Platelet Modulation of Polymorphonuclear Leukocyte Shear Induced Aggregation

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A cone and plate viscometer and Coulter Counter were used to study platelet modulation of polymorphonuclear leukocyte (PMNL) aggregation caused by controlled shear stress. As an index of aggregation, the large-particle percentage (LPP) was calculated. This represents the ratio of aggregated cell count to total cell count. PMNL suspensions in buffer (1.0 x 10^5 cells per milliliter, final concentration) did not show any aggregate formation at shear stresses below 150 dynes/cm^2 for one minute exposure time (LPP < 3%). However, there was PMNL aggregation in mixed PMNL and platelet-rich plasma suspensions in this shear stress range. Supernatant plasma from sheared platelets initiated PMNL aggregation at moderate shear stress (150 dynes/cm^2 for one minute; LPP, 20.3% ± 2.5%). In contrast, platelet release factors, such as adenosine diphosphate (2 μmol/L) and serotonin (2 μmol/L) did not cause PMNL aggregation (LPP, 2.5% ± 1.2% and 3.3% ± 0.8%, respectively). The use of a cyclooxygenase inhibitor (acetylsalicylic acid, 50 μmol/L) did not suppress the aggregation of PMNLs after shear (LPP, 20.1% ± 2.4%). However, preincubation with nordihydroguaiaretic acid (10 μmol/L), an inhibitor of C-5 and C-12 lipoygenase, and 6,9-deepoxy-6,9-(phenylimino)-6,8-prostaglandin I_2 (U-60257, 10 μmol/L), an inhibitor of C-5 lipoygenase in human leukocytes, suppressed this aggregation (LPP, 9.1% ± 2.5% and 10.4% ± 3.2%, respectively). Also, the formation of lipoygenase products (5-HETE, 12-HETE, 15-HETE, and LTB4) activated by shear stress was documented by reversed phase–high-performance liquid chromatography (RP-HPLC). These data support the possibility of a cooperation between platelets and leukocytes in shear-induced PMNL aggregation that is dependent on C-12 or C-5 lipoygenase activity, or both.

Fluid shearing is known to produce cell interactions and, in whole blood, can lead to platelet–leukocyte aggregation. However, no studies have been reported of shear-induced PMNL aggregation under controlled fluid mechanical conditions. Improved designs for artificial organs and prostheses require more detailed knowledge of thromboembolic phenomena produced by mechanical trauma and biomaterial surface exposure. Shear stresses of greater than 150 dynes/cm^2 can be generated during blood flow through hemodialysis devices, artificial heart valves, and blood oxygenators. Unfortunately, the complex and variable shear stress histories that cells experience in cardiopulmonary bypass and other circulatory support systems are hard to quantitate. Therefore, to generate an understanding of the role of leukocytes in embolic phenomena initially requires performing experiments in which the fluid forces are well known. In vitro studies of shear stress and surface interaction effects have normally used simpler flows, including stagnation point flow, flow through tubes, and flow in rotational viscometers. In this study, a rotational cone and plate viscometer was used for the purpose of applying a known uniform shear stress to the entire sample volume.

