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Research Article

Resident macrophages isolated from uninfected animals produce large quantities of arachidonic acid (AA) metabolites. Immunizing animals with protein antigens or bacteria activates macrophages and causes an 80% reduction in the cyclooxygenase and lipoxygenase metabolites relative to resident cells. Since some products have been shown to modulate immune functions, we examined how the AA metabolic enzyme activities regulate the products that are synthesized. We demonstrate that the cyclooxygenase, 5-lipoxygenase, prostacyclin synthase, and probably prostaglandin (PG) endoperoxide E-isomerase activities were decreased in activated peritoneal macrophages. In sharp contrast, thromboxane synthase activity was selectively unchanged or enhanced in the activated macrophages. Thus the immune response appears to modulate the activity of the AA and PG endoperoxide-dependent enzymes, thus dictating a major shift in the profile of metabolites synthesized by macrophages.

Thromboxane Synthase Is Preferentially Conserved in Activated Mouse Peritoneal Macrophages

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Abstract

Resident macrophages isolated from uninfected animals produce large quantities of arachidonic acid (AA) metabolites. Immunizing animals with protein antigens or bacteria activates macrophages and causes an 80% reduction in the cyclooxygenase and lipoxygenase metabolites relative to resident cells. Since some products have been shown to modulate immune functions, we examined how the AA metabolic enzyme activities regulate the products that are synthesized. We demonstrate that the cyclooxygenase, 5-lipoxygenase, prostacyclin synthase, and probably prostaglandin (PG) endoperoxide E-isomerase activities were decreased in activated peritoneal macrophages. In sharp contrast, thromboxane synthase activity was selectively unchanged or enhanced in the activated macrophages. Thus the immune response appears to modulate the activity of the AA and PG endoperoxide-dependent enzymes, thus dictating a major shift in the profile of metabolites synthesized by macrophages.

Introduction

Humes et al. (1) have demonstrated that resident macrophages produce 10–20-fold greater quantities of prostaglandin E_2 (PGE_2)[1] and prostacyclin (PGI_2), measured as 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), in response to zymosan stimulation than activated macrophages isolated from mice treated with thioglycollate, *Cornebacterium parvum* (*C. parvum*), or bacille Calmette-Guerin. Thioglycollate-elicited macrophages exhibited only 10% of the phospholipase activity, measured as [^{3}H]arachidonic acid (AA) release, seen in prelabeled resident cells. Scott et al. (2) found that the zymosan-stimulated production of leukotriene C_4 (LTC_4) and monohydroxyeicosatetrienoic acids (HETEs) as well as PGE_2 and 6-keto-$PGF_{1\alpha}$ were decreased in *C. parvum*-elicited macrophages when compared with resident cells. Paradoxically, the quantity of thromboxane A_2 (TxA_2), measured as thromboxane B_2 (TxB_2), was increased in the *C. parvum*-elicited macrophages. In this report we have evaluated the AA and prostaglandin H_2 (PGH_2)-dependent metabolic enzymes in resident and *Listeria monocytogenes*-elicited macrophages (*Listeria* macrophages) to characterize the preferential conservation of TxA_2 production by macrophages during an immune response.

Methods

Macrophages were obtained by lavage from the peritoneal cavity of adult BIO.A/Sg Sn J mice (The Jackson Laboratory, Bar Harbor, ME) that were either uninfected (resident macrophages) or infected with *Listeria monocytogenes* (*Listeria* macrophages). Mice were infected intraperitoneally with 5×10^4 live bacteria on day 1, boosted with 1×10^5 live bacteria on day 7, and peritoneal exudates were collected on day 10 by lavaging with 3 ml sterile phosphate-buffered saline (PBS). $1–2 \times 10^6$ peritoneal cells were allowed to adhere to 35-mm tissue culture dishes for 2 h (37°C, 5% CO_2) in 1 ml of α-minimal essential media (α-MEM; Gibco Laboratories, Grand Island, NY), 5% fetal calf serum, and 100 μg penicillin/streptomycin (P/S). The nonadherent cells were removed by washing with PBS, and the adherent cells were incubated with various doses of agonists, substrates, or inhibitors in α-MEM and P/S for 2 h (37°C, 5% CO_2). Zymosan (Sigma Chemical Co., St. Louis, MO) was prepared as described by Bonney et al. (3). AA (Nu-Chek Prep., Inc., Elysian, MN) was dissolved using dimethylsulfoxide and then diluted with α-MEM and P/S. Dimethylsulfoxide had no effect on cellular morphology or protein content. 30 μM AA caused a 50% reduction in cellular protein after 2 h because of cells detaching from the plate. The cells that remained on the plate excluded trypan blue.

