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Synergistic Effect of Type II Phospholipase A\textsubscript{2} and Platelet-Activating Factor on Mac-1 Surface Expression and Exocytosis of Gelatinase Granules in Human Neutrophils: Evidence for the 5-Lipoxygenase-Dependent Mechanism

Jun Takasaki,\textsuperscript{1} Yasushi Kawauchi, and Yasuhiko Masuho

Stimulation of human neutrophils with inflammatory mediators such as TNF-\textalpha or platelet-activating factor (PAF) induces translocation of adhesion molecule Mac-1 (CD11b/CD18) from secretory vesicles to the plasma membrane. Type II phospholipase A\textsubscript{2} (PLA\textsubscript{2}-II) also induces translocation of Mac-1 from secretory vesicles. However, there are more Mac-1 molecules in gelatinase granules and specific granules than in secretory vesicles. Therefore, different combinations of PLA\textsubscript{2}-II and other mediators were examined for their ability to induce gelatinase granules and specific granules to induce Mac-1 surface expression. The combination of PLA\textsubscript{2}-II and PAF synergistically increased Mac-1 surface expression, and the effect was greater than the combinations of PLA\textsubscript{2}-II with TNF-\textalpha, IL-8, or FMLP. Additionally, the combination of PLA\textsubscript{2}-II and PAF induced exocytosis of both secretory vesicles and gelatinase granules, which did not occur with either PLA\textsubscript{2}-II alone or PAF alone. The induction was accompanied by marked production of leukotriene B\textsubscript{4}, AA861, an inhibitor of 5-lipoxygenase, did not inhibit exocytosis of secretory vesicles but did inhibit exocytosis of gelatinase granules and decrease Mac-1 surface expression. It was also found that Ca\textsuperscript{2+} influx is essential for 5-lipoxygenase activation, because Ni\textsuperscript{2+}, which blocks the influx of extracellular Ca\textsuperscript{2+}, inhibited the production of leukotriene B\textsubscript{4}. These results suggest that stimulation by the combination of PLA\textsubscript{2}-II and PAF, unlike stimulation by each mediator alone, causes exocytosis of gelatinase granules via the 5-lipoxygenase pathway, resulting in a synergistic increase in neutrophil Mac-1 surface expression during inflammatory processes. The Journal of Immunology, 1998, 160: 5066–5072.

The production of eicosanoids, which are arachidonic acid metabolites derived from the lipoxygenase and cyclooxygenase pathways, is regulated by phospholipase A\textsubscript{2}. This enzyme catalyzes the hydrolysis of cell membrane phospholipids, resulting in formation of fatty acids such as arachidonic acid. The well-characterized phospholipase A\textsubscript{2} has been classified into three types, i.e., type I, type II, and cytosolic PLA\textsubscript{2} (cPLA\textsubscript{2}). Based on their primary structures and origins (1, 2), Type II phospholipase A\textsubscript{2} (PLA\textsubscript{2}-II) is detected in inflamed sites and in the plasma of patients with rheumatoid arthritis, septic shock, and pancreatitis (3–5). PLA\textsubscript{2}-II by itself induces an inflammatory reaction when injected into experimental animals (6, 7). Furthermore, PLA\textsubscript{2}-II is released into the extracellular space from platelets, mast cells, and endothelial cells (8–10). Therefore, PLA\textsubscript{2}-II plays an important role in inflammation.

The adhesion of circulating neutrophils to the vascular endothelium is an essential and early event in acute inflammatory reactions. It has been shown that interaction of \beta\textsubscript{2} integrins with intracellular adhesion molecules 1 and 2 on the endothelium promotes neutrophil adhesion (11). Mac-1, the most abundant \beta\textsubscript{2} integrin in neutrophils, resides on the membrane of intracelluar granules and vesicles. In resting neutrophils, 75% of Mac-1 is localized in gelatinase granules and specific granules, 20% in secretory vesicles, and 5% on the plasma membrane (12). Stimulation of human neutrophils with each of various inflammatory mediators such as TNF-\textalpha, PAF, FMLP, LTB\textsubscript{4}, IL-8, and C5a induces translocation of Mac-1 from the only secretory vesicles to the plasma membrane (12).

