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Research Article

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Recombinant Interferon-Gamma Primes Alveolar Macrophages Cultured In Vitro for the Release of Leukotriene B₄ in Response to IgG Stimulation

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Abstract

The capacity of interferon-gamma to regulate the generation and release of leukotriene B₄ (LTB₄) from human alveolar macrophages of normal nonsmoking individuals was evaluated. When alveolar macrophages were incubated for 60 min with heat aggregated IgG (HAIGG), they generated and released 5.7 ± 1.7 ng of LTB₄ per 10^6 cells compared to 1.9 ± 0.4 ng from cells incubated with buffer alone, $P = 0.02$. When alveolar macrophages were preincubated with interferon-gamma for 24 h before activation for 60 min with heat-aggregated IgG, the soluble IgG aggregates became a significantly more effective stimulus for LTB₄ release, 17.0 ± 3.9 ng/ 10^6 cells, $P = 0.001$, compared to cells incubated in the absence of interferon-gamma and challenged with HAIGG. Interferon-gamma did not alter the response to A23187. This effect of interferon-gamma was both time and dose dependent; it also was specific since neither interferon-alpha nor interferon-beta had a regulatory effect on the release of LTB₄ from cells in response to challenge with HAIGG. Preincubation of the alveolar macrophages with interferon-gamma augmented the density of IgG₁ receptors by $81.5 \pm 17.3\%$; neither interferon-alpha nor interferon-beta effected this parameter. Furthermore, monomeric IgG₁ blocked HAIGG induced LTB₄ release from alveolar macrophages primed with interferon-gamma. Therefore, at least one of the mechanisms by which interferon-gamma primes alveolar macrophages for the production and release of LTB₄ in response to stimulation by aggregates of IgG is that of increasing the number of receptors for this stimulus.

Introduction

Alveolar macrophages (AM)¹ reside on the epithelial surfaces of the lower respiratory tract and have been implicated as being central to the evolution of a variety of inflammatory events in the lung (1, 2). In orchestrating such reactions, human AM release numerous biologically active molecules, among which are included lipid metabolites of cellular mem-

brane origin, which are synthesized de novo after cell activation (1-4). For these cells, only zymosan and the calcium ionophore A23187 are known to activate both phospholipase A₂ to cleave arachidonic acid from some pools of membrane phospholipids and also the calcium-dependent oxidative metabolism of arachidonic acid by the 5-lipoxygenase to leukotriene A₄. Leukotriene A₄ then is catalytically hydrolyzed to leukotriene B₄ (LTB₄) (5-7). As a proinflammatory mediator, LTB₄ is of particular interest because of its numerous effects on a variety of leukocytes. These effects include potent chemokinetic and chemotactic activity for human neutrophils (8-11), eosinophils (10-13), and monocytes (8, 14) in vitro, induction of human neutrophil aggregation (9), and stimulation of rabbit and human neutrophil lysosomal enzyme release (15). In addition, LTB₄ exerts potent immunoregulatory effects on human peripheral blood lymphocytes with the net effect being immunosuppression (16-18). In order to extend the relevance of these observations to the spectrum of human inflammatory lung diseases, we used a receptor-dependent soluble activator, heat aggregated IgG (HAIGG). In addition, we assessed the effect of an immune response regulator, human interferon-gamma (IF- γ), on human AM generation and release of LTB₄.

Human IF- γ is a 20,000 mol wt glycoprotein produced by lymphocytes (19, 20) and human AM (21) in response to a variety of stimuli (22). Many of the effects of IF- γ serve to intensify immune reactions. Macrophages and monocytes in particular, are targets for the actions of this molecule. Murine macrophages possess IF- γ receptors (23), and recently, receptors for IF- γ have been found on human peripheral blood monocytes (24). Some of the effects of IF- γ on macrophages or monocytes include augmenting expression of MHC class 2 antigens and antigen presentation to lymphocytes (25), enhancing cytotoxicity for tumor cells (26) and intracellular parasites (27), and augmenting the density of IgG Fc receptors (IgGFcR) (28). Based on these observations, we hypothesized that IF- γ might increase LTB₄ release from human AM when they were challenged with the immunologically relevant stimulus, HAIGG.

Methods

Preparation of AM cultures. Human AM from nonsmoking normal volunteers were retrieved by bronchoalveolar lavage (BAL) (29). Briefly, all subjects had normal medical histories, chest radiographs, spirometry, and complete blood counts, and none admitted to any illness including a respiratory tract infection for which any medication, including nonsteroidal antiinflammatory agents, was required in the 4-wk period before participation. After topical 4% lidocaine anesthesia was applied to the oropharynx, BAL was performed through a fiberoptic bronchoscope, wedged in a segmental bronchus, by instilling sterile normal saline in 50-ml aliquots and gently aspirating after each

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1. *Abbreviations used in this paper:* AM, alveolar macrophages; BAL, bronchoalveolar lavage; HAIGG, heat aggregated IgG; IF- γ , interferon γ .

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instillate. A maximum of 300 ml was instilled into each lung of an individual volunteer. BAL aliquots were pooled, filtered through a single layer of gauze, and centrifuged at 500 g for 5–10 min at 24°C. The cells were washed twice in Ca²⁺ and Mg²⁺ free HBSS (Grand Island Biological Co., Grand Island, NY) and resuspended in McCoy's 5a medium (Grand Island) containing 5% heat-inactivated fetal bovine serum (HIFBS) (KC Biological Co., Lenexa, KS) supplemented with 100 U/ml penicillin, and 100 µg/ml streptomycin. Total cells were counted on a hemocytometer, viability was assessed by cellular exclusion of trypan blue, and differential cell counts were made on Wright-stained cytocentrifuge preparations. Lavage cells prepared in this way averaged (mean±SEM) 85.1±1.0% AM, 13.4±1.0% lymphocytes, 1.1±0.1% polymorphonuclear cells, 0.7±0.2% mast-basophiloid cells, and 0.5±0.1% eosinophils, *n* = 85. Macrophage viability averaged 88.7±0.6%. Platelet contamination, estimated from the small number of red blood cells, 9.8±1.7% of total cells recovered, present in the lavage fluid due to trauma of the airway epithelium by the tip of the bronchoscope, never exceeded one platelet per two nucleated cells. For the cell cultures used in the assessment of leukotriene release, lavage cells were adjusted to 2 × 10⁶ cells per ml and placed into sterile 7-ml Teflon chambers (Scientific Specialties, Randallstown, MD).