PMNLs may participate along with platelets in hemostasis and thrombosis. In platelets, the cyclooxygenase pathway of arachidonic acid metabolism has been implicated in these processes. Platelets also have a pathway involving lipoygenase action on arachidonic acid, which forms (12S)-12-hydroperoxy-5,8,10,14-eicosatetraenoic acid (12-HPETE) and, subsequently, the corresponding (12S)-12-hydroxy acid (12-HETE). The main pathway of arachidonic acid metabolism in neutrophils involves 5-lipoxygenation, leading to the formation of eicosanoids such as leukotrienes (LTs) and other hydroxy acids. The discovery of LTs, a new family of bioactive metabolites formed in a lipoygenase-type reaction (involving the lipoxygenase C-5 enzyme), and the detection of a C-15 lipoygenase in leukocytes have indicated the existence of several lipoygenase enzymes in blood cells. Arachidonic acid does not normally exist in free form but is usually esterified in
triglycerides and phospholipids. Stimulated phospholipases can release arachidonic acid, which is then enzymatically metabolized by the 5-lipoxygenase pathway to form LTs as well as a host of other bioactive metabolites.\textsuperscript{16-23} In addition to differences in positional specificities, the heterogeneity of these enzymes is further emphasized by different reactivities. For instance, it has been shown that, in contrast to platelet suspensions, which can efficiently transform exogenous arachidonic acid into 12-HETE and cyclo-oxygenase products, the addition of the fatty acid to preparations of human PMNLs was followed by only minor transformation of the substrate.\textsuperscript{4,13} However, the metabolism of arachidonic acid was strongly enhanced when leukocytes were incubated in the presence of arachidonic acid and divalent cation ionophore A23187, indicating clearly that the human PMNL C-5 lipoxygenase required activation for 5-HETE and LT synthesis. Maclouf et al\textsuperscript{10} have shown that platelet-derived 12-HPETE can stimulate this C-5 lipoxygenase. Recent studies suggest the involvement of LTs in hypersensitivity reactions and inflammation and point out the importance of a better understanding of the biochemical process involved in the control of the biosynthesis of these bioactive substances.\textsuperscript{13,19-23}

To begin to understand the biochemical control mechanisms of shear-induced PMNL aggregation, we have used inhibitors of cyclo-oxidase or lipoxygenase (acetylsalicylic acid [ASA], nordihydroguaiaretic acid [NDGA], U-60257), synthetic chemotactic tripeptide formyl-methionyl-leucyl-phenylalanine (FMLP), and calcium ionophore A23187. The effect of these factors in PMNL aggregation in the presence or absence of platelets and shear-activated platelet-released products suggests the occurrence of significant biochemical interactions between platelets and leukocytes in mechanically induced activation processes.

MATERIALS AND METHODS

Preparation of human PMNLs. Venous blood from normal healthy donors was drawn by venipuncture into heparin (10 U/mL, final concentration) in conical plastic tubes. PMNLs were purified using standard techniques of dextran sedimentation, centrifugation on Ficoll-Hypaque, and hypotonic lysis of erythrocytes. Final cell suspensions contained a minimum of 95% PMNLs. Viability of the isolated PMNLs was always greater than 95% as determined by trypan blue exclusion. PMNLs were labeled with 5.5 \mu mol/L of 1-\textsuperscript{13}C arachidonate added during the first wash.

Preparation of platelet-rich plasma (PRP). Blood was obtained by venipuncture of healthy volunteers. Ten milliliters of blood was quickly and gently mixed with 1.1 mL of heparin (100 U/mL) in siliconized glass tubes. The heparinized blood was centrifuged for ten minutes at 150 g. The supernatant PRP was drawn off with a Pasteur pipette and transferred to a clean siliconized glass or plastic test tube. The remaining fractions were centrifuged for five minutes at 150 g to retrieve more PRP. Finally, the red blood cell fraction was centrifuged for ten minutes at 1,000 g. The resultant supernatant was platelet-poor plasma (PPP) and was transferred to a separate test tube. Platelet counts in all PRP samples were adjusted to 3 \times 10\textsuperscript{12} cells per milliliter in PPP.