It was previously shown that stimulation of human neutrophils by human PLA\textsubscript{2}-II induces exocytosis of secretory vesicles to increase Mac-1 surface expression (13). However, neither PLA\textsubscript{2}-II nor any other inflammatory mediator alone induces translocation of Mac-1 from ether gelatinase granules or specific granules. Treatment of neutrophils with A23187, a calcium ionophore, induced the translocation of Mac-1 from not only secretory vesicles but also gelatinase granules and specific granules, thereby resulting in much greater Mac-1 expression than seen with any inflammatory mediator (13). Therefore, high Mac-1 expression, which accompanies exocytosis of gelatinase granules and specific granules, could be stimulated by a combination of different inflammatory mediators.

In this article, the combination of PLA\textsubscript{2}-II and PAF is shown to induce exocytosis of gelatinase granules as well as secretory vesicles, with a concomitant increase in Mac-1 surface expression on human neutrophils. Our findings suggest that gelatinase granule exocytosis by the combination of PLA\textsubscript{2}-II and PAF involves activation of the 5-lipoxygenase pathway and the influx of extracellular Ca\textsuperscript{2+}.
Materials and Methods

Purified recombinant human PLA2-II was obtained as described elsewhere (14). The PLA2-II preparation contained <7 ng of endotoxin in a solution containing 1 mg of protein as estimated by the Limulus ES-II test (Toyo Chemical Industries, Osaka, Japan). A mAb against Mac-1 (clone M1/70), labeled with R-phycoerythrin (R-PE-anti-Mac-1), and control R-PE-rat IgG2b were purchased from Boehringer Mannheim (Mannheim, Germany) and PharMingen (San Diego, CA), respectively. AA861 and FMLP were purchased from Wako, and PAF and human TNF-α were obtained from BACHEM (Bubendorf, Switzerland). Human endothelial IL-8 was obtained from Upstate Biotechnology (Lake Placid, NY), YM264 was chemically synthesized by methods described elsewhere (15). Rabbit Abs against human PLA2-II, and the F(ab′)2 fragment was obtained by digesting the purified IgG with pepsin. The F(ab′)2 fragment was able to neutralize the catalytic activity of human PLA2-II (13).

Isolation of human neutrophils

Neutrophils were separated from the heparinized blood of healthy volunteers. First, RBC were sedimented with dextran. Then the neutrophils were separated by using Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden), as described previously (13). The cells were suspended in a balanced salt solution (BSS) composed of 0.14 M NaCl, 5 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 1 g/l glucose, and 10 mM HEPES (pH 7.4) and kept on ice until use.

Flow cytometry for assessment of Mac-1 expression

Cells were incubated with PLA2-II in BSS or with BSS alone for 30 min at 37°C. For combination assays, cells were incubated with a second stimulus for an additional 10 min before termination of the assays. Inhibition assays were conducted under the following conditions. The cell suspension was preincubated with anti-PLA2-II (Fab′)2, AA861, or YM264 for 10 min at 37°C and then stimulated with PLA2-II and PAF.

The stimulation was terminated by placing the suspensions on ice. The cells were stained with 10 μg/ml R-PE-anti-Mac-1 in 2% FCS/calcium and sodium-free BSS and analyzed with a flow cytometer (Coulter EPIICS Prof ile II, Coulter, Miami, FL) as described previously (13).

The levels of Mac-1 expression on the cell surface were correlated with values obtained by subtracting the mean fluorescence intensity (MFI) due to staining with control Ab (R-PE-normal IgG2b) from the MFI due to staining with R-PE-anti-Mac-1. The effects of different mediators on Mac-1 expression were expressed as the percentage change compared with the value for neutrophils incubated in buffer alone at 37°C. The changes in the fluorescence of samples were calculated according to the formula relative fluorescence increase (%) = [(MFI of sample) − (MFI of buffer control)]/MFI of buffer control × 100.

Exocytosis assay

Cell suspensions were stimulated under the conditions described above. After stimulation, the cell supernatants were collected and stored at −80°C until use. The marker proteins were assayed for different types of vesicles and granules as described previously (13). In brief, myeloperoxidase activity as a marker of azurophilic granules was measured as described by Suzuki et al. (16), and the lactoferrin concentration as a marker of specific granules was measured with an ELISA kit (BIOXYTECK, Bonneuil, France). The gelatinase activity, as a marker of gelatinase-containing granules, was measured with a Type IV collagenase assay kit (YAGAI, Yamagata, Japan) after pretreatment of each sample with PMFS (Sigma, St. Louis, MO) to inhibit serine proteases and then with p-aminophenylmercuric acetate (Sigma) to activate the latent form of gelatinase. The release of these marker proteins was expressed as the percentage of the total activity released from sonicated cells.

