Production of Thromboxane A_2 and Prostaglandin I_2 Affected by Interaction of Heat Aggregated IgG, Endothelial Cells, and Platelets in Lupus Nephritis

NAOKO KANEKO, JUN-ICHI MASUYAMA, HIROYUKI NARA, DAISUKE HIRATA, MASAHIRO IWAMOTO, HITOAKI OKAZAKI, SEIJI MINOTA, and TAKU YOSHIO

ABSTRACT. ***Objective.*** To examine the role of immune complexes in the prostanoid metabolism of glomerular capillary endothelial cells (EC) and platelets in lupus nephritis. Heat aggregated IgG (HA-IgG), instead of immune complexes, was incubated using an *in vitro* coculture system with human umbilical vein EC, instead of glomerular capillary EC, and platelets. The effect of complement component C1q and a novel imidazole-type thromboxane A_2 (TXA_2) synthetase inhibitor, DP-1904, on this prostanoid metabolism change was also investigated.

Methods. EC monolayers (1.5×10^5 cells/well) were incubated with various concentrations of HA-IgG, monomeric IgG, or medium alone for 1 h at 37°C, and then incubated with platelet suspensions (1×10^8 cells/ml) for various times. Concentrations of TXB_2 and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), the stable hydrolysis products of TXA_2 and prostaglandin I_2 (PGI_2), respectively, released in the supernatants were measured by ELISA.

Results. HA-IgG bound to EC monolayers produced TXB_2 and 6-keto-$PGF_{1\alpha}$ in a concentration dependent manner and much more than monomeric IgG or medium alone did. However, the production of 6-keto-$PGF_{1\alpha}$ stimulated with HA-IgG was much lower than that of TXB_2, indicating a large imbalance between TXA_2 and PGI_2. Preincubation of HA-IgG with purified C1q partially suppressed the production of TXB_2, but not that of 6-keto-$PGF_{1\alpha}$. DP-1904 suppressed the production of TXB_2 completely, but by sharp contrast, it dramatically increased the production of 6-keto-$PGF_{1\alpha}$ from EC and platelets by HA-IgG.

Conclusion. The large imbalance of TXA_2 and PGI_2 produced by the interaction of EC, immune complexes, and platelets may be associated with alterations in glomerular pathological findings and hemodynamics mediated by immune complexes in lupus nephritis. C1q and a TXA_2 synthetase inhibitor may improve the abnormal prostanoid metabolism change of lupus nephritis. (J Rheumatol 2002;29:2106–13)

Key Indexing Terms:
LUPUS NEPHRITIS | THROMBOXANE A_2 | PROSTAGLANDIN I_2
ENDOTHELIAL CELLS | IMMUNE COMPLEXES | PLATELETS

Enhanced urinary excretion of thromboxane B_2 (TXB_2), the stable hydrolysis product of thromboxane A_2 (TXA_2), decreased excretion of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), the stable hydrolysis product of prostaglandin I_2 (PGI_2), and a higher ratio of TXB_2/6-keto-$PGF_{1\alpha}$ in the urine have been reported in patients with lupus nephritis[1-3]. In murine lupus nephritis increased renal TXB_2 production has been reported[4]. Moreover, the severity of the abnormal prostanoid metabolism correlated with the activity of renal lesions and with deteriorating renal function; a significant inverse correlation between urinary TXB_2 and creatinine clearance rate and a significant linear correlation between urinary 6-keto-$PGF_{1\alpha}$ and creatinine clearance rate[1], a negative correlation between urinary TXB_2/6-keto-$PGF_{1\alpha}$ ratio and creatinine clearance rate[2], and a positive correlation between production of TXB_2 and the amount of proteinuria[2]. Increased TXB_2 synthesis and reduction in creatinine clearance rate were at least partially corrected by administration of a TXA_2 receptor antagonist[5] or a TXA_2 synthetase inhibitor[2,3]. The abnormalities of intrarenal synthesis of TXA_2 and PGI_2 were suggested to be associated with changes in glomerular hemodynamics and play a role in the progression of lupus nephritis[2,3].

Although such abnormal prostanoid metabolism has been observed in lupus nephritis[1-5], the question of what causes the intrarenal TXA_2 increase and which cells are producing this metabolite have never been resolved. Deposits of

From the Division of Rheumatology and Clinical Immunology, Jichi Medical School, Tochigi, Japan.

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N. Kaneko, MD, Assistant; J-I. Masuyama, MD, Assistant Professor; H. Nara, MD, Assistant; D. Hirata, MD, Assistant; M. Iwamoto, MD, Assistant Professor; H. Okazaki, MD, Associate Professor; S. Minota, MD, Professor; T. Yoshio, MD, Assistant Professor.