Reagents and buffers. 1-\textsuperscript{13}C Arachidonate (52.0 mCi/mmol, specific activity), \textsuperscript{3}H 6-keto PGE\textsubscript{1}, \textsuperscript{3}H thromboxane B\textsubscript{2}, \textsuperscript{3}H PGE\textsubscript{2}, \textsuperscript{3}H PGE\textsubscript{3}, and \textsuperscript{3}H PGD\textsubscript{2} were all purchased from New England Nuclear (Boston). 5-HETE radioimmunoassay kit was purchased from SeraGen (Boston). A23187, adenosine diphosphate (ADP), ASA, NDGA, FMLP, cytochalasin B, and colchicine were purchased from Sigma Chemical Co (St Louis). A23187, NDGA, FMLP, and colchicine were dissolved in dimethylsulfoxide (DMSO). Cytochalasin B and ASA were prepared in ethanol. The 6,9-deoxy-6,9-phenylimino-6,8-prostaglandin (U-60257) was kindly provided by Dr M.K. Bach of the Upjohn Company and prepared in tris(hydroxyethyl)aminomethane (THAM). The small amount of ethanol and DMSO (final concentration of 0.1%) used as vehicles did not alter cell viability. The buffer was Dulbecco's phosphate-buffered saline (PBS) containing (mmol/L): NaCl (137), KCl (2.7), Na\textsubscript{2}HPO\textsubscript{4} \cdot 7H\textsubscript{2}O (8.1), KH\textsubscript{2}PO\textsubscript{4} (1.9), CaCl\textsubscript{2} (0.9), and MgCl\textsubscript{2} \cdot 6H\textsubscript{2}O (0.5).

Aggregation assay. The isolated PMNLs were suspended (1 \times 10\textsuperscript{12} cells per milliliter) in PBS and incubated according to the various procedures described under Results. After one minute of shearing at 150 dynes/cm\textsuperscript{2}, 20-\muL samples were taken from the suspensions. The samples were immediately diluted in 10 mL of Isoton solution (Coulter Electronics, Hialeah, Fla) and analyzed in the Coulter Counter system. The Coulter Counter was a Model ZBI and was connected to Coulter Channelizer and microcomputer. For each sample, the particle concentration was enumerated with a lower channel of 150 \mu m\textsuperscript{3} (called the total “T” particle concentration) and with a lower channel of 540 \mu m\textsuperscript{3} (called the aggregated or large “A” particle concentration).\textsuperscript{12} These names are given because with the lower channel of 150 \mu m\textsuperscript{3}, all PMNLs in a sample were counted, whereas with a lower channel of 540 \mu m\textsuperscript{3}, only particles larger than 1.8 times the size of an average unaggregated PMNL were enumerated. Results for a given sample of the PMNL suspension were reported as the large-particle percentage (LPP = A/T \times 100) or the maximum large-particle percentage. Sheared samples (and others incubated with various chemicals) were analyzed at ten-minute intervals to follow the aggregation process. The maximum large-particle percentage was the peak value of LPP obtained over the 60-minute aggregation interval.

Shear stress application. Leukocyte suspensions and PRP were subjected to shear stress in a specially modified Ferranti-Shirley Viscometer.\textsuperscript{4} The plates used gave a cone and plate geometry. Thus the entire sample experienced the same constant shear stress. After shearing PRP for one minute at 150 dynes/cm\textsuperscript{2}, the sample was removed and rapidly centrifuged to produce a platelet-free supernatant. This supernatant plasma was added to PMNL suspensions within 30 seconds.

High-performance liquid chromatography (HPLC) analysis of i.eosanoids. In preparation for reverse phase-HPLC (RP-HPLC), cell suspensions were extracted by using Sep-PAK C18 cartridges.
and 90% methanol eluates were evaporated under nitrogen. Residues were dissolved in 100 μL of methanol, and 10 μL was injected onto the HPLC column. RP-HPLC was carried out on a 4-mm ID μ-PAK C18 (5 μm) column (Varian Assoc Instrument, Palo Alto, Calif) with water–methanol–acetronitrile as the eluting solvent. Table 1 gives the mixing ratio and flow rate changes as functions of elution time. Fractions were collected, and the radiolabel content of each was determined in Hydrofluor by liquid scintillation counting. Identification of the peaks was done by comparison with standards. Under these conditions, stable cyclo-oxygenase products 6-keto prostaglandin F1α (6-keto PGF1α), thromboxane B2 (TXB2), PGF2α, PGE2, PGD2, and PGF1α were eluted at four to ten minutes; LTβ2 at 20 to 22 minutes; mono-HETEs at 40 to 52 minutes, and unreacted arachidonate at 55 to 60 minutes.