PGH_2 was prepared from sheep seminal vesicles and AA (4) and stored in dry acetone at −70°C. Immediately before addition to macrophage cultures, PGH_2 was dried under nitrogen and resolubilized in dimethylsulfoxide. Various aliquots of PGH_2 in dimethylsulfoxide were added directly to macrophage cultures containing 1 ml of αMEM and P/S. The cells were incubated for 30 min at 22–25°C, 5% CO_2.

The media was analyzed for 6-keto-$PGF_{1\alpha}$, PGE_2, TxB_2, and LTC_4 by radioimmunoassay (RIA) (5, 6). Antibodies to 6-keto-$PGF_{1\alpha}$ and LTC_4 were kindly supplied by Dr. Richard Fertel, Ohio State University, Columbus, OH, and by Dr. Alan Rosenthal, Merck Sharp & Dohme Research Laboratories, Rahway, NJ, respectively. Duplicate stimulations within a given experiment resulted in RIA values that were not discernibly different. The cross-reactivity at 50% displacement of other arachidonate metabolites with antisera were as follows: PGE_2 antiserum: 6-keto-$PGF_{1\alpha}$, 0.39%; prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 0.04%; TxB_2, 0.003%; 6-keto-$PGF_{1\alpha}$ antiserum: PGE_2, 0.57%; $PGF_{2\alpha}$, 0.14%; TxB_2, <0.08%; TxB_2 antiserum: 6-keto-$PGF_{1\alpha}$, 0.009%; PGE_2, 0.012%; $PGF_{2\alpha}$, 0.025%; and LTC_4 antiserum: leukotriene D_4, 43%; leukotriene E_4, 6%; leu-

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1. *Abbreviations used in this paper:* AA, arachidonic acid; *C. parvum, Cornebacterium parvum;* HETE, monohydroxyeicosatetrienoic acid; HPLC, high performance liquid chromatography; 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$; LTC_4, leukotriene C_4; α-MEM, α-minimal essential media; PG, prostaglandin; PGE_2, $PGF_{2\alpha}$, PGH_2, prostaglandins E_2, $P_{2\alpha}$, and H_2; PGI_2, prostacyclin; P/S, penicillin/streptomycin; TxA_2, thromboxane A_2; TxB_2, thromboxane B_2.

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kotriene B_4, PGE_2, $PGF_{2\alpha}$, AA, and 5-HETE, <0.25%. The production of LTC_4 by macrophages was also semiquantitated using high performance liquid chromatography (HPLC) and structurally verified using scanning ultraviolet spectroscopy between wavelengths 250 and 300 nm. The amounts of LTC_4 produced by the macrophage cultures quantitated by HPLC and RIA were similar. The cells were washed with PBS and solubilized in 0.62 N NaOH for protein determination using the fluorescamine assay. All protein values in a given cell preparation were within 10% of each other. $1-2 \times 10^6$ cells corresponds to 50–100 μg protein. The data is represented as the mean±SE (7).

Results

Zymosan stimulation of total endogenous AA metabolism (sum of all the measured cyclooxygenase and lipoxygenase products) was decreased by 94% in *Listeria* macrophages (Fig. 1), findings comparable to those previously reported (1, 2). *Listeria* macrophages produced 2, 10, and 4% of the amount of 6-keto-$PGF_{1\alpha}$, PGE_2, and LTC_4, respectively, compared with resident cells. However, not only was TxB_2 produced by resident mouse peritoneal macrophages but the TxB_2 production in *Listeria* macrophages was not significantly different (71%) from resident controls. In agreement with Scott et al. (2), we found that the resident macrophages produced small quantities of a product that co-migrated on HPLC with authentic 12-HETE, whereas *Listeria* macrophages did not produce any measurable 12-HETE (data not shown). No other lipoxygenase products were detected by either cell population. Thus the similar quantity of TxB_2 produced by the two macrophage populations was due to synthesis by the macrophages and not to platelets contaminating the cultures, because (*a*) zymosan does not stimulate platelet AA metabolism (8), and (*b*) the disappearance of the other potential major platelet lipoxygenase metabolite, 12-HETE, in *Listeria* macrophage preparation is not consistent with platelet contamination and stimulation.