Address reprint requests to Dr. T. Yoshio, Division of Rheumatology and Clinical Immunology, Jichi Medical School, 3311 Yakushiji, Minamikawachi-machi, Tochigi-ken 329-0498, Japan. E-mail: takuyosh@jichi.ac.jp

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immune complexes and complement components along glomerular capillary loops have been described in patients with lupus nephritis[6] and suggested to play a role in the progression of lupus nephritis[7]. Thrombosis and later sclerosis have been observed pathologically in glomerular capillary in patients with lupus nephritis, especially the diffuse proliferative form[8,9]. A correlation between reduced platelet survival and increased renal platelet localization in patients with proliferative lupus nephritis has suggested intrarenal platelet consumption[10]. In addition, platelet cationic proteins, which are released from platelets stimulated with thrombin, immune complexes, and platelet-activating factor, have been reported to be present in glomerular capillaries in patients with lupus nephritis[11]. Platelets express Fcγ receptor (FcγR) II, to which IgG immune complexes can bind[12]. Considering these reports[6-12], we suggest this possibility: in patients with lupus platelets bind to immune complexes bound on endothelial cell (EC) surfaces of glomerular capillaries, and are activated. TXA_2 (the principal metabolite of arachidonic acid in platelets[13]) produced by activated platelets causes platelet aggregation and mesangial contraction. In addition, the production of PGI_2 (the principal metabolite of arachidonic acid in EC[14]) within glomerular capillaries is affected by the interaction of EC, immune complexes, and platelets. Such prostanoid metabolism changes may be associated with alterations in glomerular pathological findings and hemodynamics of lupus nephritis. Cines, *et al* made EC derived from human umbilical vein and platelet coculture system *in vitro*, and reported that incubation of EC monolayer with heat aggregated IgG (HA-IgG) increased the adherence of platelets to EC monolayer and the procoagulant activity, and suggested that platelets augment immune vascular injury in lupus[15,16].

We therefore made an EC and platelet coculture system and investigated the prostanoid metabolism affected by interaction of HA-IgG (instead of immune complexes), EC derived from human umbilical veins (instead of glomerular capillary), and platelets *in vitro*. Moreover, we investigated the prostanoid metabolism changes on an EC and platelet coculture system with HA-IgG by complement component C1q and a novel imidazole-type TXA_2 synthetase inhibitor, DP-1904.

MATERIALS AND METHODS

Human EC culture. EC were prepared from fresh human umbilical vein and cultured as described[17]. EC were serially passed through brief exposure to 0.25% trypsin (Difco, Detroit, MI, USA) and 0.04% EDTA (Sigma, St. Louis, MO, USA). Only cells from the second or third passage were used. The cells were positive for von Willebrand factor.

Preparation of monomeric IgG and HA-IgG. Isolated IgG from normal human serum (Sigma) was dissolved with phosphate buffered saline (PBS) (final concentration of IgG 10 mg/ml), followed by centrifugation at 100,000 g for 60 min to remove insoluble aggregates, and used as monomeric IgG. HA-IgG was prepared before each experiment by heating monomeric IgG isolated from normal human serum (Sigma) for 20 min at 63°C, followed by centrifugation at 3000 g for 15 min to remove insoluble aggregates.

Binding of HA-IgG or monomeric IgG to EC. EC (1×10^5 cells/well) were seeded in 96 well flat bottom tissue culture plates (Costar Corp., Cambridge, MA, USA) precoated with 5% gelatin (Sigma). After 2–3 days in culture, EC reached confluence as a monolayer. After 2 washes with medium 199 (Nissui, Tokyo, Japan), 100 μl of HA-IgG or monomeric IgG diluted in PBS (various concentrations) were added to each well and incubated 1 h at 37°C. After 2 washes with washing buffer [PBS-1% bovine serum albumin (BSA; Sigma)] 200 μl/well, EC were fixed by incubation with 1% paraformaldehyde (Sigma) in PBS for 10 min at room temperature. After 2 washes with PBS 200 μl/well, the remaining free protein sites were blocked by adding 300 μl/well of blocking buffer [PBS-3% BSA (Sigma)] and incubated for at least 1 h at room temperature. Plates were then washed 4 times with washing buffer and 100 μl of peroxidase conjugated goat anti-human IgG (Dako, Glostrup, Denmark) diluted 1:3000 in blocking buffer were added to each well. Plates were incubated 90 min at room temperature. After 5 washes with washing buffer, 200 μl of peroxidase substrate buffer (o-phenylenediamine 0.4 mg/ml, 0.015% H_2O_2 in 25 mM citric phosphate buffer, pH 5.2) were added to each well and incubated for 3 min at room temperature. Color development was stopped by adding 50 μl of 1.0 M H_2SO_4. The color intensity of the reaction was read at an optical density (OD) of 492 nm using an ELISA reader (Titertek Multiskan; Flow Laboratories, McLean, VA, USA). OD value was used as an expression of binding of HA-IgG or monomeric IgG.

Platelet preparation. Venous blood was drawn from male donors who had taken no aspirin or related antirheumatic drugs for 2 weeks or ethanol in the last 24 h. Freshly drawn blood was collected into polypropylene tubes containing ACD-A solution [2.2% (wt/vol) sodium citrate, 0.8% citric acid, 2.2% glucose, pH 4.5~5.5; Terumo Co. Ltd., Tokyo, Japan] (3 vol:20 vol venous blood), and centrifuged at 250 g for 10 min to obtain platelet-rich plasma. The platelet-rich plasma was collected and centrifuged at 1500 g for 10 min to obtain platelet pellets. The platelet pellet was washed 3 times in CBS buffer (6.85 mM citric acid, 130 mM sodium chloride, 4 mM potassium chloride, 5.5 mM glucose, pH 6.5), and finally resuspended in modified Tyrode's buffer, pH 7.3 (Sigma), containing 5 mM HEPES and 0.2% BSA. The platelets were then counted in triplicate in a Coulter counter and adjusted to 1×10^9 cells/ml using the same buffer. The platelets were kept at room temperature until used in experiments of the EC and platelet coculture system. Before addition to each well, platelets were adjusted to 1×10^8 cells/ml using PBS.