RESULTS

Chemotactic factor and ionophore-induced PMNL aggregation. FMLP, as previously described by others,2,3 aggregated human PMNLs. This effect was reflected in an increase in the percentage of large particles formed after adding 1 μmol/L FMLP to the suspension of PMNLs (Fig 1). Cytochalasin B, by itself, did not aggregate the cells but did significantly enhance FMLP-induced aggregation (Fig 1). The calcium ionophore A23187 (10 μmol/L) aggregated PMNLs (Fig 1), and the time course of aggregation was similar to that of shear-induced PMNL aggregation in the presence of supernatant from sheared PRP (see below). The kinetics of FMLP-induced aggregation reached nearly a maximum within two to three minutes. However, the time course of ionophore A23187- and shear-induced aggregation was much slower, and a maximum value was not attained for over 30 minutes.

Shear-induced PMNL aggregation. PMNL suspensions in PBS (1 x 10^7 cells per milliliter, final concentration) did not show any aggregate formation at shear stresses below 150 dynes/cm² for one minute (Figs 2 and 3). However, when PMNLs were incubated in the presence of PRP, a strong stimulation of PMNL aggregation occurred in this shear stress range. PMNLs incubated with supernatant plasma from sheared platelets (1:1 by volume) would initiate

Fig 1. The kinetics of the chemotactic factor and ionophore-induced PMNL aggregation. PMNLs (1 x 10^6 cells per milliliter) were incubated with 1 μmol/L FMLP (A) and 10 μmol/L A23187 (B) for the specific time periods. PMNLs were preincubated with cytochalasin B (5 μg/mL) for five minutes and then incubated with 1 μmol/L FMLP (Φ).

Fig 2. The kinetics of the shear-induced PMNL aggregation. PMNLs (A). PMNLs mixed with platelet-free plasma (PFP) (B). PMNLs mixed with supernatant plasma of sheared platelets (2.5 to 4.0 x 10^6 cells per milliliter) (C), and the last sample preincubated with 10 μmol/L NDGA (D) were sheared at 150 dynes/cm² for one minute. After shearing, samples were analyzed at ten-minute intervals for one hour to follow the aggregation process.

PMNL aggregation after exposure to moderate shear stress (150 dynes/cm² for one minute, Figs 2 and 3). The effect of plasma protein on the shear-induced PMNL aggregation was examined by using the PMNL suspensions mixed with platelet-free plasma (PFP) (1:1 by volume, Figs 2 and 3). Platelet release factors, 2 μmol/L ADP or 2 μmol/L serotonin, did not induce PMNL aggregation at this shear stress (Fig 3).

To define the nature of the factor(s) responsible for the effect of shear-induced platelet supernatants on leukocyte aggregation activity, experiments with inhibitors were performed. The inhibition of the cyclo-oxygenase pathway by 50 μmol/L ASA did not suppress the aggregation of PMNLs after shear (Fig 4). However, preincubation with NDGA (10 μmol/L), an inhibitor of C-5 and C-12 lipoxygenase, and U-60257 (10 μmol/L), an inhibitor of C-5 lipoxygenase in human leukocytes, suppressed the aggregation of PMNLs (Figs 2 and 4).
Fig 4. Effect of cyclo-oxygenase and lipoxygenase-inhibitors on PMNL shear-induced aggregation. PMNLs were mixed with supernatant plasma of sheared platelets and incubated with no inhibitors (A), ASA (50 μmol/L) (B), NDGA (10 μmol/L) (C), U-60257 (10 μmol/L) (D). In (E), PRP was incubated with imipramine (1 μmol/L) and sheared, and subsequently, the PMNLs were mixed with this supernatant. Sheared samples (150 dynes/cm² for one minute, closed bar) were compared with control samples (open bar). Data represent the mean ± SEM for three to nine separate experiments.

4). Supernatant of sheared PRP, which had been preincubated with NDGA (10 μmol/L), also suppressed subsequent shear-induced PMNL aggregation (data not shown).

