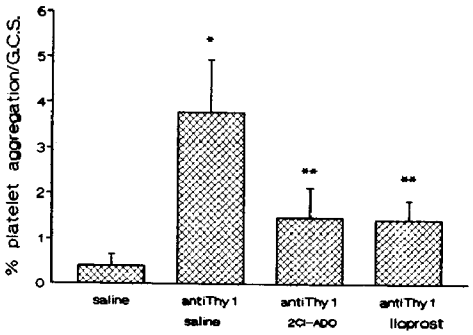

Fig. 2: Intraglomerular platelet aggregation in rats injected with saline or anti-Thy1 IgG. Immunohistological staining was quantitatively evaluated using computerized image analyzing techniques in 6 rats per group. Results are expressed as mean percent platelet aggregation per GCS (±SD) which represents the mean glomerular area stained with PL1. Significant platelet accumulation can be observed in rats with anti-Thy1 GN (= $p < 0.005$ as compared to saline, Wilcoxon), which is reduced in rats treated with 2Cl-ADO or Iloprost (** = $p < 0.005$ as compared to untreated anti-Thy1 nephritis).*

In addition to this platelet accumulation, anti-Thy1 GN was also characterized by a profound influx of neutrophils (table 1). No significant influx of monocytes, as reflected by non-specific esterase staining in glomeruli, could be observed in nephritic rats (table 1). This inflammatory cell influx was not affected by 2Cl-ADO or Iloprost: neither HIS48 staining

nor the number of non-specific esterase positive cells was significantly changed in nephritic rats treated with 2Cl-ADO or Iloprost as compared to rats with anti-Thy1 GN receiving no treatment (table 1). Also, no change in deposition of anti-Thy1 IgG could be observed in glomeruli of different groups of rats with anti-Thy1 nephritis (table 1).
Staining of kidney sections for O_2^- producing activity showed abundant presence of O_2^- producing cells in glomeruli of nephritic rats (1.89 $\pm$ 0.65 DAB positive cells per GCS), whereas virtually no O_2^- producing cells were found in glomeruli of rats injected with saline (0.1 $\pm$ 0.04 DAB positive cells per GCS; $p < 0.005$, Wilcoxon). Histochemical staining for O_2^- production in glomeruli of rats receiving 2Cl-ADO after the anti-Thy1 injection was reduced as compared to nephritic rats treated with saline (fig.3). Treatment with Iloprost did not influence intraglomerular staining for O_2^-.

Table 1: *Mean number ($\pm$ SD) of granulocytes (HIS48), monocytes (non-specific esterase positive cells) and IgG staining (fluorescence) per glomerulus in different groups, 24 hr after injection of anti-Thy1 IgG or saline.*

Group	*HIS48* †	*non-specific esterase* †	*fluorescence intensity (units)*†
saline	*0.20 $\pm$ 0.08*	*0.15 $\pm$ 0.04*	*0.3 $\pm$ 1.5x10^3*
anti-Thy1 + saline	*2.55 $\pm$ 0.84**	*0.09 $\pm$ 0.09*	*14.4 $\pm$ 1.9x10^3**
anti-Thy1 + 2Cl-A	*2.56 $\pm$ 0.25**	*0.08 $\pm$ 0.04*	*15.2 $\pm$ 3.3x10^3**
anti-Thy1 + Iloprost	*2.16 $\pm$ 0.74**	*0.14 $\pm$ 0.06*	*13.6 $\pm$ 4.1x10^3**

† *50 glomeruli per kidney section were evaluated.*
* = *$p < 0.005$ as compared to saline (ANOVA)*

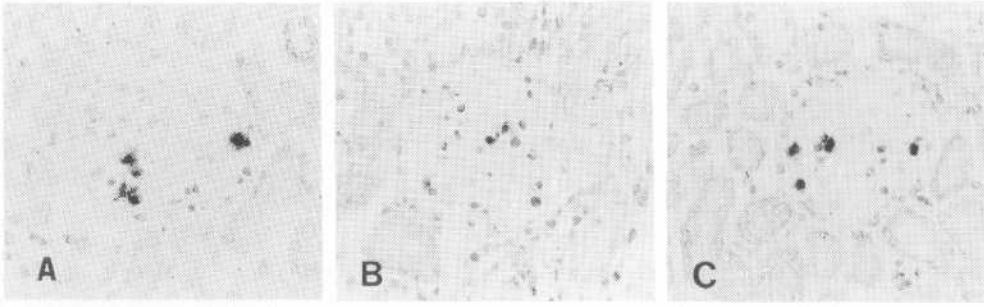

Fig.3: Micrographs of glomeruli stained for O_2^- producing activity, (dark staining), as detected histochemically in cryostat sections, 24 hr after induction of anti-Thy1 GN. Rats were treated with either saline (A), 2Cl-ADO (B) or Iloprost (C) after induction of nephritis. Reduced staining for O_2^- producing activity can be observed in rats treated with 2Cl-ADO after injection of anti-Thy1 IgG. Magnification 350x.