TXA_2 and PGI_2 release from EC and platelet coculture system. EC (1.5×10^5 cells/well) were seeded in 48 well flat bottom tissue culture plates (Costar) coated with 5% gelatin (Sigma). EC formed a confluent monolayer after 2 to 3 days in culture, then the medium was removed and EC were washed twice with medium 199. One milliliter of HA-IgG (various concentrations), 1 ml of monomeric IgG (various concentrations), or 1 ml of PBS alone were added to each well and EC were incubated 1 h at 37°C. The supernatants in each well were removed and EC were washed twice with medium 199. Then 1 ml of platelet suspension (1×10^8 cells/ml) was added to each well and EC were incubated for various times at 37°C. The supernatants were collected and disodium EDTA (Sigma) and indomethacin (Sigma) added to stop prostaglandin cascades (final concentration 4.64 mM and 0.182 mM, respectively), and centrifugation was carried out at 3000 g for 10 min to remove platelets and EC. After removal of platelets and EC the supernatants were stored at –20°C for assay of TXA_2 and PGI_2.

Preincubation of HA-IgG with purified C1q. HA-IgG (2000 μg/ml) was incubated with an equal volume of purified human complement component C1q (Sigma) (various concentrations) for 1 h at 37°C. HA-IgG preincubated with C1q (final concentration of HA-IgG 1000 μg/ml) or C1q solutions (various concentrations) without IgG in PBS were added to each well at the time of addition of HA-IgG (1000 μg/ml) or PBS alone to each well.

Effect of DP-1904 on TXA_2 and PGI_2 release from EC and platelet coculture system. The crystalline powder form of DP-1904 [6-(imidazolylmethyl)-5,6,7,8-tetrahydro-naphthalene-2-carboxylic acid hydrochloride hemihydrate] was kindly provided by Daiichi Pharmaceutical Co., Tokyo,

Japan. The powder was dissolved in sterile distilled water just before each experiment. At the time of addition of platelet-suspension to each well, dissolved DP-1904 (various concentrations) was added to each well at final concentrations of 10^{-3}~10^{-7} M.

Enzyme immunoassay for TXA_2 and PGI_2. The concentrations of TXB_2 and 6-keto-$PGF_{1\alpha}$, the inactive stable metabolites of TXA_2 and PGI_2, respectively, were measured in cell supernatants by specific ELISA (Amersham, Buckinghamshire, UK).

Statistical analysis. All samples were tested in triplicate. The results except for TXB_2/6-keto-$PGF_{1\alpha}$ ratio were expressed as means ± SD of triplicate data and were representative of experiments performed 3 or 4 times. Comparisons of mean levels were carried out using analysis of variance with Student's unpaired t test (for single comparisons) to assess statistical significance between any 2 groups.

RESULTS

Binding of HA-IgG or monomeric IgG to EC (Figure 1). The binding of HA-IgG was dependent upon the concentration of IgG added. IgG was detected on EC following the addition of 7.8 μg/ml HA-IgG. Monomeric IgG also bound to EC in a dose dependent manner, but greater than 10-fold more protein than HA-IgG was required for the same detection of OD values at monomeric IgG concentrations greater than 250 μg/ml.

Relationship between concentration of IgG and TXA_2 production (Figure 2A). Various concentrations of HA-IgG, monomeric IgG, or medium alone were first added to EC monolayers, and then EC were incubated with platelet suspensions for 2 h at 37°C. The amounts of TXB_2 produced by EC and platelets in the presence of HA-IgG (EC + HA-IgG + platelets) were compared with those produced by EC and platelets in the presence of monomeric IgG (EC + monomeric IgG + platelets) and EC and platelets in the absence of IgG (EC + platelets). The amounts of TXB_2 produced by EC + HA-IgG + platelets were dependent upon the concentrations of HA-IgG. EC + HA-IgG + platelets

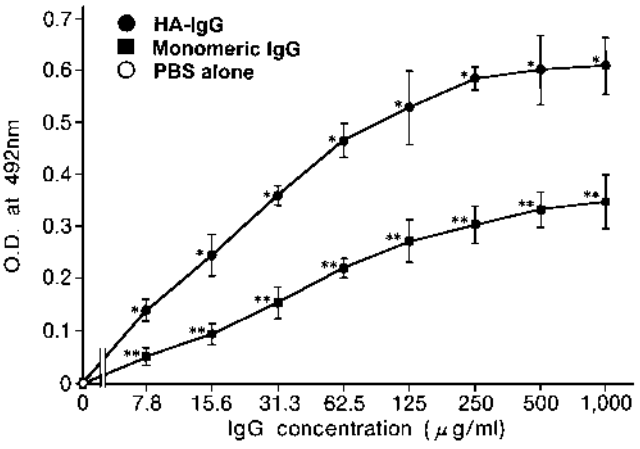

Figure 1. Binding of HA-IgG, monomeric IgG, and PBS alone to human umbilical vein endothelial cells using peroxidase conjugated anti-human IgG and determined by ELISA. OD value of PBS alone was adjusted to zero. *p < 0.01 vs PBS alone, and p < 0.01 vs monomeric IgG at each concentration of IgG. **p < 0.01 vs PBS alone.