Preparation of HAIgG. The gamma-globulin fraction from 30–50 ml of fresh human serum was obtained by precipitation with one-third saturated ammonium sulfate, extensively dialyzed against 0.05 M Tris-HCl, pH 8.0, and was partially purified by ion exchange column chromatography on diethylaminoethyl cellulose (DEAE 52, Whatman; H. Reeve Angel, Inc., Clifton, NJ). This was followed by dialysis against PBS 0.1 M, pH 7.2, and 0.1% sodium azide, with subsequent filtration on Sephadex G-200-gel bed volume 150 ml (Pharmacia Fine Chemicals, Piscataway, NJ) that had been previously calibrated with the following standards: blue dextran, 11s-IgA (kindly provided by Dr. H. Y. Reynolds, Yale University School of Medicine), IgG, and albumin. The IgG containing eluates from each column were detected by absorbance at 280 nm and their known elution times for these columns. After the initial isolation of IgG from the G-200 column, the IgG was concentrated by positive pressure ultrafiltration through a UM010 membrane (Amicon Corp., Lexington, MA). HAIgG was prepared by incubating the IgG (10 mg/ml) at 63°C until slight opalescence appeared and then centrifuging at 3,000 rpm for 10 min (30). The supernatant then was refiltered over the Sephadex G-200 column and the void volume containing the IgG aggregates was retained, concentrated by positive pressure ultrafiltration, and dialyzed into Dulbecco's PBS, pH 7.2 (Gibco Laboratories, Grand Island, NY). The presence and purity of IgG aggregates was verified by immunoprecipitation diffusion-in-gel analysis using specific rabbit anti-human albumin, transferrin, whole serum, and immunoglobulin G and A antisera (Calbiochem-Behring Corp., San Diego, CA) and calculated to be > 99% pure. After sterilization by filtration through a 0.2-µm membrane, the amount of HAIgG was quantified by the method of Lowry (31) using bovine gamma globulin to generate the standard curve (Bio-Rad Laboratories, Richmond, CA). The presence of endotoxin in the HAIgG preparations and cell culture supernatants was quantitated using a Limulus assay sensitive to 1.0 ng/ml, as described (32).

Incubation of lavage cells with recombinant interferons-alpha (IF-α), beta (IF-β), or gamma (IF-γ). Bronchoalveolar lavage cells were incubated for 1 or 24 h at 37°C in a humidified atmosphere containing 5% CO₂ with 10, 100, or 1,000 U/ml of recombinant IF-γ or without interferon (Hoffmann-La Roche, Nutley, NJ). The units of the stock solution were determined by a WISH/VSV microtiter assay that utilized National Institutes of Health (NIH) standard Gg 23-901-530. The cells then, were centrifuged at 500 g for 5 min and resuspended in HBSS containing Ca²⁺ and Mg²⁺ immediately before stimulation. In those experiments that utilized IF-α and IF-β (Hoffmann-La Roche), the cell cultures were incubated under identical conditions at a single interferon concentration, 1,000 U/ml.

Stimulation of human AM. Bronchoalveolar lavage cells, after incubation with IF-γ as described above, were incubated for 30 min at 37°C, in an atmosphere of air and 5% CO₂ and with media containing

2 µM A23187 (Calbiochem-Behring Corp.), which was dissolved in dimethylsulfoxide and diluted to 0.1% (vol/vol) in the cell cultures. Then, the cells were centrifuged at 500 g for 5 min and the supernatants were harvested and stored at -70°C until assayed by RIA for LTB₄. Preliminary observations in our laboratory indicated that storage of macrophage supernatants under these conditions for as long as one year did not adversely affect the recovery of immunoreactive LTB₄. When reverse-phase high performance liquid chromatography (RP-HPLC) was to be performed for resolution of LTB₄, the supernatants were mixed with 4 vol of ethanol before being stored at -70°C. Just before chromatography, the samples were warmed to 4°C and centrifuged at 2,000 g for 10 min at 4°C to remove precipitated proteins. The supernatants were evaporated to dryness under reduced pressure in a Speed Vac Concentrator (Savant Products, Hicksville, NY) and resuspended in either RIA buffer for LTB₄ determination or mobile phase solvent for RP-HPLC.

Some of the selected cell cultures, which were to be stimulated by HAIgG, were first treated with IF-γ, then washed and resuspended in HBSS and challenged with 25–50 µg/ml of HAIgG for 1 h at 37°C, under 5% CO₂. The cell cultures were processed for LTB₄ by RIA with or without prior RP-HPLC resolution, as described for the cells activated with A23187. In some experiments lipopolysaccharide (LPS), type Salmonella Minnesota R595 (List Biological Laboratories, Campbell, CA) was added to IF-γ treated cell cultures in place of or in addition to HAIgG, to assess whether or not endotoxin was involved in the release of LTB₄. In addition, experiments were performed in which a monoclonal anti-IF-γ antibody (Meloy Laboratories, Springfield, VA) was added to AM cell cultures at the same time as the recombinant IF-γ. The AM then were cultured for 24 h before washing and stimulation with HAIgG. In other experiments monomeric IgG₁ was added at a final concentration of 50–100 µg/ml to AM cell cultures 10 min before the addition of HAIgG.

Quantitation of LTB₄ and LTC₄. LTB₄ was quantitated by either of two radioimmunoassays using immune rabbit plasmas provided by Dr. E. Hayes, Merck & Co., Rahway, NJ (33). The first was a double-antibody RIA. Briefly, 200 µl of immune rabbit serum, diluted to give 50% binding of [³H]LTB₄, were added to a mixture of 200 µl of PBS/BSA/azide containing ~ 7,000 cpm of [³H]LTB₄ (New England Nuclear, Boston, MA) and 100 µl of sample or a synthetic LTB₄ standard (J. Rokach, Merck Frosst, Québec, Canada). This mixture was incubated for 2 h at 4°C before the addition of 500 µl of a 1:5 dilution of goat anti-rabbit IgG (Bio-Rad Laboratories), and the incubation was continued for 18–20 h. The immune precipitates were sedimented at 12,000 g for 3 min and 800 µl of the solution phase added to 10 ml of scintillation cocktail, and assessed for radioactivity in a liquid scintillation counter. There was no difference in standard curves run in the presence or absence of 1,000 U/ml of IF-γ. The minimum quantities of LTB₄ detectable with this assay were 1.5 ng/ml. The second and more sensitive RIA (minimum detectable levels of 0.5 ng/ml) employed dextran-coated charcoal to separate antibody-bound LTB₄ from unbound LTB₄ (34). Results from the two assays were compared to each other and to results obtained with RP-HPLC on aliquots of cell supernatants. LTC₄ was quantitated using a charcoal RIA which was able to detect a minimum of 0.5 ng/ml and was purchased from New England Nuclear. Quantities of leukotriene in nanograms per milliliter were adjusted to nanograms/10⁶ viable alveolar macrophages.