In anti-Thy1 nephritic rats, 75.6% ± 14.3 of the HIS48 positive cells appeared to produce O_2^- (fig.4). Treatment of nephritic rats with 2Cl-ADO reduced this percentage to 50.7% ± 4.8 ($p < 0.05$ as compared to rats receiving anti-Thy1 IgG alone). However, treatment with Iloprost caused no reduction in the percentage of O_2^- positive granulocytes (73,2% ± 21.4). In vitro, both 2Cl-ADO and Iloprost inhibited O_2^- production of activated PEC significantly in a dose dependent manner (fig.5). This inhibition occurred in suspensions of STZ-activated as well as in PMA-stimulated PEC.

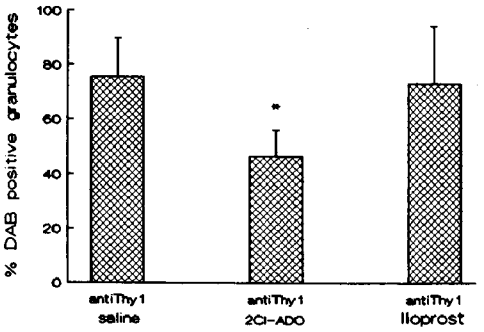

Fig. 4: Mean percentage (± SD) of granulocytes (HIS48 positive cells) producing O_2^- per 50 GCS in different groups of rats with anti-Thy1 GN (n=6). The percentage of granulocytes producing O_2^- in situ, 24 hr after induction of anti-Thy1 GN, is significantly reduced after treatment with 2Cl-ADO but not after treatment with Iloprost (=$p < 0.05$ as compared to rats receiving anti-Thy1 IgG alone, Wilcoxon).*

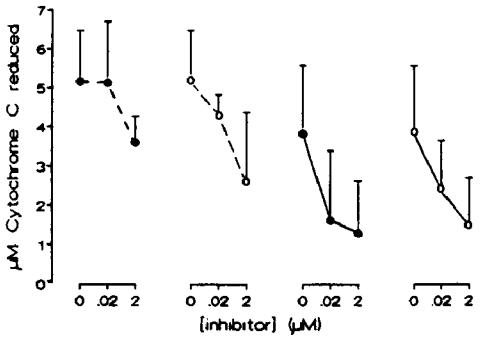

Fig.5: In vitro O_2^- production of PEC stimulated with either STZ (- - - -) or PMA (————) and incubated in the presence of 2Cl-ADO (closed symbols) or Iloprost (open symbols). It can be seen that both 2Cl-ADO and Iloprost inhibit O_2^- production of neutrophils in vitro upon stimulation with STZ as well as after stimulation with PMA.

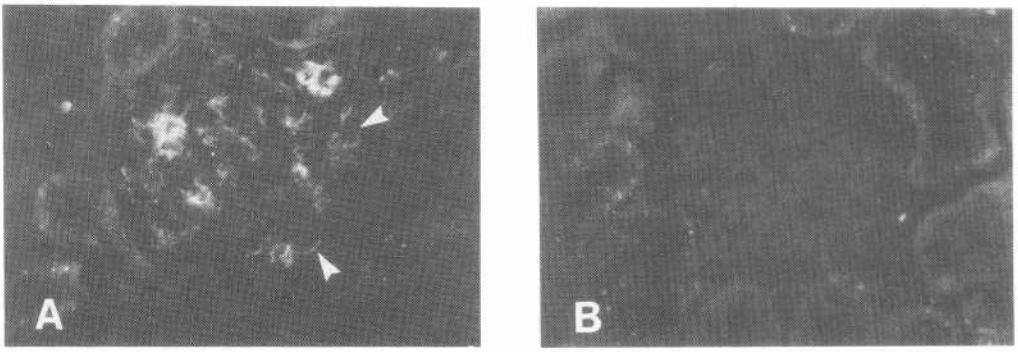

Fig. 6: Intraglomerular staining for MPO, 24 hour after induction of anti-Thy1 GN (A) or after saline injection (B), as detected using indirect immunofluorescence methods with rabbit serum against rat MPO. Focal staining for MPO can be observed in infiltrating cells as well as along capillary walls (arrows) in glomeruli of rats with antiThy1 GN (A) but not in control glomeruli (B). Magnification 300x.