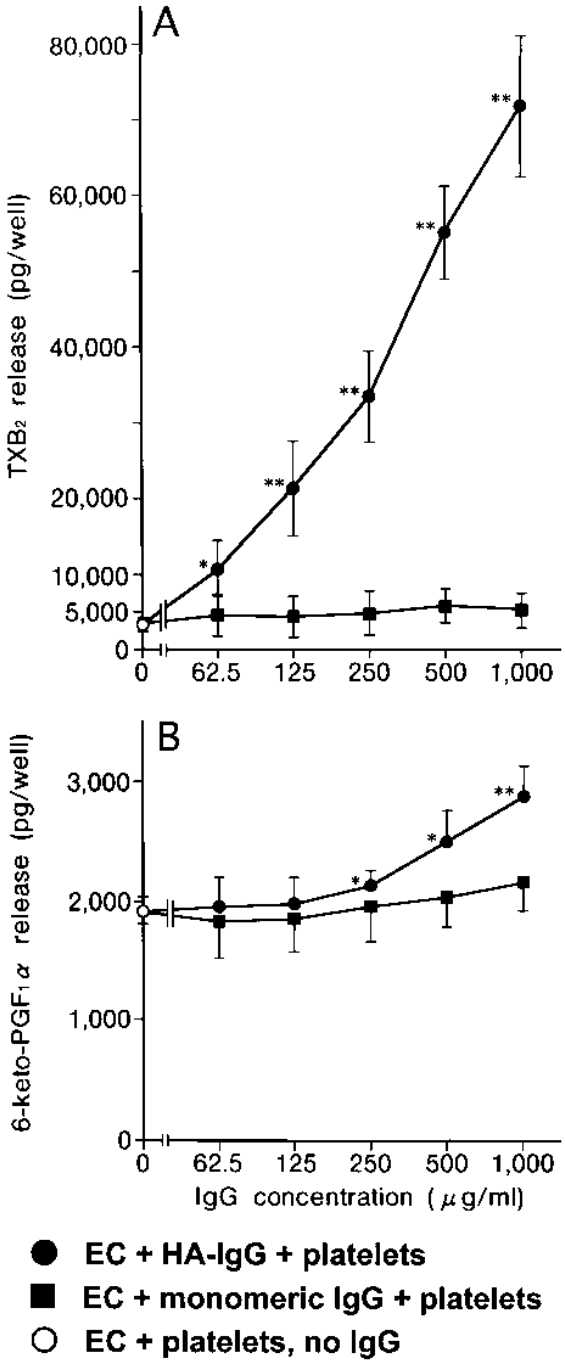

Figure 2. Relationship between concentration of IgG and TXA_2 (A) and PBI_2 (B) production in the EC and platelet coculture system. Various concentrations of HA-IgG, monomeric IgG, or medium alone were first added to EC monolayers, and then EC were incubated with platelet suspensions for 2 h at 37°C. The amounts of TXB_2 (A) and 6-keto-$PGF_{1\alpha}$ (B) produced by EC + HA-IgG + platelets, EC + monomeric IgG + platelets, and EC + platelets in the absence of IgG were measured. (A) *p < 0.05 vs EC + platelets in the absence of IgG, and p < 0.05 vs EC + monomeric IgG 62.5 μg/ml + platelets. **p < 0.01 vs EC + platelets in the absence of IgG, and p < 0.01 vs EC + monomeric IgG + platelets at each concentration of IgG. (B) *p < 0.05 vs EC + platelets in the absence of IgG. **p < 0.01 vs EC + platelets in the absence of IgG, and p < 0.05 vs EC + monomeric IgG 1000 μg/ml + platelets.

produced TXB_2 in quantity significantly greater than EC + platelets at all concentrations of IgG added. The amount of TXB_2 produced by EC + HA-IgG 1000 μg/ml + platelets was about 20 times higher than that by EC + platelets. EC + HA-IgG + platelets produced TXB_2 in quantity significantly greater than EC + monomeric IgG + platelets at each concentration of IgG. The amount of TXB_2 produced by EC + HA-IgG + platelets was about 13 times higher than that produced by EC + monomeric IgG + platelets at IgG concentration of 1000 mg/ml. The amounts of TXB_2 produced by EC + monomeric IgG + platelets were not significantly different from those produced by EC + platelets at all concentrations of monomeric IgG.

Relationship between concentration of IgG and PGI_2 production (Figure 2B). Various concentrations of HA-IgG, monomeric IgG, or medium alone were first added to EC monolayers, and then EC were incubated with platelet suspensions for 2 h at 37°C. The amounts of 6-keto-$PGF_{1\alpha}$ produced by EC + HA-IgG + platelets were compared with those produced by EC + monomeric IgG + platelets and EC + platelets. The amounts of 6-keto-$PGF_{1\alpha}$ produced by EC + HA-IgG + platelets were much less than amounts of TXB_2 at each HA-IgG concentration in the EC and platelet coculture system. The amounts of 6-keto-$PGF_{1\alpha}$ produced by EC + HA-IgG + platelets were dependent upon the concentrations of HA-IgG. EC + HA-IgG + platelets produced 6-keto-$PGF_{1\alpha}$ in significantly greater amounts than EC + platelets did at HA-IgG concentration > 125 μg/ml. EC + HA-IgG + platelets produced more 6-keto-$PGF_{1\alpha}$ than EC + monomeric IgG + platelets, but the significant difference was observed only at IgG concentration of 1000 μg/ml. The amounts of 6-keto-$PGF_{1\alpha}$ produced by EC + monomeric IgG + platelets were not significantly different from those produced by EC + platelets at all concentrations of monomeric IgG.