RP-HPLC. RP-HPLC was performed for some experiments using a Waters system with a NOVA PAK C18 column (3.9 × 150 mm) and for other experiments on an Altex-Beckman system (35) with a 10-µm C-18 Ultrasil ODS column (4.6 × 250 mm) (Altex Rainin, Berkeley, CA) and eluted isocratically with a mobile phase consisting of methanol/water/acetic acid (67;33;0.08, vol/vol; pH adjusted to 5.85 with ammonium hydroxide) at a flow rate of 1 ml/min with continuous online monitoring at 280 nm (36). The performance of the two chromatography systems was essentially equivalent. Synthetic LTC₄, LTD₄, and LTE₄ standards were provided by J. Rokach.

IgG rosette assay. Rosette assays were performed as described by Spiegelberg and co-workers (37). Briefly, ox erythrocytes (Colorado

Serum, Denver, CO) were washed thoroughly in PBS and were sensitized with that subagglutinating concentration of rabbit IgG anti-ox red blood cell antibody (Cappel Laboratories, Cochranville, PA) which resulted in rosette formation of ~ 40% of fresh human alveolar macrophages. The rosette assays were performed by mixing 50 μ l of 1% ox erythrocytes, suspended in McCoy's 5a medium supplemented with 5% HIFCS, with 50 μ l of lavage cells (5×10^6 /ml) suspended in the same medium. The mixture was centrifuged at 200 *g* for 1 min and incubated on ice for 1 h. The cells then were agitated to gently resuspend them, and the number of cells bearing three or more erythrocytes counted as IgG receptor positive. Assays performed using ox erythrocytes not coated with anti-ox cell antibody served as controls.

Purification and iodination of IgG₁ monomer. IgG₁ monomer was prepared from the serum of a patient with monoclonal IgG₁ myeloma as summarized previously (38). Briefly, serum gamma globulin was twice precipitated, first with the addition of dry weight ammonium sulfate to 30% concentration, and then the supernatant was adjusted to 50% concentration. The precipitates were dialyzed extensively against 0.01 M PBS/azide, pH 7.4, to remove ammonium sulfate and then against 0.02 M Tris-HCl in preparation for anion exchange column chromatography on DEAE-52. The IgG was eluted with a logarithmic gradient of 0.5 M NaCl in 0.02 M Tris-HCl. The IgG-rich fractions were gel filtered on Sephadex G-200. The fraction that eluted after an 11S-IgA standard was collected, concentrated, and iodinated with ¹²⁵I (New England Nuclear) by the lactoperoxidase-glucose oxidase method (Enzymobeads; Bio-Rad). Briefly, 15 μ l of 11.0 mg/ml monomeric IgG₁ were added to 50 μ l of preswollen enzymobeads and 50 μ l of 0.2 M phosphate buffer, pH 7.2. After adding 1.0 mCi of ¹²⁵I, the reaction was initiated by adding 50 μ l of 1% B-D-glucose, and continued for 30 min at room temperature; the reaction was terminated by the addition of 50 μ l of 1% sodium azide. Iodinated IgG₁ was separated from unbound ¹²⁵I by filtration over a Bio-Gel P-6DG (Bio-Rad) column. In all instances over 95% of the radioactivity was precipitable with 20% TCA. Ultracentrifugation of a trace quantity of the iodinated IgG₁ on a 10–40% sucrose density gradient at 35,000 *g* for 18 h verified that the IgG₁ was monomeric and that the iodination procedure did not result in aggregation of the ligand. IgG₁ concentrations in the final preparations were determined by the method of Lowry (31) using an IgG standard (Bio-Rad). The mean \pm SEM specific activity was 1.43 ± 0.37 μ Ci/ μ g IgG₁. ¹²⁵I-IgG₁ was stored at 4°C and was used within 2 wk of preparation.

Binding studies of monomeric ¹²⁵I-IgG₁ to AM. The binding of radioligand to alveolar macrophages was carried out in PBS (Dulbecco's; Gibco) pH 7.2, supplemented with 0.1% sodium azide, 0.1% EDTA, and 0.1% human serum albumin (39). For Scatchard analysis, duplicate suspensions of alveolar macrophages (200,000–400,000 cells per sample) were incubated with radioligand concentrations ranging from 1 to 250 nM in polypropylene microfuge tubes at 37°C for 1 h, since preliminary kinetic studies demonstrated that equilibrium was established at these conditions. At the end of the incubation period the tubes were mixed gently and 50- μ l aliquots centrifuged through 200 μ l of a 4:7 (vol/vol) mixture of dioctyl phthalate/dibutyl phthalate oil (Aldrich Chemical Co., Milwaukee, WI) for 1 min at 10,000 *g* in a microfuge (Fisher Scientific Corp., Springfield, NJ).

The tubes then were frozen in methanol, which was cooled with dry ice, and the tubes were cut above the cell pellet. Both cell pellet and supernatant were counted in a gamma counter. Nonspecific binding to cells always was determined at the same time by incubating duplicate cell suspensions with radioligand and a 100-fold excess of unlabeled IgG₁. Binding of ¹²⁵I-IgG₁ was calculated in molecules per macrophage present in the cell suspensions. The binding of radioligand to lymphocytes was ignored because lymphocyte contamination of the macrophage cultures used for binding studies was limited to $15.5 \pm 3.4\%$, *n* = 9, but more notably because lymphocytes possess minimal numbers of IgG receptors (39). Binding data were calculated as described by Scatchard (40). Linear regressions (TI-55; Texas Instruments, Lubbock, TX) were performed by plotting the ratio of bound/free ¹²⁵I counts per minute against the nanomolar amount of bound ¹²⁵I-IgG₁.

The number of binding sites per cell was calculated from the *x* axis intercept. The equilibrium association constant (*K_a*), expressed in liters/mol (*M*⁻¹), was determined from the negative slope.

Determination of AM cell volume. Macrophage cell volume was determined by incubating a 1-ml aliquot of lavage cells (1×10^6 cells) with 2.5 μ Ci [³H]H₂O (New England Nuclear) and 0.5 μ Ci of [¹⁴C]-inulin (Amersham Corp., Arlington Heights, IL) for 1 min at room temperature. 50, 100, and 200 μ l aliquots were placed in 400- μ l eppendorf microfuge tubes, which contained 120 μ l of a mixture of paraffin and silicone oil (16:84 vol/vol) layered over 35 μ l of 15% TCA in water. The cells were centrifuged into the TCA in a Beckman model B microfuge at 10,000 *g* for 1 min. The difference between the total pellet volume of the water (³H-related dpm) and the extracellular volume (¹⁴C-related dpm) was taken as the intracellular macrophage volume (41).