PRP incubated with imipramine (1 μmol/L), an inhibitor of serotonin uptake, for 15 minutes was sheared and centrifuged. In this case, serotonin that was released from sheared PRP was in the supernatant. PMNLs incubated with this supernatant did not show any change in the shear-induced PMNL aggregation (data not shown). PRP incubated with 10 μmol/L NDGA did not induce aggregation, with or without shear stress. Fig 5 shows the effect of shear stress magnitude on PMNL aggregation for three different samples.

Effect of platelet-PMNL ratios. When PMNL and PRP (1:1 by volume) samples were sheared at 150 dynes/cm² for one minute, there were differences in the aggregation profiles dependent on the ratio of platelets to PMNLs (Fig 6). When the ratio was approximately 30, the aggregation response of PMNL was maximum. At this ratio, the total volume of platelets was nearly equivalent to that of PMNLs. Also, this ratio is nearly the same as that found physiologically.

HPLC experiments. Studies of platelet or PMNL arachidonate metabolites, identified by HPLC, were used to examine the details of arachidonic acid metabolism under shear stress. Results of a group of experiments analyzed by RP-HPLC are shown in Figs 7 through 10.

PMNL suspensions mixed with 5.5 μmol/L 1-14C arachidonic acid and supernatant of sheared PRP generated a significantly increased quantity of LTB₄ after shearing, which appeared as a distinct peak (retention time, 21 minutes), as shown in Fig 7. When PMNL suspensions were preincubated with 10 μmol/L NDGA, there was no significant increase of LTB₄ formation after shearing (Fig 8). Only 12-HETE formation was increased when PRP and 5,5

Fig 5. Effect of shear stress magnitude on the maximum LPP. Three shear stresses (50, 100, and 150 dynes/cm²) were used to shear PMNLs in buffer (A), PMNLs incubated with 2 μmol/L ADP (B), and PMNLs mixed with supernatant plasma of sheared platelets (C).

Fig 6. Effect of platelet concentration on shear-induced PMNL aggregation. Different platelet concentrations in PRP were sheared and centrifuged. This supernatant plasma was added to PMNL suspensions (1 × 10⁶ cells per milliliter) and, subsequently, sheared at 150 dynes/cm² for one minute.

Fig 7. RP-HPLC chromatogram of the metabolites of arachidonic acid formed in human PMNLs incubated with 5.5 μmol/L 1-14C arachidonic acid in the presence of supernatant of sheared PRP. Control (◊) and after shearing at 150 dynes/cm² for one minute (△).
μmol/L 1-14C arachidonic acid were exposed to shear stress (Fig 9). That this metabolite was 12-HETE, not 5-HETE, was confirmed by using thin-layer chromatography (data not shown). Little cyclo-oxygenase products were detected after shear stress stimulation of platelets. The amount of TXB2 formation after shear stress stimulation of platelets measured by radioimmunoassay was ≤10 ng/10^8 platelets. The counts per minute (cpm) range corresponding to this concentration was nearly the same as the background cpm.

The amount of shear-induced 5-lipoxygenase products (LTB4, 5-HETE) in sheared PMNL suspensions alone was much less than when supernatant of sheared PRP was present (data not shown). In fact, LTB4 production under these conditions was negligible. Shear-induced 5-HETE production in the PMNL suspensions has also been quantitated using radioimmunoassay with labeled antibody purchased from Seragen. The formation of 5-HETE increased proportionally with the shear stress increase (data not shown). PMNLs and 5.5 μmol/L 1-14C arachidonic acid were incubated with 10 μmol/L ionophore A23187 in the presence of sheared PRP supernatant. In this case, production of LTB4 by PMNLs after shearing increased even more dramatically. It is noteworthy that 5-HETE production was increased and 15-HETE formation was decreased (Fig 10).