Time course of TXA_2 release from EC and platelets by HA-IgG (Figure 3A). The amounts of TXB_2 produced by EC + HA-IgG 1000 μg/ml + platelets, EC + monomeric IgG 1000 μg/ml + platelets, and EC + platelets were measured according to time course of incubation after the addition of platelet suspensions. Addition of suspensions without platelets was also analyzed. Upon addition of suspension without platelets, no TXB_2 production was detected in 3 combinations (EC + HA-IgG 1000 μg/ml; EC + monomeric IgG 1000 μg/ml; and EC + medium alone) until after 6 h, but after 12 h almost the same small amounts of TXB_2 were detected in these 3 combinations (820.0 ± 20.0, 813.3 ± 32.1, and 833.3 ± 11.5 pg/well, respectively) (data not shown in Figure 3). EC + HA-IgG + platelets produced the largest amount of TXB_2 rapidly between 0.5 and 1 h, and reached a plateau at 2 h; the amount of TXB_2 produced between 2 and 24 h was not markedly greater than at 2 h. The amount of TXB_2 produced by EC + HA-IgG + platelets was significantly greater than that produced by EC + monomeric IgG + platelets and by EC + platelets at each time point. The combination of EC + monomeric IgG + platelets tended to produce more TXB_2 than EC + platelets, but no significant difference was observed at any time during the time course.

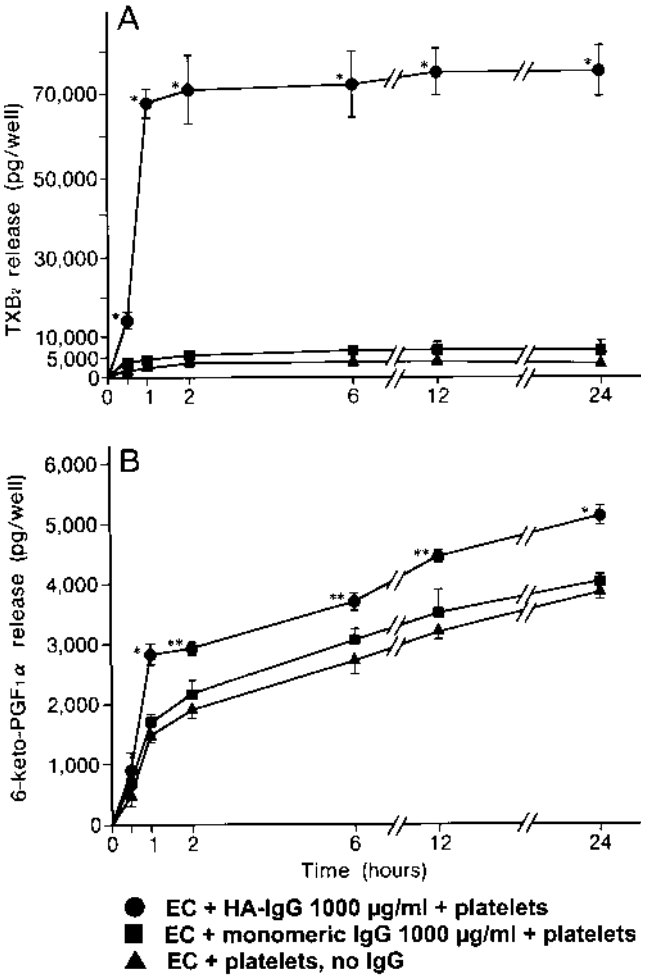

Figure 3. Time course of TXA_2 (A) and PBI_2 (B) release from EC and platelets by HA-IgG. According to time course of incubation after addition of platelet suspensions, amounts of TXB_2 (A) and 6-keto-$PGF_{1\alpha}$ (B) produced by EC + HA-IgG 1000 μg/ml + platelets, EC + monomeric IgG 1000 μg/ml + platelets, and EC + platelets in the absence of IgG were measured. *p < 0.01 vs EC + monomeric IgG 1000 μg/ml + platelets at each time, and p < 0.01 vs EC + platelets in the absence of IgG at each time. **p < 0.05 vs EC + monomeric IgG 1000 g/ml + platelets at each time, and p < 0.01 vs EC + platelets in the absence of IgG at each time.

Time course of PGI_2 release from EC and platelets by HA-IgG (Figure 3B). The time course of PGI_2 release was analyzed in the same way as for TXA_2 release. EC + HA-IgG 1000 μg/ml + platelets produced 6-keto-$PGF_{1\alpha}$ rapidly by 1 h, and then gradually between 1 and 24 h. This combination produced significantly more 6-keto-$PGF_{1\alpha}$ than EC + monomeric IgG 1000 μg/ml + platelets and EC + platelets between 1 and 24 h. No significant difference of 6-keto-

$PGF_{1\alpha}$ production was observed between EC + monomeric IgG + platelets and EC + platelets at any time point.