Statistical analyses. All data are expressed as the mean \pm SEM. For normally distributed data statistical calculations were performed using a paired *t* test (two tailed). For nonparametric data, the Wilcoxon's signed rank test or the sign test were used for paired data. Comparisons between groups of subjects were made using the Wilcoxon two-group rank sum test. All analyses were performed using the NIH sponsored CLINFO project. *P* values \leq 0.05 were considered significant.

Results

LTB₄ release from human AM stimulated with calcium ionophore or HAIgG. Human AM cultures stimulated for 30 min at 37°C with 5 μ M A23187 released a mean \pm SEM of 86.9 ± 14.9 ng of immunoreactive LTB₄/10⁶ viable AM, range 24–178 ng, (Fig. 1). Preliminary A23187 dose-response experiments determined that maximal LTB₄ release occurred with between 2 and 10 μ M A23187. Previous experiments in our laboratory using rat AM (42) demonstrated that maximal LTB₄ release occurs between 10 and 30 min after challenge with A23187; hence the 30-min time point was used in our experiments. As assessed by the double-antibody RIA, incubation with 25–50 μ g/ml of HAIgG for 60 min resulted in the release of 1.6 ± 0.5 ng/10⁶ viable AM, which was not significantly different than that released from cells incubated in medium alone, 1.2 ± 0.8 ng/10⁶ viable AM (*n* = 12). However, when LTB₄ in cell supernatants of AM from a second group of volunteers was quantitated using the more sensitive dextran-coated charcoal RIA, HAIgG was found to elicit the release of LTB₄, 8.9 ± 2.8 ng/10⁶ cells, that was significantly greater (*P* < 0.01) than that seen in supernatants from alveolar macro-

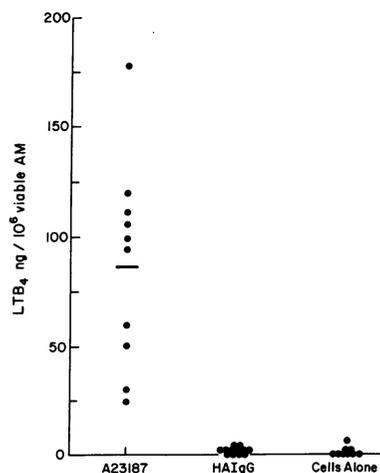


Figure 1. Human alveolar macrophage release of immunoreactive LTB₄, as assessed by the double-antibody RIA, in response to the addition of A23187, HAIgG or medium alone. Each point represents the mean of duplicate determinations for a single experiment.

phages incubated with medium alone, 2.5 ± 0.4 ng/ 10^6 cells ($n = 15$).

To determine if these differences were due to variances in measurements by the two RIAs, we compared LTB₄ concentrations in aliquots of 10 additional supernatants from cells stimulated with A23187, HAIgG, or buffer alone. We found no significant difference ($P < 0.6$) in the amounts of LTB₄ determined with either RIA. The correlation coefficient between values obtained with the two assays was excellent, $r = 0.967$, $P < 0.001$. More importantly, LTB₄ concentrations quantitated using the more sensitive dextran-charcoal RIA were within a mean of $33.0 \pm 6.3\%$ of the determinations made with the double-antibody RIA. The differences in LTB₄ determined with each RIA were similar at both high and low LTB₄ ranges. When the results of all supernatants from both groups of subjects were combined without regard to the RIA used, AM challenged with HAIgG generated and released 5.7 ± 1.7 ng/ 10^6 viable AM compared with cells incubated with buffer alone, which released 1.9 ± 0.4 ng/ 10^6 viable AM, $P = 0.02$, ($n = 27$). Thus, HAIgG is an effective stimulus for the release of LTB₄ by AM from some normal nonsmoking subjects.

Release of LTB₄ from human AM preincubated with IF- γ . The time course for release of immunoreactive LTB₄ from AM after incubation with 1,000 U/ml of IF- γ for 24 h at 37°C and subsequent to challenge with HAIgG was assessed using the double-antibody RIA (Fig. 2). When macrophage cell cultures were challenged at 37°C with 25–50 μ g/ml of HAIgG for times varying between 2.5 min and 3 h, net release of immunoreactive LTB₄ occurred in some instances at the earliest time point measured, 2.5 min. Maximal release of LTB₄ varied between experiments, occurring between 2.5 min and 1 h after incubation with HAIgG. Therefore, a 1-h incubation period was utilized for all subsequent experiments involving HAIgG challenge. Detection of immunoreactive LTB₄ decreased progressively in all experiments by 2 h. The decline in the quantities of LTB₄ detected over time in the macrophage supernatants was not due to absorption of leukotriene onto the Teflon chamber because, when 5,000 cpm of [³H]LTB₄ were added to 1 ml of macrophage culture medium containing either A23187 or HAIgG and the mixtures were incubated at 37°C for 90 min,

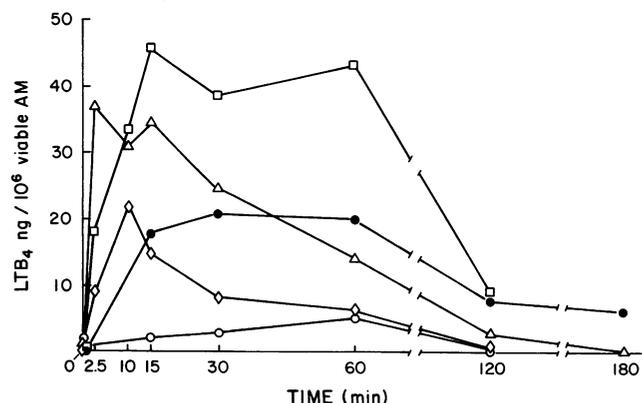


Figure 2. Time-response curve for release of immunoreactive LTB₄, as assessed by the double-antibody RIA, from AM preincubated with 1,000 U/ml of IF- γ for 24 h and then challenged with HAIgG. Each symbol represents determinations made from a separate subject. Each point represents the mean of duplicate determinations.

> 90% of the counts were recovered in the supernatants of mixtures in all instances. In addition, when 40,000 cpm of [³H]LTB₄ were incubated for either 15 min or 2 h with IF- γ primed AM, no conversion to either 20-hydroxy-LTB₄ or 20-carboxy-LTB₄ was observed. However, after 2 h, > 50% of the counts were converted to two products more polar than the omega oxidation metabolites of LTB₄. Further investigations into the identities of these metabolites currently are being performed.