The cell surface expression of alkaline phosphatase as a marker of secretory vesicles was measured as described by DeChatelet et al. (17), with modifications, as described previously (13). The surface expression of alkaline phosphatase was expressed as the percentage of the total activity measured with Triton X-100-treated cells.

Measurement of leukotriene B4

After stimulation as described above, the cellular supernatants were collected and stored at −80°C until use. The leukotriene B4 (LTB4) concentrations in those supernatants were measured with an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI).

Results

Augmentation of Mac-1 expression on human neutrophils by combination of PLA2-II with different mediators

Certain inflammatory mediators, including PLA2-II (0.1–10 μg/ml), induce cell surface expression of Mac-1 on human neutrophils, as described previously (13). However, the Mac-1 induced by each of those mediators separately originates by translocation from secretory vesicles, which contain only 20% of the total intracellularly stored Mac-1 molecules. To investigate possible synergistic effects of PLA2-II and other mediators on Mac-1 expression, neutrophils were incubated with 10 μg/ml PLA2-II for 30 min and then stimulated with the optimal concentration of PAF, TNF-α, IL-8, or FMLP for an additional 10 min. Mac-1 expression on the cell surface was assessed by flow cytometry using R-PE-anti-Mac-1 mAb. As shown in Table I, stimulation with only one of those inflammatory substances increased Mac-1 expression by 100 to 260% within 10 min, compared with unstimulated cells. Prior stimulation with 10 μg/ml PLA2-II augmented the increase in Mac-1 expression which was induced by those mediators. Among the various combinations, PLA2-II plus PAF showed the greatest enhancement of induction of Mac-1 expression.

Mac-1 expression on the cell surface was assessed at various concentrations of PLA2-II and PAF (Fig. 1). The surface expression of Mac-1 by PAF increased in a dose-dependent manner and reached a maximum at 100 nM PAF. Preincubation with 10 μg/ml PLA2-II, which is within the range of physiologic concentration, resulted in a synergistic effect at 100 and 1000 nM PAF. Since the synergy was greater with 10 μg/ml PLA2-II and 100 nM PAF, these conditions were used in additional experiments to investigate the mechanism of Mac-1 induction.

Exocytosis of gelatinase granules and secretory vesicles was induced by the combination of PLA2-II and PAF

It has been demonstrated that 75% of Mac-1 is localized in gelatinase granules and specific granules, and 20% is localized in secretory vesicles (12). Exocytosis of azurophilic, specific, and gelatinase granules as well as secretory vesicles was examined after neutrophils were stimulated with 10 μg/ml PLA2-II alone, 100 nM PAF alone, or PLA2-II plus PAF (Fig. 2). Exocytosis of azurophilic granules, specific granules, gelatinase-containing granules, and secretory vesicles was assessed by measuring the release of myeloperoxidase, lactoferrin, and gelatinase from cells and the increase in cell surface alkaline phosphatase, respectively. Either PLA2-II alone or PAF alone induced maximal exocytosis of secretory vesicles (90% of total content), but neither of them induced significant exocytosis of other granules. Interestingly, stimulation with PLA2-II plus PAF caused the release of 60% of the total

| Table I. Mac-1 induction by PLA2-II in combination with different mediators* |
|-----------------|-----------------|-----------------|
| None (%) | 10 μg/ml PLA2-II (%) |
| None | 0 | 120 ± 66 |
| 50 U/ml TNF-α | 100 ± 29 | 228 ± 105 |
| 100 ng/ml IL-8 | 140 ± 36 | 242 ± 96 |
| 10 nM FMLP | 261 ± 85 | 350 ± 62 |
| 100 nM PAF | 259 ± 60 | 502 ± 74 |

* Neutrophils were treated by PLA2-II, TNF-α, IL-8, FMLP, PAF, or PLA2-II in combination with a second stimulus as described in Materials and Methods. Induction of Mac-1 expression is indicated as the mean ± SD of relative fluorescence increase over neutrophils incubated with buffer at 37°C in four independent experiments.
gelatinase content. It has recently been shown that 50% of the total cellular gelatinase content is localized in so-called gelatinase granules and 50% in specific granules, which contain lactoferrin as well (18). PLA2-II plus PAF did not induce the release of lactoferrin. Therefore, stimulation with this combination probably induces exocytosis of most gelatinase granules but not of specific granules.

These results suggest that the increased Mac-1 surface expression induced by PLA2-II and PAF is due to exocytosis of gelatinase granules as well as secretory vesicles.