Since the phospholipase(s) activity had been shown to be decreased in bacterial-elicited macrophages (1), we bypassed the need for receptor activation or stimulation of phospholipase(s) by incubating exogenous AA with each macrophage population. Total exogenous AA metabolism by the cyclooxygenase pathway (sum of PGE_2, TxB_2, and 6-keto-$PGF_{1\alpha}$) in *Listeria* macrophages was only 13% of that by resident cells, indicating that the cyclooxygenase activity was substantially decreased in the bacteria-elicited macrophages. Again, the quantity of 6-keto-$PGF_{1\alpha}$ produced by *Listeria* macrophages was only 5 and 27%, respectively, while 71% of the TxB_2 production was still maintained (Fig. 2).

Exogenous AA conversion by the 5-lipoxygenase pathway (LTC_4) was also decreased by 87% in *Listeria* macrophages (Fig. 2). However, zymosan stimulation produced tenfold more LTC_4 than exogenous AA. Thus differences in the 5-lipoxygenase activity between the two cell populations is more easily detected using zymosan as an agonist. Furthermore, zymosan-stimulated LTC_4 production should be enhanced in the presence of indomethacin, which inhibits cyclooxygenase and blocks PGE_2, PGI_2, and TxA_2 production. Pretreatment of resident macrophages with indomethacin diverted endogenous AA into the lipoxygenase pathway after zymosan stimulation (Fig. 3). However, *Listeria* macrophages still did not exhibit an increase in the 5-lipoxygenase metabolite LTC_4 (Fig. 3). When the samples were analyzed for other 5-lipoxygenase products (5-HETE and 5,12-diHETEs, data not shown) by HPLC, none of these nonenzymatic products of the 5-lipoxygenase could be detected even in the presence of indomethacin, though more substrate was available for conversion by this pathway. Thus the 5-lipoxygenase activity must be suppressed in *Listeria* macrophages since the amount of 5-lipoxygenase product LTC_4 is decreased and no other nonenzymatic products are detectable.

The synthesis of PGE_2, PGI_2, and TxA_2 each requires the sequential action of three enzymes: phospholipase, cyclooxygenase, and the appropriate prostaglandin (PG) endoperoxide-dependent enzyme. When exogenous PGH_2 was used as substrate for PGI_2 synthase, *Listeria* macrophages still demonstrated only 5% of the 6-keto-$PGF_{1\alpha}$ production seen with resident macrophages (Fig. 4). The enzymatic production of PGE_2 could not be measured due to the extensive nonenzymatic

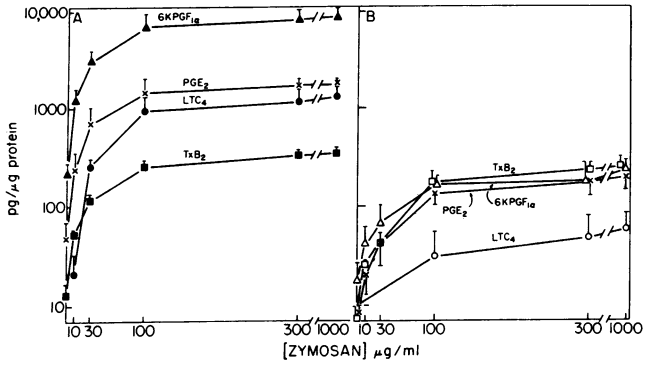

Figure 1. Comparison of zymosan stimulation of AA metabolism in resident and *Listeria* macrophages. Resident (*A*) or *Listeria* (*B*) mouse peritoneal macrophages were cultured and stimulated with varying concentrations of zymosan as described in Methods. The media was analyzed by RIA for 6-keto-$PGF_{1\alpha}$ (6KPGF$_{1\alpha}$) (▲), TxB_2 (■), PGE_2 (×), and LTC_4 (●). Resident macrophages are represented by closed symbols and *Listeria* macrophages by open symbols. The data represent the mean±SE of four different macrophage preparations.