HAIgG dose-response curves were performed using concentrations ranging from 2.5 ng to 250 μ g/ml on AM preincubated with 1,000 U/ml of IF- γ (Table I). The HAIgG dose-response of LTB₄ release was progressive up to a concentration of 25 μ g/ml and remained constant at the highest concentration, 250 μ g/ml.

In paired experiments, the effects of incubating alveolar macrophages without IF- γ or with 10 U/ml, 100 U/ml, and 1,000 U/ml of IF- γ for either 1 h or 24 h before stimulation with either A23187 or HAIgG were assessed using the double-antibody RIA. None of these concentrations of IF- γ alone could elicit release of immunoreactive LTB₄ after 1 or 24 h in two separate experiments. The results (not shown) reveal that preincubation of macrophages for 1 h in the absence or presence of IF- γ (1,000 U/ml) did not affect the release of immunoreactive LTB₄ induced with either HAIgG ($n = 7$) 3.0 ± 2.7 vs. 3.6 ± 2.3 ng/ 10^6 AM $P = 0.5$ or with A23187 ($n = 8$) 69.4 ± 19.4 vs. 105.2 ± 26.0 ng/ 10^6 viable AM without or with IF- γ , respectively. In contrast, HAIgG augmented LTB₄ release when macrophages were preincubated for 24 h with IF- γ , Fig. 3 A. This effect of IF- γ on macrophage release of immunoreactive LTB₄ was dose dependent. A statistically significant increase in immunoreactive LTB₄ release was observed from cells incubated with as little as 10 U/ml of IF- γ ($P = 0.04$). The effect of IF- γ was maximal at the highest concentration utilized in this study, 1,000 U/ml, ($n = 12$), 11.3 ± 3.1 ng vs. 1.6 ± 0.5 ng/ 10^6 viable AM for cells not primed with IF- γ , Fig. 3 A. In 9 of the 12 experiments the increase in LTB₄ release from primed AM exceeded 100%. In an additional 15 experiments where AM were cultured either without IF- γ or with 1,000 U/ml of IF- γ , and LTB₄ was assayed with the dextran-coated charcoal RIA, HAIgG induced the release of 8.9 ± 2.8 and 21.6 ± 6.5 ng of LTB₄/ 10^6 viable AM, respectively (data not shown, $P = 0.01$). In these later experiments concentrations of LTB₄ rose by > 100% in 8 of the 15 experiments. The ability of IF- γ to augment HAIgG induced LTB₄ release was not due to a stimulus-independent increase in the overall capacity of the cell to generate LTB₄, because A23187-induced release of LTB₄ from unprimed AM, 86.9 ± 15.0 ng/ 10^6 viable AM, did not change statistically after macrophages were incubated with IF- γ under similar conditions, 60.9 ± 11.9 ng/ 10^6 viable AM ($n = 12$) (Fig. 3 B).

Because stimulation of polymorphonuclear leukocytes with unopsonized zymosan results in the cellular retention of ~ 80% of the generated LTB₄ (35), we assessed the cellular and extracellular distribution of LTB₄ generated from macrophages incubated with 1,000 U/ml of IF- γ for 24 h and subsequently challenged with HAIgG or A23187. In two experiments A23187 stimulated macrophages released into the supernatants 110.0 and 102.7 ng of immunoreactive LTB₄/ 10^6 viable AM, whereas 4.7 ng (4%) and 5.3 ng (5%) remained cell associated (assessed by the double-antibody RIA). Similarly, cells incubated with IF- γ and stimulated with HAIgG released

Table I. Dose-dependent Effect of HAIgG on the Release of Immunoreactive LTB₄ by AM Primed for 24 h with 1,000 U/ml of IF-γ*

Macrophage donor	HAIgG (μg/ml)					
	0.0025	0.025	0.25	2.5	25	250
1	0	0	0.9	0	0.92	1.9
2	0	0	1.1	3.3	40.0	10.8
3	0	0	0	0	8.5	3.3
4	ND [‡]	0	0	0	10.7	11.5
5	ND	ND	1.2	6.9	37.2	22.0
Mean±SEM	0	0	0.6±0.3	2.0±1.4	19.5±7.9	9.9±3.6

* LTB₄ expressed in ng/10⁶ viable AM and assessed by either the double-antibody or dextran-charcoal RIA. ‡ ND not determined.

13.5 and 10.3 ng of immunoreactive LTB₄/10⁶ viable AM into the supernatants with 0.4 ng (3%) and 0.7 ng (7%) remaining cell associated.

Cell supernatants of macrophages cultured for 24 h in the absence or presence of IF-γ (1,000 U/ml) and subsequently challenged with A23187 or HAIgG were examined for the presence of LTC₄ by a charcoal RIA and RP-HPLC. LTC₄ was not detected by RP-HPLC and was detected in only minimal quantities by RIA in supernatants from A23187 stimulated cells preincubated in the absence of IF-γ, 0.9±0.6 ng, or presence of IF-γ, 0.7±0.6 ng LTC₄/10⁶ viable AM (*n* = 9) and in only minimal quantities in supernatants from macrophages stimulated with HAIgG after preincubation in the absence of IF-γ, 0.3±0.2 ng or presence of IF-γ, 0.2±0.1 ng/10⁶ viable AM (*n* = 9), respectively.

Effect of cytochalasin B and endotoxin on HAIgG-induced LTB₄ release from AM cultured with IF-γ. Experiments were performed to determine whether phagocytosis of HAIgG was required for LTB₄ release. In three paired experiments, alveolar macrophages were incubated with or without cytochalasin B, 5 μg/ml, for 10–15 min at room temperature after culture with IF-γ for 24 h and before challenge with HAIgG. In pre-

liminary experiments we had observed that this concentration of cytochalasin B eliminated the capacity of human AM to ingest three or more IgG coated ox red blood cells. Cytochalasin B at this concentration and for this incubation period did not, however, decrease the quantity of immunoreactive LTB₄ released. Rather, cells cultured with HAIgG in the presence of cytochalasin B released 88.6±47 ng of immunoreactive LTB₄/10⁶ viable AM, whereas cells not treated with cytochalasin B released 12.1±2.8 ng/10⁶ viable AM as assessed by the double-antibody RIA. A similar effect of cytochalasin B on leukotriene release from human peripheral blood monocytes (43) and leukocytes (44) has been observed.

