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A POSSIBLE REQUIREMENT FOR ARACHIDONIC ACID LIPOXYGENATION IN THE MECHANISM OF PHAGOCYTIC DEGRANULATION BY HUMAN NEUTROPHILS STIMULATED WITH AGGREGATED IMMUNOGLOBULIN G

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SUMMARY: Aggregated immunoglobulin G (AggIgG) caused a concentration-dependent extracellular release of granule-associated lysozyme and myeloperoxidase (MPO) from human neutrophils. Generation of the 5-lipoxygenase product of arachidonic acid (AA) metabolism, 5(S),12(R)-dihydroxy-6,14-cis,8,10-trans-eicosatetraenoic acid [leukotriene B_4 (LTB_4)], by neutrophils exposed to AggIgG occurred in the presence but not absence of exogenous AA. U-60,257B (piriprost potassium), an inhibitor of leukotriene synthesis, caused a dose-related suppression of LTB_4 production and granule exocytosis by AggIgG-treated cells. These data suggest that a lipoxygenase product of AA metabolism may mediate AggIgG-induced phagocytic release of granule constituents from neutrophils.

The principal role of the neutrophil in host-defense mechanisms is to internalize and kill foreign substances including pathogens. However, during the phagocytic uptake of particulate material by neutrophils, cytoplasmic granules have been demonstrated to fuse with the developing phagosome while it is still open to the extracellular environment (1,2), resulting in the discharge of granule constituents. We have recently reported on several characteristics of phagocytic degranulation by human neutrophils exposed to heat aggregates of immunoglobulin G (2). The purpose of this study was to investigate a requirement for AA lipoxygenation in the expression of the neutrophil granule exocytotic response to AggIgG in that this immunologic phagocytic stimulus also induces the 5-lipoxygenation of AA by neutrophils.

METHODS AND MATERIALS

Preparation of Neutrophils. Blood from normal donors was drawn by venipuncture in one-tenth volume of 3.8% citrate in conical plastic tubes.

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Neutrophils were purified employing standard techniques of dextran sedimentation, centrifugation on Hypaque/Ficoll, and hypotonic lysis of red blood cells. Final cell suspensions contained a minimum of 98% neutrophils. Cell viability always exceeded 99% as determined by trypan blue exclusion. Neutrophils from one donor were used in each experiment.

Incubation Conditions. Neutrophils (2.5 x 10^6/ml) suspended in phosphate-buffered saline (PBS), pH 7.4, containing 138 mM NaCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 2.7 mM KCl, 0.6 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 0.1% glucose were incubated at 37°C in a Dubnoff shaker set at 120 cycles per min. according to the various procedures described under RESULTS. After incubation the samples were centrifuged at 750 g (4°C) for 3 min., and the clear supernatants were assayed for enzyme activities. The net percent of MPO and lysozyme activities released was calculated by substracting the percent release in buffer or vehicle alone from that attributable to a given test agent. The release of the granule constituents is expressed as the percent of total activity released by 0.05% Triton X-100 in simultaneously run duplicate reaction mixtures.

Generation, Isolation and Quantification of Leukotriene B_4. Neutrophils (5 x 10^6/ml) in PBS were incubated with AggIgG according to the procedures described under RESULTS. After incubation the supernatants were assessed for LTB_4 content with a specific and sensitive radioimmunoassay for this 5-lipoxygenase product. The data are expressed as picograms of LTB_4 per 2 x 10^6 cells.

Preparation of Aggregated Immunoglobulin G. Heat aggregated immunoglobulin G was prepared as described previously (2).

Enzyme Assays. MPO (EC 1.11.1.7), lysozyme (EC 3.2.1.17) and lactate dehydrogenase (LDH:EC 1.1.1.27) activities were determined as described previously (3-5).

Source and Preparation of Reagents. Immunoglobulin G was purchased from Miles Scientific, Naperville, IL. LTB_4 (prepared by Dr. G. L. Bundy of The Upjohn Company) and U-60,257B (prepared by Dr. H. W. Smith of The Upjohn Company) were dissolved in methanol and DMSO, respectively. Arachidonic acid (Nu-Chek-Prep, Inc., Elysian, MN) was dissolved in methanol. H^3-LTB_4 (5 µCi/0.25 ml/vial; 220 Ci/mmole) was purchased from Amersham Corporation (Arlington Heights, IL).

RESULTS

AggIgG-Stimulated Granule Enzyme Release from and LTB_4 Production by Human Neutrophils. AggIgG elicited a concentration-dependent selective extracellular release of MPO and lysozyme from and generation of LTB_4 by neutrophils (Figure 1). An electron microscopic analysis of AggIgG-treated neutrophils revealed degranulation to result predominantly from the release of constituents from cytoplasmic granules which had fused with developing phagosomes which were still open to the extracellular milieu (2). Maximum degranulation and LTB_4 production were demonstrated with an AggIgG concentration of 400 µg/ml and the EC_{50} (concentration of AggIgG stimulating 50% of the maximal secretory and LTB_4 generating responses) is approximately 150-200 µg/ml.