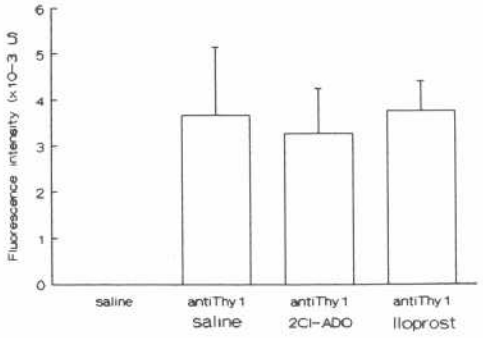

Fig. 7: Mean fluorescence intensity (units) in glomeruli of different experimental rats with anti-Thy1 GN after staining for MPO. 50 GCS per kidney are evaluated and columns represent arithmetic means (± SD) of 6 rats per group. Fluorescence staining is related to mean glomerular staining intensity in kidney sections of control rats. A significant increase in staining intensity can be detected in glomeruli of rats with anti-Thy1 GN. No statistical differences in staining intensity for MPO is observed between groups of nephritic rats receiving different treatments (ANOVA).

In glomeruli of rats with anti-Thy1 GN, strong focal immunofluorescence staining for MPO could be detected. Staining appeared to be localized in infiltrating cells and along capillary walls (fig.6). Intraglomerular MPO depositions were not changed in nephritic rats receiving 2Cl-ADO or Iloprost; distribution as well as staining intensity (fig. 7) was similar to staining in nephritic rats receiving no treatment.

Longitudinal studies of rats with anti-Thy1 GN showed a transient proteinuria from day 1 till day 11, with a maximum at day 3. In contrast, rats receiving 2Cl-ADO or Iloprost after induction of GN displayed no proteinuria at this time interval (fig. 8). In both groups of rats, urinary protein excretion did not increase significantly above control levels between day 3 and 11.

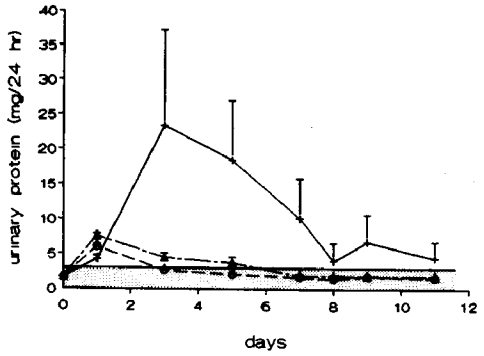

Fig. 8: Mean urinary protein excretion (± SEM) of rats with anti-Thy1 GN (——+——) and rats with anti-Thy1 GN receiving 2Cl-ADO (--o--) or Iloprost (——— -▲). Shaded area represents range in protein excretion of non-nephritic rats treated with saline.

DISCUSSION

In the present study, the anti-thrombotic and anti-inflammatory activities of Iloprost and 2Cl-ADO were examined in vivo in the acute phase of anti-Thy1 nephritis. As can be seen in figure 2, both Iloprost and 2Cl-ADO are able to inhibit intraglomerular platelet aggregation in vivo during anti-Thy1 GN to a similar level.

In addition to platelet inhibiting effects of Iloprost and 2Cl-ADO, potential anti-inflammatory activity of these pharmaca is also studied in the present model of acute GN by quantification of inflammatory cell influx as well as by assessment of in situ activity of granulocytes. Inflammatory cell influx, which can be considered as the resultante of intraglomerular antibody deposition and chemotactic activity, was quantified to examine whether 2Cl-ADO or Iloprost influenced the initial phase of the disease. No change in neutrophil or monocyte influx could be detected in nephritic rats treated with Iloprost or 2Cl-ADO as compared to nephritic rats treated with saline (table 1), indicating that these drugs do not affect antibody deposition and chemotactic activity. Furthermore, direct quantitative evaluation of glomerular IgG staining support this notion since no significant differences could be detected between experimental groups (table 1). Although application of a light exposure meter does not provide information about the actual amount of IgG deposits in glomeruli, the method is highly sensitive and reproducible for the detection of differences in fluorescence staining