Undetectable quantities (< 1.0 ng/ml) of endotoxin were present in 25–50 μg/ml dilutions of two separate HAIgG preparations. The highest concentration of LPS detected in macrophage supernatants tested after challenge with HAIgG was 100 ng, *n* = 12. Therefore, we assessed whether or not LPS contaminating our cultures was responsible in part, or totally for HAIgG-induced LTB₄ release. In three sets of paired experiments AM preincubated with IF-γ and challenged with HAIgG released 14.0±7.0 ng of immunoreactive LTB₄/10⁶ viable AM, while macrophages preincubated with IF-γ and challenged with HAIgG plus 100 ng LPS released 8.7±10.5 ng/10⁶ viable AM; macrophages incubated with IF-γ and challenged with 100 ng of LPS alone for 60 min released minimal quantities of LTB₄, 0.7±0.5 ng/10⁶ viable AM. To assess whether or not LPS present in the cell cultures served as a cofactor with IF-γ in priming AM for enhanced LTB₄ release, five paired experiments were performed in which AM were preincubated for 24 h with IF-γ (1,000 U/ml) in the presence or absence of a monoclonal anti-IF-γ antibody. That portion of the LTB₄ response augmented by preincubation with IF-γ was completely eliminated in three of the five experiments and was decreased by 44% and 70% in the remaining two, suggesting, but not proving, that IF-γ alone was responsible for the priming effect observed.

Reverse-phase HPLC. RP-HPLC was utilized to verify that the immunoreactive material in macrophage supernatants was LTB₄. For these experiments aliquots of supernatants from noninterferon and IF-γ (1,000 U/ml)-treated cell cultures which had been challenged with HAIgG, *n* = 3, were pooled, extracted with methanol, and subjected to RP-HPLC (Fig. 4). Chromatographic peaks which coeluted with synthetic LTB₄ standard were observed in both aliquots. As determined by double-antibody RIA, 17.0 ng of LTB₄ from supernatants

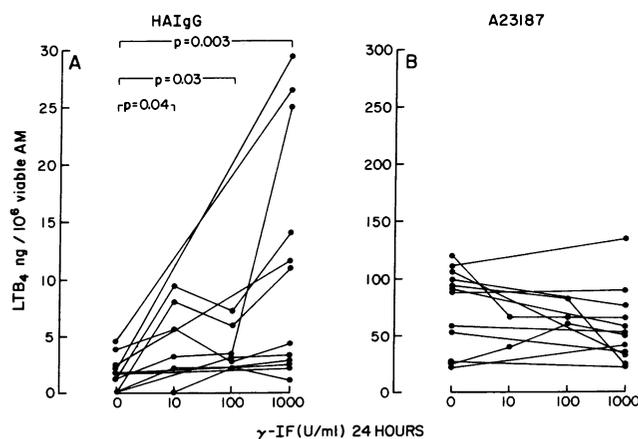


Figure 3. Dose-dependent effect of IF-γ on release of immunoreactive LTB₄, as assessed by the double-antibody RIA, from AM in response to HAIgG or A23187. AM were cultured in the presence of 0–1,000 U/ml of IF-γ for 24 h before challenge with HAIgG (A) or with A23187 (B). Each point represents the mean of duplicate determinations.

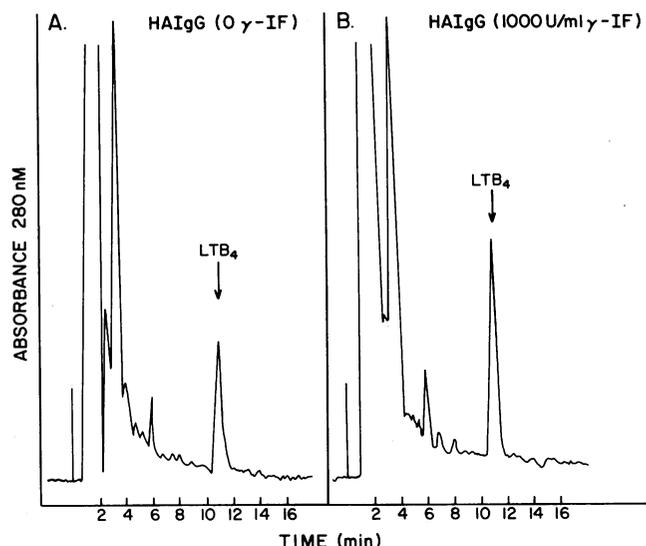


Figure 4. RP-HPLC resolution of methanol-extracted aliquots of supernatants from cell cultures incubated for 24 h in the absence of IF- γ before challenge with HAIGG (A), or in the presence of 1,000 U/ml of IF- γ (B) before challenge with HAIGG. The retention times of pure synthetic LTB₄ standard are indicated by the arrow.

from macrophages that were not treated with IF- γ and 32.4 ng of LTB₄ from IF- γ -treated macrophages were injected. 74.8% and 63.7% of these amounts, respectively, were recovered, as determined by optical density at 280 nM. No LTC₄ or LTD₄ was observed by ultraviolet absorbance after elution from RP-HPLC.

Specificity of effect for IF- γ . To assess the specificity of the effect of IF- γ on macrophage release of LTB₄ in response to HAIGG, paired experiments were performed in which macrophages were incubated alone or with 1,000 U/ml of either IF- α , IF- β , and with IF- γ for 24 h and then challenged with

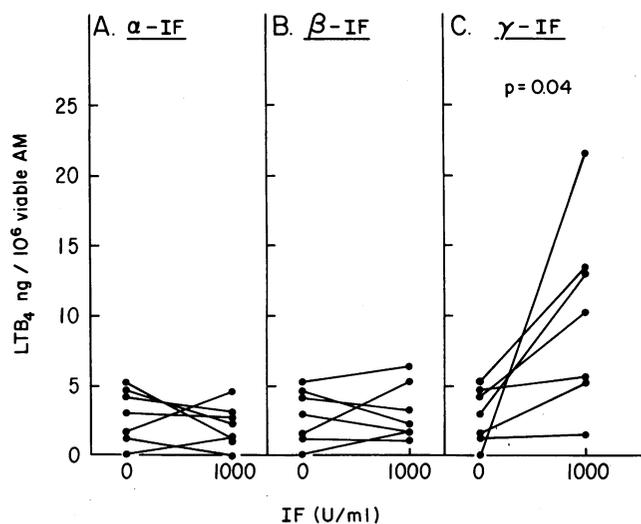


Figure 5. Specificity of the effect of IF- γ . Comparison of the effect of IF- α (A), IF- β (B) and IF- γ (C) on AM release of immunoreactive LTB₄, as assessed by the double-antibody RIA, in response to stimulation with HAIGG. Each point represents the mean of duplicate determinations.