FIGURE 1. Augmentation of Mac-1 expression by PLA2-II in combination with PAF. Neutrophils were incubated with buffer (■), 1 μg/ml PLA2-II (▲) for 30 min at 37°C and then stimulated with various concentrations of PAF for an additional 10 min before stopping the reaction. The neutrophils were then stained with R-PE-anti-Mac-1 mAb and analyzed by flow cytometry, as described in Materials and Methods. Induction of Mac-1 expression is indicated as the mean ± SD of relative fluorescence increase over neutrophils incubated with buffer at 37°C in five independent experiments.

Effect of a 5-lipoxygenase inhibitor on Mac-1 expression and exocytosis
To further investigate the relationship between Mac-1 expression and LTB4 generation, a 5-lipoxygenase inhibitor, AA861, was tested for effects on Mac-1 expression and exocytosis from secretory vesicles and gelatinase granules. As shown in Figure 3, AA861 at a concentration of 10 μM did not affect either Mac-1 expression or exocytosis of secretory vesicles induced by PLA2-II or PAF. Therefore, exocytosis of secretory vesicles and translocation of Mac-1 from secretory vesicles do not require activation of the 5-lipoxygenase pathway.

In contrast with single stimulation, 10 μM AA861 decreased Mac-1 expression induced by the combination of PLA2-II and PAF from 540% to 290%, which is comparable with that induced by PAF alone (Figs. 3 and 4). It was confirmed that AA861 inhibited LTB4 generation induced by the PLA2-II plus PAF combination in a dose-dependent manner (Fig. 5). AA861 also decreased exocytosis of gelatinase granules in proportion to the decrease in LTB4 generation, whereas it did not affect exocytosis from secretory vesicles.

PAF acetylhydrolase is a phospholipase A2 that hydrolyzes phospholipids in which the sn-2 fatty acid is an acetyl group (22, 23). The enzyme digests PAF to fatty acids and biologically inactive lyso-PAF. It is possible that exocytosis of gelatinase and LTB4 generation by PLA2-II plus PAF (Figs. 4 and 5). Therefore, these results suggest that the synergistic effects of PAF occur through a PAF receptor-mediated process but not through degradation products of PAF.

FIGURE 2. Exocytosis induced by PLA2-II in combination with PAF. Neutrophils were incubated with 10 μg/ml PLA2-II, 100 nM PAF, or the combination of PLA2-II and PAF under conditions described in Materials and Methods. The concentration or activity of myeloperoxidase (open bar), lactoferrin (hatched bar), gelatinase (cross-hatched bar), and alkaline phosphatase (closed bar) were measured. Each bar indicates the mean ± SD of four independent experiments.

Table II. LTB4 induction by PLA2-II in combination with different mediators

<table>
<thead>
<tr>
<th>Mediator</th>
<th>None (pg/ml)</th>
<th>10 μg/ml PLA2-II (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>31.1 ± 1.4</td>
<td>59.7 ± 13</td>
</tr>
<tr>
<td>50 U/ml TNF-α</td>
<td>45.1 ± 7.8</td>
<td>106 ± 17</td>
</tr>
<tr>
<td>100 ng/ml IL-8</td>
<td>72.5 ± 12</td>
<td>226 ± 13</td>
</tr>
<tr>
<td>10 nM FMLP</td>
<td>125 ± 9.0</td>
<td>284 ± 57</td>
</tr>
<tr>
<td>100 nM PAF</td>
<td>120 ± 41</td>
<td>900 ± 70</td>
</tr>
</tbody>
</table>

*The cell supernatants of the experiments described in Table I were collected. LTB4 in each cell supernatant was measured with an EIA kit. Data are the mean ± SD.
These results suggest that exocytosis of gelatinase granules requires activation of the 5-lipoxygenase pathway by the synergistic effect of PLA₂-II and PAF.

Involvement of extracellular Ca \(^{2+}\) on LTB₄ generation by a combination of PLA₂-II and PAF