There was no significant release of cytoplasmic LDH (<6% of total cell activity) during the entire incubation period, which indicates activation of neutrophils with AggIgG to be a selective, noncytotoxic process.

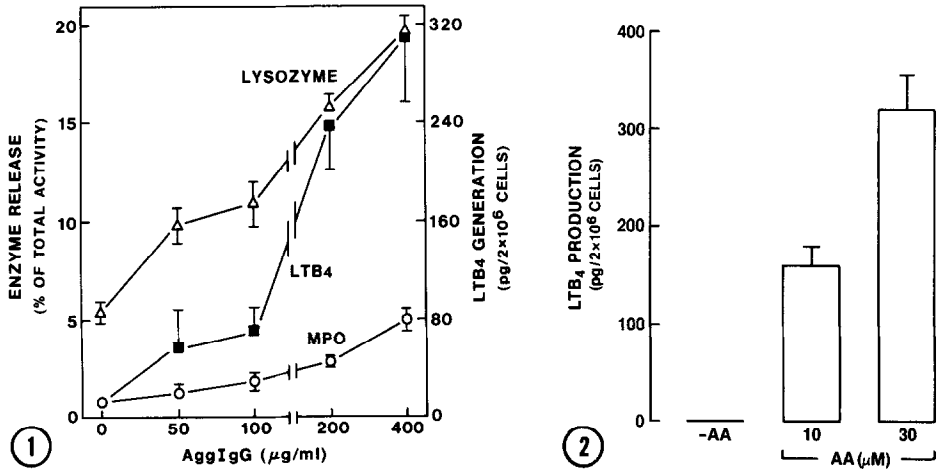

Figure 1: AggIgG-Stimulated Granule Enzyme Release from and Leukotriene B_4 Production by Human Neutrophils

Neutrophils (5×10^6) were incubated with the stipulated concentrations of AggIgG for 15 (LTB_4 generation) or 30 (degranulation) min. In the experiments involving LTB_4 production the cells were incubated with AggIgG in the presence of AA (30 μM). Total cell enzyme activities were 1.13 ± 0.01 ΔABS 460nm for MPO and 28.2 ± 0.2 μg lysozyme std./3 min./5 x 10^6 cells for lysozyme. LTB_4 generation by cells alone was nondetectable as determined by RIA. Data represent the mean ± SEM of four experiments performed in duplicate.

Figure 2: Concentration Dependence of the Effect of Arachidonic Acid on Leukotriene B_4 Generation by Human Neutrophils exposed to AggIgG

Neutrophils (5×10^6) were incubated with AggIgG (400 μg/ml) in the presence and absence of the designated concentrations of AA for 15 min. at 37°C. Date report the mean ± SEM of three experiments performed in duplicate.

AggIgG-Induced LTB_4 Generation by Human Neutrophils in the Presence and Absence of Arachidonic Acid. The data in Figure 2 indicate that AggIgG stimulated the production of 160.5 ± 20.5 and 318.3 ± 38.7 pg $LTB_4/2 \times 10^6$ cells in the presence of 10 and 30 μM, respectively, exogenous AA (Figure 2). However, the amount of LTB_4 generated by neutrophils activated with AggIgG in the absence of AA was nondetectable by radioimmunoassay.

Effect of Piriprost Potassium on AggIgG-Stimulated LTB_4 Generation by Human Neutrophils. Piriprost potassium caused a concentration-dependent inhibition of AggIgG-induced LTB_4 production by neutrophils (Figure 3). The IC_{50} (concentration causing 50% inhibition of the maximal LTB_4 generating response) for piriprost potassium is approximately 5 μM.

Effect of Piriprost Potassium on AggIgG-Induced Neutrophil Degranulation. Exposure of neutrophils to piriprost potassium resulted in a concentration-

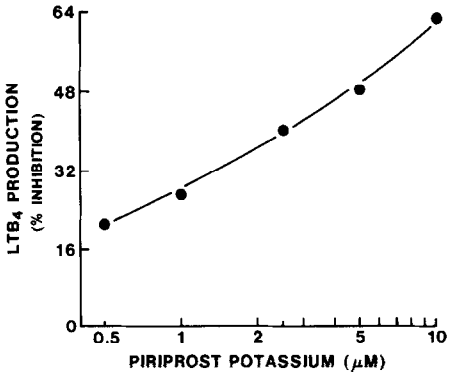

Figure 3: Effect of Piriprost Potassium on AggIgG-Induced Leukotriene B_4 Generation by Human Neutrophils

Neutrophils (5 x 10^6) were preincubated with or without piriprost potassium for 5 min. followed by a 15 min. incubation with AggIgG (400 μg/ml) in the presence of AA (30 μM). Data represent the mean ± SEM of five experiments performed in duplicate.

dependent inhibition of the phagocytic secretory response of these cells to AggIgG (Table 1). The IC_{50} for piriprost potassium is approximately 70 μM.