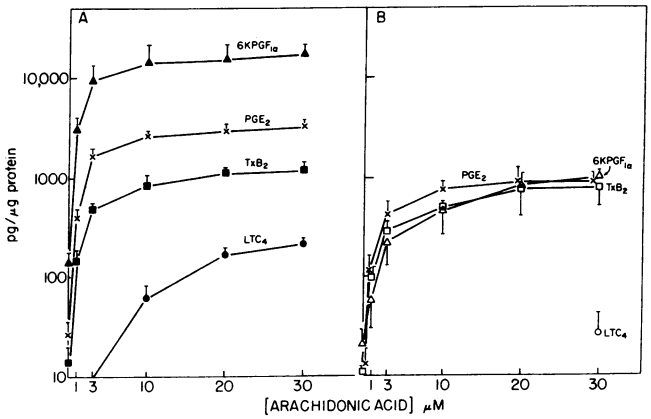

Figure 2. Comparison of exogenous AA metabolism by resident and *Listeria* macrophages. Cultured resident (*A*) and *Listeria* (*B*) macrophages were stimulated with various concentrations of AA. The media was analyzed by RIA for 6-keto-$PGF_{1\alpha}$ (6KPGF$_{1\alpha}$) (▲), TxB_2 (■), PGE_2 (×), and LTC_4 (●). Resident macrophages are represented by open symbols and *Listeria* macrophages by closed symbols. The data represent the mean±SE of four separate macrophage cultures.

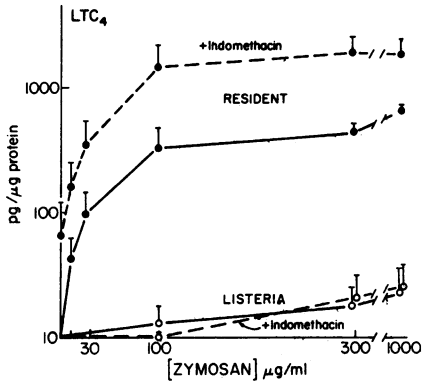

Figure 3. The synthesis of LTC_4 by *Listeria* macrophages cannot be enhanced with indomethacin, Macrophage cultures were obtained and stimulated with zymosan as described, except that after adherence macrophages were preincubated for 15 min (37°C, 5% CO_2) in α-MEM and P/S in the presence (-▾---) or absence (——) of 5 μg/ml indomethacin (Merck Sharp & Dohme Research Laboratories). The stimulation of the cyclooxygenase products in the absence of indomethacin was comparable to that seen in Fig. 1 but was inhibited to basal values (no zymosan) in the presence of indomethacin. Resident macrophages are represented by closed symbols and *Listeria* macrophages by open symbols. The data represent the mean±SE of three macrophage culture preparations.

conversion of PGH_2 to PGE_2 obtained in aqueous solution (no cells). However, using PGH_2 as substrate for the direct measurement of thromboxane synthase activity unmasked a 250% increase in TxB_2 production in *Listeria* macrophages. This increase in thromboxane synthase activity accounts for the relatively unchanged TxB_2 production in response to endogenous (zymosan-stimulated) and exogenous AA metabolism by *Listeria* macrophages. Furthermore, if the cyclooxygenase activity were the same in both cell populations, then this increased TxB_2 production should have been seen with exogenous AA. Since TxB_2 was slightly decreased in *Listeria* macrophages in response to exogenous AA (Fig. 2 *B*) but increased in response to exogenous PGH_2 (Fig. 4), then the cyclooxygenase activity must be decreased in the *Listeria* cells, limiting the amount of PGH_2 available for conversion by the thromboxane synthase. Although the PG endoperoxide E-isomerase activity could not be measured directly with PGH_2 stimulation, this enzyme is also likely to be decreased in *Listeria* macrophage since the PGE_2 production was greatly decreased in these cells in response to zymosan or exogenous AA (Figs. 1 and 2). Thromboxane was the only metabolite conserved in *Listeria* macrophages.