**DISCUSSION**

In this paper, we have shown that a substance(s) derived from platelet activation in a shear field may activate the C-5 lipoxygenase of human blood PMNLs in vitro. However, there is an activation (shearing) requirement for PMNL aggregation even though the cells have been incubated with supernatant plasma of sheared PRP (Fig 3B). PMNLs incubated with plasma alone show some shear-induced aggregation (Fig 3C). This may be due to the plasma protein binding and, to a lesser extent, viscosity changes. The difference between B and C in Fig 3 indicates that a substance(s) derived from shear activated platelets plays an active role in this PMNL aggregation.

Sheared platelets release ADP and serotonin, which are potent platelet-aggregating agents. However, 2 μmol/L ADP and 2 μmol/L serotonin do not cause PMNL aggregation (Fig 3D and E, respectively). Redl et al.26 recently demonstrated that platelets augment granulocyte aggregation by a serotonin and thromboxane A2-independent mechanism, and it was not necessary for platelets to be intact for them to augment granulocyte aggregation. Indeed, platelet lysates and the supernatant fluids therefrom proved equally efficacious. NDGA (10 μmol/L) and U-60257 (10 μmol/L) totally suppressed the shear-induced PMNL aggregation to that level seen in a mixture of PMNL and PFP (Fig 4C and D, respectively). It is hypothesized that the platelet-derived PMNL activating substance may be 12-HPETE. Shear-induced aggregation of platelets produces the release of arachidonic acid from cellular phospholipids and the subsequent metabolism of the arachidonic acid by cyclooxygenase and lipoxygenase systems. It is interesting that in
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shear activation, a substantial portion of the free arachidonic acid is transformed by a lipoxygenase to 12-HPETE and, subsequently, 12-HETE (Fig 9). This 12-HPETE may activate the C-5 lipoxygenase in PMNLs and therefore lead to increased release of C-5 lipoxygenase products, such as 5-HPETE and LTs. Figure 11 shows a hypothetical scheme of the platelet–PMNL interactions that may lead to increased release of leukocyte C-5 lipoxygenase products.

It has been shown that the C-15 lipoxygenase also interacts with the C-5 lipoxygenase to produce a (5S, 15S)-5,15-dihydroxy-6,8,11,13-eicosatetraenoic acid (5S, 15S diHETE).\textsuperscript{10} The formation of the 5S, 12S diHETE and of the 5S, 15S diHETE reflects the interactions between lipoxygenase enzymes in different blood cells. Marcus et al\textsuperscript{27} have shown a new metabolite of arachiconid acid, formed during interaction between thrombin- or collagen-stimulated platelets and unstimulated neutrophils. That was confirmed to be 12S, 20-dihydroxyicosatetraenoic acid (12S, 20 diHETE). Their experiments showed that platelet stimuli known to occur in vivo might initiate metabolic interactions between different cell types by way of the arachidonic acid pathway. Leukotriene B\textsubscript{4} (LTB\textsubscript{4}) is a potent chemotactic factor and induces PMNL aggregation. The stimulation of LT synthesis in human blood leukocytes by 12-HPETE has been observed.\textsuperscript{10} Plasma protein binding in our system may stabilize 12-HPETE, prolonging its activity over that seen in buffers alone. LT biosynthesis requires enzymes that are distributed among different subcellular compartments, and it appears that their biosynthesis involves movement of intermediates between these compartments.\textsuperscript{28} In the case of LTB\textsubscript{4} formation, the action of a soluble cytosolic hydrolase has been proposed to be the rate-limiting step.\textsuperscript{17} Consequently, intermediate substrates such as 5-HPETE or LTA\textsubscript{4} must accumulate, decay by way of alternate enzymatic and nonenzymatic processes, or be released from cells, such as PMNLs. In the latter instance, a plasma enzyme capable of metabolizing extracellular LTA\textsubscript{4} to LTB\textsubscript{4} seems reasonable. In fact, recent studies suggest that an extracellular enzymatic transformation of LTA\textsubscript{4} into the potent chemotaxin LTB\textsubscript{4} may be involved.\textsuperscript{29,30} Plasma enzymatic activity responsible for LTB\textsubscript{4} production may play a general role in modulating the inflammatory response.