TXA_2 and PGI_2 release from EC and platelets by HA-IgG preincubated with C1q (Figure 4). Before addition of HA-IgG to EC monolayers, HA-IgG were incubated with various concentrations of C1q. The following procedure was done in the same assays for TXA_2 and PGI_2 release from the EC and platelet coculture system. EC were incubated with platelet suspensions for 2 h at 37°C. The amounts of TXB_2 and 6-keto-$PGF_{1\alpha}$ released from EC and platelets by HA-IgG 1000 μg/ml preincubated with various concentrations of C1q were compared with amounts released by HA-IgG 1000 μg/ml without preincubation (EC + HA-IgG 1000 μg/ml + platelets). The amount of TXB_2 released from EC and platelets by HA-IgG 1000 μg/ml preincubated with C1q (final concentration 25 μg/ml) was not significantly different from that released by EC + HA-IgG 1000 μg/ml + platelets, whereas amounts released by HA-IgG 1000 μg/ml preincubated with C1q (final concentrations 50, 100, and 200 μg/ml) decreased significantly to about 60% of that released by EC + HA-IgG 1000 μg/ml + platelets. The amount of 6-keto-$PGF_{1\alpha}$ released from EC and platelets by HA-IgG 1000 μg/ml preincubated with C1q was not significantly different from that released by EC + HA-IgG 1000 μg/ml + platelets at all concentrations of C1q. The amounts of TXB_2 and 6-keto-$PGF_{1\alpha}$ released from EC preincubated with C1q and platelets in the absence of IgG were not significantly different from those released by EC + platelets at all concentrations of C1q (data not shown in Figure 4).

Effect of DP-1904 on TXA_2 and PGI_2 release from EC and platelets by HA-IgG (Figure 5). After incubation of EC monolayer with HA-IgG 1000 μg/ml or medium alone, 1 ml of platelet suspension was added to each well with or without DP-1904 (various concentrations), and then EC were incubated 2 h at 37°C. The following procedure was done in the same assays for TXA_2 and PGI_2 release from the EC and platelet coculture system. DP-1904 (final concentration 10^{-3}~10^{-6} M) strikingly suppressed the amount of TXA_2 produced by EC + HA-IgG 1000 μg/ml + platelets. The amounts of TXB_2 produced by this combination with DP-1904 (final concentration 10^{-3}~10^{-6} M) were almost the same as those produced by EC + platelets in the absence of IgG with DP-1904 (final concentration 10^{-3}~10^{-7} M) and without DP-1904. By contrast, DP-1904 (final concentration 10^{-4}~10^{-7} M) significantly increased the amounts of 6-keto-$PGF_{1\alpha}$ produced by EC + HA-IgG 1000 μg/ml + platelets compared to this combination without DP-1904. DP-1904 (final concentration 10^{-3}~10^{-7} M) did not significantly change the amounts of 6-keto-$PGF_{1\alpha}$ produced by EC + platelets in the absence of IgG. The ratio of TXB_2/6-keto $PGF_{1\alpha}$ by EC + HAG 1000 μg/ml + platelets at all concentrations of DP-1904 decreased strikingly compared to this combination without DP-1904, but those by EC + platelets in the absence of IgG changed little (data not shown in Figure 5).

DISCUSSION

No *in vitro* study has been reported that analyzed prostanoid metabolism changes using 3 components such as EC, immune complexes, and platelets. We attempted to analyze this prostanoid metabolism change *in vitro*. Although the platelet count used in this study was near that *in vivo*, EC derived from human umbilical veins and HA-IgG were used instead of glomerular capillary EC and immune complexes,

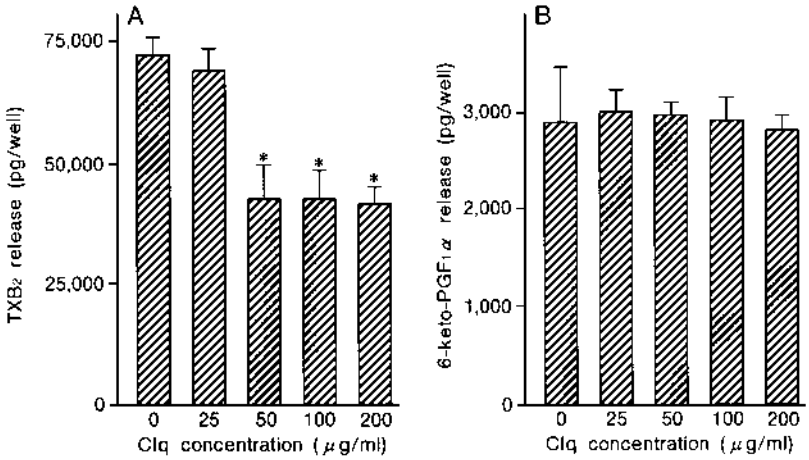

Figure 4. The amounts of TXA_2 (A) and PGI_2 (B) released from EC and platelets by HA-IgG 1000 μg/ml preincubated with C1q. Before the addition of HA-IgG to EC monolayers, HA-IgG were incubated with various concentrations of C1q. The following procedure was done in the same assay for TXA_2 and PGI_2 release from the EC and platelet coculture system. EC were incubated with platelet suspensions for 2 h at 37°C. Illustrated here are the amounts of TXB_2 and 6-keto-$PGF_{1\alpha}$ released from EC and platelets by HA-IgG without preincubation with C1q (EC + HA-IgG + platelets), shown as 0, and HA-IgG preincubated with various concentrations of C1q (final concentration 25, l50, 100, 200 μg/ml). *$p < 0.01$ vs EC + HA-IgG + platelets.