Maximal increase in Mac-1 expression and the high level formation of 5-lipoxygenase metabolites by calcium ionophores in human neutrophils both require extracellular Ca\(^{2+}\) influx (24, 25). Therefore, the role of extracellular Ca\(^{2+}\) in the PLA₂-II and PAF synergism was examined. In human neutrophils, agonist-stimulated extracellular Ca\(^{2+}\) influx is inhibited by EGTA, EDTA, and inorganic Ca\(^{2+}\) antagonists such as La\(^{3+}\), Ni\(^{2+}\), and Co\(^{2+}\) (26, 27). Thus, to define the role of extracellular Ca\(^{2+}\) influx in the LTB₄ generation by PLA₂-II and PAF, the effect of EGTA, EDTA, and inorganic Ca\(^{2+}\) antagonists was assessed. However, because PLA₂-II enzymatic activity is dependent on Ca\(^{2+}\), the effect of these reagents on PLA₂-II enzymatic activity was first tested. Ni\(^{2+}\) at concentrations between 1 and 10 mM did not affect the enzymatic activity, whereas EDTA, EGTA, La\(^{3+}\), and Co\(^{2+}\) inhibited it completely (data not shown). In this study, Ni\(^{2+}\) was used to determine the effect of Ca\(^{2+}\) influx on the LTB₄ generation. Pretreatment with NiCl₂ decreased LTB₄ generation by PLA₂-II and PAF to the level of control BSS-treated cells (Fig. 6). These results indicate that the influx of extracellular Ca\(^{2+}\) is essential for LTB₄ generation, which causes the exocytosis of gelatinase granules and consequent Mac-1 induction.

Discussion

Stimulation of human neutrophils with a combination of PLA₂-II and PAF induces exocytosis of gelatinase granules as well as secretory vesicles, resulting in a synergistic increase in Mac-1 expression on the cell surface. The biologic functions of neutrophils are regulated locally in the microenvironment and systemically by various mediators including cytokines, biologically active lipids, and neuroendocrine hormones (28). In interaction of neutrophils with endothelial cells, selectin-dependent neutrophil rolling is essential to subsequent events in transmigration process, including

![Figure 3](http://www.jimmunol.org/)

**FIGURE 3.** Mac-1 induction, exocytosis of secretory vesicles and gelatinase granules by PLA₂-II or PAF. Neutrophils were incubated with 10 μg/ml PLA₂-II for 30 min at 37°C or 100 nM PAF for 10 min. Each bar indicates the mean ± SD of four independent experiments. Data for the control BSS-treated cells were as follows: alkaline phosphatase, 38.2 ± 3.4; gelatinase, 6.5 ± 4.8.

![Figure 4](http://www.jimmunol.org/)

**FIGURE 4.** Mac-1 induction and exocytosis of secretory vesicles and gelatinase granules by PLA₂-II and PAF. Neutrophils were pretreated with AA861 or YM264 for 10 min. The neutrophils were then incubated with 10 μg/ml PLA₂-II for 30 min and 100 nM PAF for an additional 10 min. Each bar indicates the mean ± SD of five independent experiments. Data for the control BSS-treated cells were as follows: alkaline phosphatase, 33.9 ± 10; gelatinase, 4.5 ± 3.3.
Therefore, neutrophils would probably be activated by the PLA2-II produces PAF, which remains firmly bound to the cell surface (31). Activated endothelium also produces PAF, which remains firmly bound to the cell surface (31). Therefore, neutrophils would probably be activated by the PLA2-II and PAF combination and consequently express more Mac-1 molecules, which cause firm and stationary adhesion to the endothelium at the inflamed site.

Sengeløv et al. (12) fractionated granules and vesicles of human resting neutrophils and found that 75% of the total Mac-1 molecules were in the fractions containing both gelatinase granules and specific granules, 20% in the secretory vesicle fractions, and 5% in plasma membrane fractions. Since PLA2-II alone and PAF alone caused exocytosis of 80 to 100% of secretory vesicles but no or only slight exocytosis of both gelatinase granules and specific granules, these factors effect translocation of <~20% of the total intracellular Mac-1 content to the cell surface. Stimulation with A23187 was previously shown to induce a ~70% release of gelatinase in addition to ~75% exocytosis of specific granules and ~100% exocytosis of secretory vesicles, resulting in a 750% increase in Mac-1 expression (13). Since stimulation with PLA2-II plus PAF increased Mac-1 expression by ~500%, a major part of intracellular Mac-1 seems to be translocated to the cell surface by this stimulation.

It is biologically important that Mac-1 molecules are stored in two different compartments that include different molecules and that exocytosis of those compartments is regulated by different mediators or signals. The translocation of gelatinase granules facilitates extravasation of neutrophils into perivascular tissues by means of degradation of the extracellular matrix by gelatinase enzyme activity. Actually, gelatinase granules as well as secretory vesicles are exocytosed during the course of in vivo neutrophil exudation (32).