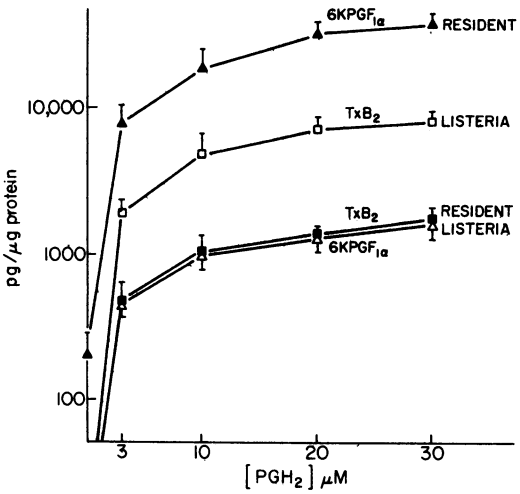

Figure 4. Comparison of exogenous PGH_2 metabolism by resident and *Listeria* macrophages. Cultured resident and *Listeria* macrophages were incubated with varying concentrations of PGH_2 as described. The media was analyzed for 6-keto-$PGF_{1\alpha}$ (6KPGF$_{1\alpha}$) (▲) and TxB_2 (■) by RIA. The PGE_2 cross-reactivity of the 6-keto-$PGF_{1\alpha}$ and TxB_2 antibodies represents 3% or less of the metabolite production. Resident macrophages are represented by closed symbols and *Listeria* macrophages by open symbols. The data represent the mean±SE of four separate macrophage cultures.

Discussion

Macrophages isolated from *Listeria monocytogenes*-treated mice have been shown to be cytocidal (9) and to express Ia antigen (10). Here we report that AA metabolism by *Listeria* macrophages has been altered in such a way that PGE_2 and PGI_2 production was greatly decreased while TxA_2 production was maintained. In cultured *Listeria* macrophages, exogenous PGI_2 and dibutyrl cyclic AMP (cAMP) have been shown to inhibit Ia expression. In addition, in vivo treatment with PGE_1 and PGE_2 blocks lymphokine-stimulated macrophage Ia expression (11). PGE_2 inhibits macrophage cytolytic activity (12) and interleukin-1 secretion (13) in vitro as well. Snyder et al. (11) have demonstrated that in vitro treatment of macrophages with TxB_2 (the inactive metabolite of TxA_2) antagonizes PGE_2 inhibition of Ia expression, but TxB_2 itself has no effect on the expression of Ia. Thus it has been proposed that increased immune function can result by a decreased production of the negative immunomodulator PGE_2. However, PGI_2 is also produced in large quantities in resident macrophages but decreased by 95% in activated cells. PGI_2 has also been shown to be a more potent stimulator of cAMP in platelets than PGE_2 (14) and therefore may be an important negative immunomodulator as well.

Finally, γ-interferon has been shown to modulate macrophage Ia expression as well as macrophage phospholipase activity. Boraschi et al. (13, 15) have reported that resident macrophages cultured with α-, β-, and γ-interferons have decreased phospholipase activity, but they do not exhibit changes in AA metabolic enzyme activities within 24 h. Beller and Ho (16) have demonstrated that γ-interferon increases Ia expression over a 4–8-d culture period. Furthermore, Johnson and Torres have reported that leukotrienes may regulate the production of γ-interferon by T cells (17). In this report we report that immune activation of macrophages in vivo suppressed all of the AA and PG endoperoxide-dependent enzymes with the exception of thromboxane synthase. The result of these AA metabolic enzyme alterations was that the ratio of $TxA_2/PGI_2/PGE_2$ production (when stimulated with an agonist) by *Listeria* macrophages was 1:1:1 compared with 1:25:5 in resident cells. Since TxA_2 and PGI_2/PGE_2 have been shown to have opposing biologies on the vasculature and platelet aggregation, the ratio of product determined the final biology expressed. We propose that γ-interferon might be responsible for the modulation of the AA metabolic enzymes described in this paper and that the regulation of Ia expression by γ-

interferon might be mediated through the stimulatory effects of TxA_2 production, as well as the removal of the inhibitory effects of PGE_2 and PGI_2.

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