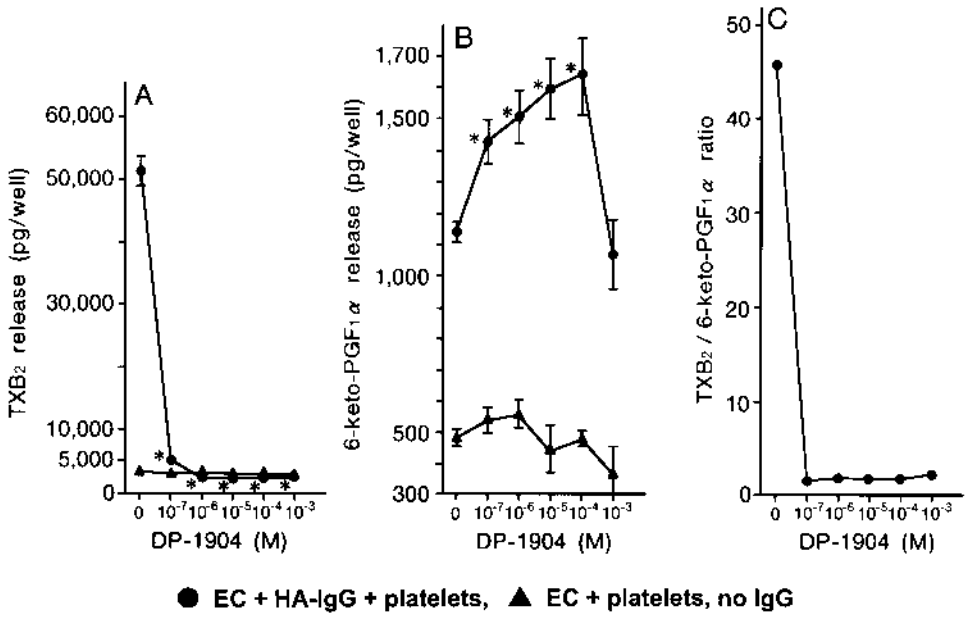

Figure 5. Effects of DP-1904 on TXA_2 and PGI_2 release from the EC and platelet coculture system. After incubation of EC monolayers with HA-IgG 1000 μg/ml or medium alone, platelet suspensions were added to each well with or without various concentrations of DP-1904 (final concentrations 10^{-3}~10^{-7} M), and then EC were incubated with platelet suspensions for 2 h at 37°C. The other procedure was done in the same assay for TXA_2 and PGI_2 release from the EC and platelet coculture system. Illustrated here are the amounts of TXB_2 (A) and 6-keto-$PGF_{1\alpha}$ (B) released by EC + HA-IgG + platelets and EC + platelets in the absence of IgG, and the TXB_2/6-keto-$PGF_{1\alpha}$ ratio (C) by EC + HA-IgG + platelets. *$p < 0.01$ vs EC + HA-IgG + platelets without DP-1904.

respectively. Monocytes/macrophages that infiltrate the glomeruli can be activated to synthesize cyclooxygenase-2 (COX-2) and TXA_2 and may contribute to the development of lupus nephritis[18]. We did not use monocytes/macrophages, thus these *in vitro* results may not entirely reflect the abnormal prostanoid metabolism that occurs within glomerular capillaries in patients with lupus nephritis.

Cines, *et al* reported that not only HA-IgG but also monomeric IgG was bound to EC monolayers, but more than 10-fold more protein was required for binding monomeric IgG than for HA-IgG[15]. Although it is controversial whether FcγR are present on the EC surface, the study by Cines, *et al*[15] and our study strongly suggest the presence of FcγR such as FcγRI to which not only immune complexes but also monomeric IgG can bind via Fc portions on the EC surface. The possibility of the formation of *in situ* immune complexes within the glomerular capillary may not require the presence of FcγR on the EC surface.

The amounts of TXB_2 and 6-keto-$PGF_{1\alpha}$ produced by EC + monomeric IgG + platelets were not significantly different from those produced by EC + platelets at all concentrations of monomeric IgG. Since platelets are not able to bind to monomeric IgG of which Fc portions bound to FcγRI on the EC surface and are not exposed, platelets may not be activated. The combination of EC + HA-IgG + platelets produced a large amount of TXA_2 rapidly. Platelets may bind to exposed Fc portions of HA-IgG on the EC surface via platelet FcγRII, and such cross-linking of platelet FcγRII recognizing the Fc portions of immune complexes may induce platelet activation. The activated platelets may produce COX mediated PGH_2 and convert PGH_2 to TXA_2 much more than inactivated or rested platelets do. Bustos, *et al* showed that porcine aortic EC stimulated with xenoreactive antibodies and complement released TXA_2 and PGE_2 in addition to increased amounts of PGI_2 by inducing COX-2 and thromboxane synthetase, but TXB_2 could not be detected 8 h after stimulation and the amount of TXB_2 released was very small[19]. In our study the combination HA-IgG + EC without platelets did not release TXB_2 by 6 h and at 12 h only small amounts of TXB_2 were detected. These results suggest that TXB_2 measured in an EC and platelet coculture system are derived from platelets and EC do not contribute to production of TXA_2. At the same time the production of PGI_2 from EC was also increased. EC adjacent to platelets have been shown to be capable of utilizing platelet derived PGH_2 for PGI_2 production very efficiently[20]. Our study also suggests that EC adjacent to platelets produce PGI_2 by utilizing PGH_2 produced by platelets stimulated with HA-IgG. But the amount of PGI_2 production was much smaller than that of TXA_2. TXA_2 reduces the glomerular filtration rate by causing mesangial contraction and aggregation of platelets. PGI_2 has opposing effects. TXA_2 may act preferentially within the glomerular capillary