The 5-lipoxygenase is present in PMNL in an inactive form and must be activated to catalyze the dioxygenation of arachidonic acid. Evidence that the lipoxygenase pathway might be involved in PMNL activation comes from the studies showing that rabbit exudate PMNLs are stimulated directly by exogenous arachidonic acid to release HETEs and LTB\textsubscript{4}\textsuperscript{4,14} and to aggregate.\textsuperscript{31} In a recent study, it was shown that human PMNLs, isolated from normal individuals, are stimulated when given a combination of arachidonic acid and ethanol, which apparently activates the 5-lipoxygenase pathway for PMNLs. However, the addition of arachidonic acid alone to human blood PMNL suspensions does not induce significant synthesis of 5-lipoxygenase products.\textsuperscript{4}

The divalent cation ionophore A23187 is known to be a potent activator of the synthesis of LTs. Its action probably involves the stimulation of Ca\textsuperscript{2+} flux and the subsequent activation of the Ca\textsuperscript{2+}-dependent 5-lipoxygenase and phospholipases. It thus appears that the release of arachidonic acid (after phospholipase stimulation) and the activation of the 5-lipoxygenase are prerequisites for the synthesis of 5-lipoxygenase products in human blood PMNLs. One possible mode of action of stress-induced mechanical activation is a membrane mechanical stretching induced increased calcium flux, leading to increased intracellular enzymatic activity. Also, Volpi et al\textsuperscript{32} have shown that the addition of arachidonic acid to neutrophils will cause a rapid and significant increase in the permeability of the plasma membrane to calcium. This hypothesis is supported by the similarity of A23187-induced (Fig 1) and shear-induced (Fig 2) PMNL aggregation time course curves.

Quantitative confirmation of lipoxygenase involvement in the shear-induced aggregation process was obtained using RP-HPLC. The formation of LTB\textsubscript{4} was greatly increased after shearing PMNL in the presence of supernatant plasma from sheared platelets (Figs 7 and 10). When the leukocytes were stimulated both by ionophore A23187 and shearing, LTB\textsubscript{4} and 5-HETE production increased dramatically. However, preincubation of 10 μmol/L NDGA with PMNL suspensions totally suppressed the increment of LTB\textsubscript{4} formation after shearing (Fig 8). This again supports the hypothesis that these 5-lipoxygenase products induce PMNL aggregation.

Small amounts of diHETEs were detectable when PMNL suspensions were sheared in PFP or in buffers. Sheared platelets exhibited greatly increased synthesis of 12-HETE (Fig 9) with surprisingly little thromboxane B\textsubscript{2} production. This indicates that shear stress stimulation of platelets may produce quite different arachidonic acid metabolites than that seen with direct chemical stimuli, such as thrombin or the ionophore A23187. This platelet-derived 12-HETE (12-HPETE) may be the mediator that stimulates the 5-lipoxygenase activity in human blood PMNLs during stress-induced aggregation. The fact that preincubation of PRP with a 12-lipoxygenase inhibitor (NDGA, 10 μmol/L) before shearing blocked the effect of sheared-platelet supernatant on PMNL aggregation strengthens our hypothesis.

The platelet-PMNL ratio appears to be important (Fig 6). When the ratio is approximately the same as found under physiological conditions, the aggregation response of the PMNL is maximum. It is possible that, in addition to the platelet-PMNL ratio, the cell concentration in the incubation medium is an important parameter. This has been observed in studies examining the possibility of platelet-
derived endoperoxides serving as substrates for endothelial cell prostacyclin synthase.\textsuperscript{33}

In summary, our studies suggest important roles for the platelet and PMNL C-12 and C-5 lipoxygenases in shear-induced PMNL aggregation. These data support the concept of platelet–PMNL interactions in shear-induced PMNL aggregation and add new data concerning the potential role of release products from one cell type in modulating the biological and mechanical response of another blood cell.

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