The combination of PLA2-II and PAF showed remarkable synergistic effects on LTb4 generation, and a 5-lipoxygenase inhibitor, AA861, decreased Mac-1 expression to the level induced by PAF alone. Additionally, AA861 inhibited the exocytosis of gelatinase granules that was caused by PLA2-II plus PAF, whereas it had no effect on exocytosis of secretory vesicles. Therefore, translocation of Mac-1 from gelatinase granules but not secretory vesicles appear to be regulated by a mechanism dependent on 5-lipoxygenase. There is additional support that PAF enhances both LTb4 generation and neutrophil adherence which are induced by FMLP, PMA, and A23187 and that the enhancement is canceled by lipoxygenase inhibitors (33). The 5-lipoxygenase products LTb4 and 5-hydroxy-6,8,11,14-eicosatetraenoic acid represent biologically active lipid mediators. In addition, human neutrophils can convert 5-hydroxyeicosanoids to 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxoETE) by the action of a specific dehydrogenase (34). Recently, Powell et al. (35) reported that 5-oxo-ETE, in addition to LTb4 and 5-hydroxy-6,8,11,14-eicosatetraenoic acid, is able to increase the surface expression of Mac-1 on human neutrophils. These authors also have shown that LTb4 and 5-oxo-ETE induces rapid actin polymerization in advance of Mac-1 expression. In neutrophils, chemotaxis, phagocytosis, and degranulation are all motile events that depend on modulation of the actin cytoskeleton (36, 37). This modulation is characterized by an initial rapid actin polymerization followed by a slower depolymerization (38). Modulation of the cytoskeleton by 5-lipoxygenase products may cause exocytosis of gelatinase granules. Taken together, biologically active lipid mediators produced by 5-lipoxygenase may work in an autocrine manner to modulate exocytosis of gelatinase granules and consequent Mac-1 induction in human neutrophils stimulated by PLA2-II and PAF.

5-Lipoxygenase is activated by its translocation from the cytosol to the nuclear membrane and interaction with a 5-lipoxygenase-activating protein (39). This process is regulated by the cystolic Ca2+ level (39). Another key enzyme involved in the activation of 5-lipoxygenase pathway would be cPLA2, which

**FIGURE 5.** Induction of LTb4 generation by PLA2-II in combination with PAF. Neutrophils were pretreated with AA861 or YM264. The neutrophils were then incubated with 10 μg/ml PLA2-II for 30 min and 100 nM PAF for an additional 10 min. LTb4 in the cellular supernatant was measured with an enzyme immunoassay kit. Data are the means ± SD of three independent experiments. Control BSS-treated cells, 75.3 ± 7.8 pg/ml.

**FIGURE 6.** Effect of Ni2+ on LTb4 generation by PLA2-II and PAF. Neutrophils pretreated with NiCl₂ for 10 min. The cell suspensions were stimulated with 10 μg/ml PLA2-II for 30 min and 100 nM PAF for an additional 10 min. The LTb4 concentration in the supernatant was measured with an enzyme immunoassay kit. Data are the means ± SD of four independent experiments. Control BSS-treated cells, 75.3 ± 16 pg/ml.
produces arachidonic acid which is metabolized by 5-lipoxygenase (40). cPLA2 is also activated by Ca2+-dependent translocation from the cytosol to the nuclear membrane and its phosphorlation on serine residues. (40, 41). Human neutrophils generate 5-lipoxygenase metabolites in only low amounts after phosphorylation on serine residues. (40, 41). Human neutrophils containing 5-lipoxygenase translocate Mac-1 only from secretory vesicles. Exocytosis of PLA2-II alone or PAF alone. The combination translocates Mac-1 to the cell surface of human neutrophils. The mechanism is very different from calcium influx, cPLA2 activation, and 5-lipoxygenase activation.

In conclusion, stimulation of neutrophils by a combination of PLA2-II and PAF synergistically induces expression of Mac-1 on the cell surface of human neutrophils. The mechanism is very different from the mechanism of Mac-1 induction expressed by PLA2-II alone or PAF alone. The combination translocates Mac-1 to the cell surface by exocytosis of gelatinase granules as well as secretory vesicles, whereas either PLA2-II or PAF separately translocate Mac-1 only from secretory vesicles. Exocytosis of gelatinase granules requires activation of the 5-lipoxygenase pathway, whereas exocytosis of secretory vesicles does not. Moreover, influx of extracellular Ca2+ is necessary for the activation of the 5-lipoxygenase pathway by the combination of PLA2-II and PAF. These findings support the idea that expression of Mac-1 on the cell surface of neutrophils is regulated by different inflammatory stimuli and different mechanisms that work at the proper time and place.

Acknowledgments

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References