between experimental groups, as is demonstrated in figure 1.
In contrast to inflammatory cell influx as such (table 1), 2Cl-ADO does influence O_2^- production of neutrophils in situ (figure 3). This is also reflected by a reduction in the percentage of radical producing neutrophils in nephritic rats treated with 2Cl-ADO as compared to rats with acute GN treated with saline (figure 4). Following Iloprost treatment, no reduction in the percentage of activated granulocytes in glomeruli can be detected.
However, in vitro, both 2Cl-ADO and Iloprost are able to inhibit oxygen free radical production of activated PEC irrespective of the neutrophil activating agenet used (figure 5). Although the reason for this discrepancy between in vivo and in vitro results is unknown, differences in tissue distribution between pharmaca, receptor expression upon target cells and half-life in vivo may account for this difference.
Since proteases from leukocytes also contribute to the induction of glomerular damage (25), intraglomerular enzyme release by neutrophils is studied as well. Degranulation of neutrophils in vivo is assessed by measuring staining intensity for MPO in glomerular cross sections. Focal staining for MPO deposits along capillary walls can be observed in rats with anti-Thy1 GN (figure 6). Although it has been suggested that intraglomerular degranulation will cause binding of cationic MPO to anionic sites within the GBM (26), this has up to now never been actually observed during experimental nephritis. Since intraglomerular influx of granulocytes is not different between experimental groups (table 1), differences in MPO staining would reflect changes in degranulation of granulocytes. Quantitative evaluation of glomerular staining for MPO reveals no difference between experimental groups with anti-Thy1 GN (figure 7), suggesting that 2Cl-ADO nor Iloprost influences degranulation of neutrophils in this in vivo model. These results are in accordance with in vitro results about the effect of 2Cl-ADO upon lysosomal enzyme release by neutrophils (19), but are in contrast with reports about inhibiting effects of Iloprost on several neutrophil responses (5,27).
In summary, from the present data it can be concluded that in vivo, both Iloprost and 2Cl-ADO exert potent platelet inhibiting activity during anti-Thy1 GN, whereas 2Cl-ADO displays additional anti-inflammatory activity by inhibiting in situ O_2^- production of intraglomerular neutrophils.
Since the ultimate glomerular damage induced by the inflammatory process is reflected by proteinuria, the effect of both platelet inhibiting pharmaca upon urinary protein excretion is examined in subsequent experiments. As can be seen in figure 8, rats with anti-Thy1 GN display a transient proteinuria. Proteinuria of rats, although in this PVG strain not as high as has been described for Wistar rats with anti-Thy1 GN (28), could be completely inhibited by both Iloprost and 2Cl-ADO. Thus, treatment of nephritic rats with Iloprost or 2Cl-ADO significantly protects the kidney from damage during acute GN.
The mechanism by which both pharmaca inhibit proteinuria is not clear. Both 2Cl-ADO and Iloprost have potent vasodilatory effects (29,30) and influence renal blood flow (31). Since both pharmaca more or less reduce glomerular filtration rate (31), these hemodynamic changes may be responsible for the reduced proteinuria following treatment. However, the complete inhibition of proteinuria during the first 11 days of nephritis suggests a diminution of glomerular damage as the causal factor, rather than a transient hemodynamic effect of the pharmaca, administered at day 1. Pilot studies from our lab showed reduced platelet

responsiveness in vitro of 2Cl-ADO and Iloprost treated rats, at day 1 (99.7% and 64.8% reduction of collagen-induced aggregation for 2Cl-ADO and Iloprost respectively) whereas at day 2 inhibitory effects were greatly reduced (44.7% and 36.6% reduction in collagen-induced aggregation for 2Cl-ADO and Iloprost respectively). This suggests that direct effects of the pharmaca are only transient, which is in agreement with in vivo studies of other authors, showing that Iloprost levels in plasma are negligible 24 hr after administration of the drug (32).

However, the results do suggest a significant role for platelets as a pathogenetic factor for proteinuria since the anti-platelet drug Iloprost inhibits proteinuria completely, despite the absence of a detectable effect upon the inflammatory component of anti-Thy1 GN. This notion is supported by results of studies in other models of nephritis, showing reduced proteinuria after platelet depletion (33,34). The mechanism of this platelet activity is unknown but neutralization of anionic charges by cationic platelet products (35-37) or release of proteinases from platelets (38) have been suggested as possible mechanisms, whereas platelet-neutrophil interactions may also account for this phenomenon (14,39,40). As a whole, our data suggest that platelets may act as inflammatory cells (38).

Other studies also demonstrated an important role for platelets during anti-Thy1 Gn by demonstrating that platelets may be involved in the mesangial cell proliferation, characteristic for a later stage of this type of GN (41).

In conclusion, the present studie demonstrates the importance of platelet inhibiting mechanisms within the intact glomerulus. Results also implicate that generation of adenosine by glomerular nucleotidases might play a significant role as such a platelet inhibitor during nephritis in rat kidneys in addition to intraglomerular PGI_2 production. Adenosine can be generated within the inflammatory microenvironment by hydrolysis of extracellular ATP and ADP, released by activated platelets and damaged cells (42,43). Hydrolysis of ATP and ADP, with pro-inflammatory activities (14-16), is accomplished by nucleotidases, present in glomeruli (10-13,17). In conjunction with ecto-5'-nucleotidase upon neutrophils (44), ADO can subsequently be generated. In this way, cooperation between the vessel wall and neutrophils may provide the anti-inflammatory activity.

Studies dealing with antithrombotic and anti-inflammatory mechanisms have focussed mainly upon arachidonic acid metabolites in the last decades. However, evidence is accumulating that extracellular adenine nucleotides and nucleotidases also may be important players in the inflammatory processes.

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