HAIGG (Fig. 5). As assessed by the double-antibody RIA, macrophages challenged with HAIGG after culture with IF- α released 2.2 ± 0.6 ng of immunoreactive LTB₄/10⁶ viable AM and after culture with IF- β , 3.2 ± 0.8 ng/10⁶ viable AM, which was not significantly different from that released from cells challenged with HAIGG after culture in the absence of either interferon, 2.8 ± 0.8 ng/10⁶ viable AM, $n = 7$. In contrast, in these same experiments macrophages incubated with IF- γ and challenged with HAIGG released 10.2 ± 2.6 ng/10⁶ viable AM, which was significantly different from that released without interferon, 2.9 ± 0.8 ng/10⁶ viable AM, ($P = 0.04$), $n = 7$.

Effect of interferons on alveolar macrophage IgG receptors. In three experiments we assessed the effect of IF- γ on the percentage of alveolar macrophages forming rosettes with IgG-coated ox red blood cells. After incubation in the absence of IF- γ for 24 h at 37°C, $39.3 \pm 10.6\%$ of macrophages were IgGFcR+, whereas incubation of macrophages with 1,000 U/ml of IF- γ for 24 h resulted in a statistically significant increase in the percent of IgGFcR+ macrophages to $61.3 \pm 8.3\%$, $P < 0.04$. These data suggested that either the number or the density of IgGFcR receptors on the cell surface increased or that the binding affinity of receptor for ligand increased in response to incubation with IF- γ .

These possibilities were explored further using Scatchard analyses of radioligand ¹²⁵I-IgG₁ binding. Insufficient numbers of cells were retrieved by BAL from the lungs of nonsmoking normal volunteers to permit the performance of radioligand binding studies on cells cultured in the absence of interferon and with each class of interferon in a single experiment. Therefore, paired experiments were performed on cells cultured in the absence or presence of IF- γ . Additional paired experiments then were performed on AM cultured without interferon and with cells incubated with IF- α or IF- β . Scatchard analysis of ¹²⁵I-IgG₁ binding to AM revealed a single binding site on IF- γ treated cells and on control cells (Fig. 6). The mean K_a for cells incubated in the absence of IF- γ was $4.5 \pm 1.1 \times 10^8$ M⁻¹, which was not significantly different from that of cells incubated with 1,000 U/ml of IF- γ for 24 h, $3.3 \pm 0.6 \times 10^8$ M⁻¹ (Table II). Similarly, no change in the K_a was observed for cells cultured with either IF- α or IF- β (Table II). However, the number of IgG₁FcR on macrophages which were not pre-treated with interferon, $168,700 \pm 16,995$ increased approxi-

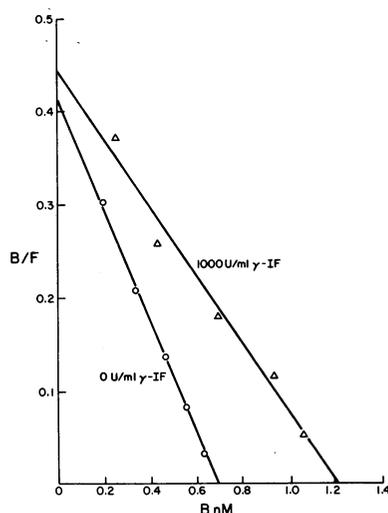


Figure 6. Representative Scatchard plot of data from a single experiment for ¹²⁵I-IgG₁ binding to AM. Human AM were cultured in the presence (Δ) or absence (\circ) of 1,000 U/ml of IF- γ for 24 h and the binding characteristics were determined.

Table II. Normal Human Alveolar Macrophage IgG₁ Receptor Data*

IF (U/ML)	K _a (M ⁻¹)	IgGFcR/cell	Cell volume ($\mu\text{l} \times 10^{-7}/\text{cell}$)
Gamma- (<i>n</i> = 7) [§]			
0	4.5±1.1 × 10 ⁸	168,700±16,995	0.71±0.2
1,000	3.3±0.6 × 10 ⁸	391,340±51,982 [‡]	0.90±0.1
Alpha- (<i>n</i> = 5)			
0	2.9±0.4 × 10 ⁸	107,810±14,948	—
1,000	2.6±0.3 × 10 ⁸	128,450±19,177	—
Beta- (<i>n</i> = 5)			
0	2.9±0.4 × 10 ⁸	107,810±14,948	—
1,000	3.1±0.3 × 10 ⁸	110,680±21,891	—

* Expressed as mean±SEM.

[‡] *P* < 0.02.

[§] (*n*) number of experiments.

mately twofold to 319,340±51,982 after incubation for 24 h with IF- γ (*P* < 0.02). Culture of macrophages with IF- α or IF- β at 1,000 U/ml did not induce a statistically significant increase in IgG₁FcR on the AM (Table II). This increase in receptor number represented an increase in surface IgG₁ receptor density because the relative macrophage cell volumes did not change after incubation with IF- γ (Table II).

To assess whether or not HAIgG induced LTB₄ release from IF- γ primed AM occurred via binding to the IgG₁FcR on AM, experiments were performed in which IF- γ prepared AM were stimulated with HAIgG (25 $\mu\text{g}/\text{ml}$) in the presence or absence of 50–100 $\mu\text{g}/\text{ml}$ of IgG₁ monomer (Table III). In 6/6 experiments IgG₁ monomer inhibited HAIgG-induced LTB₄ release, supporting the contention that HAIgG effected LTB₄ release as assessed by the dextran-coated charcoal RIA, in large part through coupling with AM IgG₁FcR.

Discussion

The results of this study demonstrate that soluble aggregates of human IgG are an effective stimulus for LTB₄ release from normal human AM, and that release due to HAIgG is enhanced significantly when these cells are preincubated with

Table III. IgG₁ Monomer Inhibition of HAIgG-induced LTB₄ Release*

Macrophage donor	IF- γ 1,000 U/ml		HAIgG
	HAIgG	HAIgG + IgG ₁	
1	68.6	39	1.9
2	38.7	22.9	3
3	14.8	3.7	5.6
4	18.9	15.3	9.2
5	8.0	2.2	3.5
6	18.5	6.7	15.1

* LTB₄ expressed in ng/10⁶ viable AM, as assessed by the dextran-charcoal RIA.