in patients with lupus nephritis as compared with PGI_2. Platelet aggregation may result in thrombosis within the glomerular capillary. A potent and continuous mesangial contraction and thrombosis within the glomerular capillary may cause glomerular sclerosis. Since thrombosis and later sclerosis have been observed pathologically in glomerular capillaries in patients with lupus nephritis, especially the diffuse proliferative form[8,9], this large imbalance between TXA_2 and PGI_2 may be associated with alterations in glomerular pathological findings and the hemodynamics of lupus nephritis. Yoshida, *et al* reported that patients with lupus nephritis had significantly higher urinary excretion of TXB_2 and a higher ratio of TXB_2/6-keto $PGF_{1\alpha}$ in the urine than patients without nephritis and healthy controls[2,3]. They suggested that TXA_2 was derived from activated platelets in the glomerular capillary[2,3]. The urinary findings reported by Yoshida, *et al*[2,3] support the possibility that the abnormal prostanoid metabolism changes that we observed may arise within glomerular capillaries.

C1q has 2 functional domains, globular domains that bind Fc portions of immune complexes and collagen-like domains capable of binding to cell surface receptors (cC1qR) on platelets and EC[21,22]. C1q normally circulates in a complex with C1r2 and C1s2, forming the first component of complement, C1[23], and thus the collagen-like domains of C1q are not exposed and C1q is not able to bind to cC1qR[24]. Upon activation of C1 by circulating immune complexes, C1r2 and C1s2 are disassembled by C1 inactivator[25], leaving immune complex bound C1q with its collagen-like domains exposed and potentially available for binding to cC1qR[26]. Since immune complexes may activate the classical complement pathway, leaving C1q bound immune complexes within the glomerular capillary in lupus nephritis, we utilized C1q to analyze the effect of complement components on prostanoid metabolism changes by immune complexes. In our study, preincubation of HA-IgG with C1q significantly suppressed the production of TXA_2 from an EC and platelet coculture system. The suppression of TXA_2 production by C1q may partially reduce platelet aggregation and vasoconstriction. Peerschke and Ghebrehiwet showed that C1q augmented platelet aggregation by aggregated IgG and suggested that this augmentation was due to a cooperative interaction between platelet FcγRII and cC1qR[27]. On the other hand, Sloand, *et al* showed that C1q inhibited platelet aggregation by aggregated IgG, and suggested 3 possible mechanisms of inhibition as follows[28]. (1) There is a negative signal to platelets by simultaneous stimulation of both FcγRII and cC1qR. (2) C1q bound immune complexes may bind exclusively to platelet cC1qR rather than to FcγRII. (3) C1q bound immune complexes may bind to platelets by both FcγRII and cC1qR but cross-linking of adjacent FcγRII required to induce platelet aggregation is adversely affected by C1q mediated alteration in Fc-FcγRII geometry. The mechanism of partial suppression of TXA_2 production by C1q in the EC and platelet coculture system in this study is unclear, but may be in accord with the possibilities suggested by Sloand, *et al*[28]. But the preincubation of HA-IgG with C1q had very little effect on the production of PGI_2 in the EC and platelet coculture system. C1q may partially suppress the production of COX mediated PGH_2 by HA-IgG, and EC adjacent to platelets may be able to utilize less PGH_2 for PGI_2 production than without the pretreatment of C1q, resulting in no increase of PGI_2. Complement components activated by immune complexes generally increase the inflammatory condition, but our study suggests the possibility that C1q may have protective action to suppress the inflammatory condition as in lupus nephritis.

A TXA_2 synthetase inhibitor, DP-1904, has been reported to inhibit the conversion of PGH_2 to TXA_2 by platelet thromboxane synthetase[29]. After an oral dose of 200 mg of DP-1904 was administered to fasting healthy Japanese men, a mean maximum DP-1904 concentration in plasma of 4.66 $\pm$ 0.58 μg/ml (1.54×10^{-5} M equivalent) was reached within 1 h[29]. The DP-1904 concentration of 10^{-5} M used in our study reflects the *in vivo* condition. The addition of DP-1904 strongly suppressed TXA_2 release from EC and platelets by HA-IgG as a direct action, and at the same time the amount of PGI_2 release was significantly increased. DP-1904 may indirectly influence vascular PGI_2 production by the following mechanism: platelets activated by HA-IgG produce a large amount of PGH_2, but DP-1904 inhibits the complete conversion of PGH_2 to TXA_2 by platelet thromboxane synthetase. EC adjacent to platelets produce PGI_2 by utilizing a part of this PGH_2. Yoshida, *et al* reported that administration of DP-1904 improved urinary TXB_2 concentrations and the urinary ratio of TXB_2/6-keto $PGF_{1\alpha}$ in patients with lupus nephritis[2]. The improved urinary findings[2] support the possibility that both the direct and indirect actions of DP-1904 we observed may occur within the glomerular capillary, and improve alterations in glomerular pathological findings and the hemodynamics of lupus nephritis.

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