Recovery of total MPO activity in excess of 98% from piriprost potassium-treated neutrophils indicates that this agent is exerting a specific inhibitory effect on the mechanism of phagocytic secretion and not MPO itself.

DISCUSSION

We report here that the interaction of AggIgG, a stimulus of pathogenetic significance, with human neutrophil cell membrane-associate Fc receptors results in the phagocytic release of granule enzymes from these cells. Further, degranulation is accompanied by the generation of a major 5-lipoxygenase product

TABLE 1

EFFECTS OF PIRIPPOST POTASSIUM ON AggIgG-INDUCED GRANULE EXOCYTOSIS FROM HUMAN NEUTROPHILS

EXPERIMENTAL CONDITION[a]	MPO RELEASE (% OF TOTAL ACTIVITY)[b]
CONTROL	7.2 ± 0.8[c]
PIRIPROST POTASSIUM	
40μM	5.3 ± 0.9 (28.1)[d]
50μM	4.8 ± 1.1 (35.3)
70μM	3.5 ± 0.7 (52.3)
100μM	2.6 ± 0.4 (65.1)

[a] NEUTROPHILS (5 x 10^6) WERE PREINCUBATED WITH OR WITHOUT PIRIPROST POTASSIUM FOR 5 MIN FOLLOWED BY A 30 MIN INCUBATION WITH AggIgG (400μg/ml)

[b] TOTAL CELL MPO ACTIVITY WAS: 1.09±0.1 ΔABS 460nm.

[c] DATA INDICATES THE MEAN ± SEM OF THREE EXPERIMENTS RUN IN DUPLICATE.

[d] NUMBERS IN PARENTHESES REPRESENT THE PERCENT INHIBITION OF MPO RELEASE.

of AA metabolism in neutrophils, LTB_4. This observation constitutes the first report of the capacity of AggIgG to stimulate AA lipoxygenation in human neutrophils. Consistent with this finding is the induction of LTB_4 generation by neutrophils exposed to other particulate stimuli (6,7).

The role of LTB_4 as an inflammatory mediator is well documented (8). Indeed, we and others have described neutrophil degranulation elicited with LTB_4 (9-11). However, whether or not LTB_4 or another endogenous lipoxygenase product of AA metabolism is an integral component of the phagocytic secretory process remains to be determined. In this regard, we have shown that AggIgG will stimulate LTB_4 production by neutrophils in the presence, but not the absence of exogenous AA. A similar finding was reported for FMLP and C5a-induced LTB_4 generation (12,13). This observation implies that while AggIgG-induced degranulation is a receptor-mediated event, the receptor-associated signal transduction mechanism is not necessarily coupled to phospholipase A_2 which is responsible for cleaving AA from cell membrane phospholipids. Neutrophils in contact with various stimuli have been demonstrated to release free AA (14-15). Therefore, at an inflammatory site the AA released by cells could be incorporated into other cells or indeed the cells releasing the AA and serve as a substrate to amplify the AA lipoxygenation capacity of neutrophils as well as the phagocytic secretory activity of these cells. Alternatively, it is conceivable that AggIgG stimulates AA lipoxygenation in the absence of AA, and that a lipoxygenase product(s) does mediate the phagocytic secretory response. However, in this case, LTB_4 would not be an expression of this particular pathway of AA metabolism.

In another series of experiments, piriprost potassium, a selective inhibitor of 5-lipoxygenase activity (16) and leukotriene synthesis (17,18), was found to suppress AggIgG-induced LTB_4 production. Furthermore, we have also demonstrated piriprost potassium to inhibit AggIgG stimulated neutrophil degranulation. The capacity of piriprost potassium to inhibit AggIgG-induced degranulation constitutes the first report of a 5-lipoxygenase inhibitor suppressing phagocytic degranulation, and is consistent with the inhibitory effect of this agent on neutrophil granule exocytosis induced with soluble stimuli (16). Therefore, our

finding with AggIgG implies that it stimulates the 5-lipoxygenation of AA via a pathway that results in the generation of a metabolite which is a component of the underlying sequence of events leading to neutrophil activation with this immunologic stimulus.

AggIgG-induced neutrophil degranulation does indeed represent a model of "Stimulus-Response Coupling" described for neutrophil activation; and neutrophils localized in rheumatoid arthritic joints contain aggregates of immunoglobulins and immune complexes. To the extent that the granule-associated enzymes released from neutrophils in contact with phagocytic stimuli contribute to the pathogenesis of inflammatory joint disease, the data reported here implicate lipoxygenase products of AA metabolism in the expression of a cell function which is associated with neutrophil-mediated tissue injury.

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