IF- γ . Under the conditions employed in our experiments, HAIgG was a potent cell activator. HAIgG induced the generation and release of 5.7±1.7 ng (*n* = 27) of LTB₄ from 10⁶ viable and not purposefully activated AM. After incubation with IF- γ for 24 h, 1 × 10⁶ AM released a mean of 17.0±3.9 ng of LTB₄ (*n* = 27), *P* = 0.001, with a range of 1 to 88 ng and corresponding to a concentration range of $\sim 6 \times 10^{-9} - 5.6 \times 10^{-7}$ M. These concentrations are functionally significant, considering that LTB₄-mediated chemokinesis of neutrophils has an EC₅₀ in the range of 1 to 10 × 10⁻¹⁰ M (45), and only slightly higher concentrations, 3 × 10⁻⁹ M, are chemotactic for neutrophils (46). LTB₄ also demonstrates chemokinetic and chemotactic activity for eosinophils (10–13) and monocytes (8, 14) at concentrations found in the supernatants of 10⁶ viable AM. Furthermore, concentrations of LTB₄ in the picomolar to nanomolar range exert profound immunosuppressive effects on immunoglobulin synthesis, which is due to induced differentiation of T-suppressor cells (16, 18). Therefore, it is reasonable to conclude that IgG immune complexes can, under appropriate conditions, stimulate human AM release of LTB₄ in sufficient quantities to profoundly influence lung inflammatory events.

The suspensions of BAL cells used in our experiments contained predominately AM and much smaller relative numbers of lymphocytes, neutrophils, mast-basophiloid cells and eosinophils. It is unlikely that these cells contributed significantly to the LTB₄ present in the cell supernatants because lymphocytes synthesize comparatively little LTB₄ (47) and because the other cells were not present in sufficient numbers to contribute significant quantities of LTB₄.

Other investigators have reported that human peripheral blood monocytes, precursors to alveolar macrophages, also will release LTB₄ by IgG dependent mechanisms (43, 48). In these experiments, on nanogram/10⁶ cells basis, the quantities of product released in response to an IgG agonist were in the range of 2 ng, which is approximately one-half of that which we observed from 10⁶ AM that were not primed with IF- γ or one-eighth of that released from IF- γ primed cells. Human AM produce ~ 10 times the amount of LTB₄ as peripheral blood monocytes when stimulated with A23187 (49). Therefore, it is possible that the amounts of LTB₄ generated by AM stimulated with HAIgG is large relative to peripheral blood monocytes because of a greater expression of the enzymes of the 5-lipoxygenase pathway or a more efficient processing of substrates.

“Spontaneous” release of LTB₄ into each milliliter of culture media by 1 × 10⁶ unprimed and not purposely activated AM averaged 1.2±0.8 ng, range 0–7 ng (Fig. 1). This is much higher than the amounts reported to be released from unstimulated peripheral blood monocytes (43), and suggests that in the “resting state,” AM are in a higher state of activation with respect to LTB₄ production than are peripheral blood monocytes, a conclusion supported by others (49). Assuming that the differences are not due to differences in the methods of cell harvesting, this would not be surprising in view of the fact that alveolar macrophages are constantly in contact with gasses, fumes, and particulate matter present in the atmosphere and inhaled each day.

Release of LTB₄ by HAIgG from IF- γ primed AM was generally rapid but the total immunoreactive LTB₄ had decreased by 2 h in the supernatants of AM from all of five subjects (Fig. 2). This was not due to a decrease in cell viability,

nor was it because of loss of immunoreactive LTB₄ in the cell supernatants due to adherence of product to the culture chamber surfaces. Analysis of AM supernatants with added [³H]LTB₄ by RP-HPLC did not reveal omega-hydroxy-LTB₄ or omega carboxy-LTB₄ but did reveal two more polar unidentified radiolabeled peaks. Thus, whereas human peripheral blood monocytes do not catabolize LTB₄ over similar time periods to a significant degree (43, 48), human AM do. The identities of the unknown metabolites presently are being sought.

Endotoxin was present in low quantities in our cell cultures. Concentrations as low as 20 ng/ml have been reported to elicit the release of small quantities of LTC₄ from human polymorphonuclear leukocytes (50) and bacterial lipopolysaccharide (LPS) can stimulate LTC₄ release by mouse peritoneal macrophages (51). However, the concentrations of endotoxin present in our cell suspensions were not directly responsible for the LTB₄ synthesis and release from AM challenged with soluble IgG complexes. Our results obtained from AM preincubated with IF- γ and a specific antibody to IF- γ before stimulation with HAIgG suggest, but do not prove, that endotoxin played no significant role as a cofactor with IF- γ to prepare our cells for release of LTB₄. Species differences or other undefined conditions may account for the observation that preincubation of mouse peritoneal macrophages with LPS has been shown to prime these cells for the release of prostaglandins and LTC₄ by IgG-coated beads (52).

The production of LTB₄ in response to HAIgG was not blocked by cytochalasin B, suggesting that phagocytosis of cell bound IgG aggregates was not necessary for LTB₄ synthesis and release. The effect of cytochalasin B on LTB₄ release in response to IgG aggregates was rather one of enhancement, which is similar to its observed effect on release of eicosanoids from human peripheral blood monocytes by immunoglobulin aggregates (43) and to that on polymorphonuclear cell generation of LTB₄ in response to FMLP (44). Neither this effect nor that of IF- γ priming of the AM for LTB₄ release in response to IgG aggregates is completely and satisfactorily explained by a single mechanism. By analogy with the proposed mechanism for cytochalasin B enhancement of the superoxide generating response of neutrophils to FMLP, it is possible that cytochalasin B maintains IgGFcR in a high activity state for signal transduction (53).

Along the same lines, several of our observations suggest that the IF- γ effect was mediated, in part, by the increase in density of cell surface IgG receptors. The ability of IF- γ to prime AM for enhanced LTB₄ release in response to IgG aggregates was not apparent after one hour but was readily detectable by 24 h. This time-related difference is consistent with an effect that requires protein synthesis. Because IF- γ -induced augmentation of peripheral blood monocyte IgGFcR is inhibitable by cycloheximide (Guyre, P., personal communication), it is likely that the expression of immunoglobulin receptors on AM surfaces is at least partially dependent on the synthesis of new receptors. IF- γ primed alveolar macrophages for enhanced LTB₄ release and simultaneously induced are approximately twofold increase in the density of AM IgG₁ receptors. In addition, IgG₁ monomer inhibited HAIgG-induced AM release of LTB₄. Finally, neither the priming effects nor the receptor-inducing effects were observed in cells preincubated with IF- α or with IF- β . Thus, it is probable that the

IF- γ prepared the AM by providing more sites for ligand binding. It is also likely that additional post-IgGFcR mechanisms could be induced by IF- γ , since the several other macrophage functions, which are altered by IFs are not obviously regulated by a single mechanism (54).

Peripheral blood monocytes, precursors to alveolar macrophages, release both LTC₄ and LTB₄ when challenged with A23187 (43, 48). Several investigators now have observed that human AM release large quantities of LTB₄ (5-7) but only minimal quantities of LTD₄ (55). While IF- γ clearly primed AM for enhanced release of LTB₄ in response to HAIgG, it did not augment release of LTC₄ from AM challenged with either A23187 or HAIgG.

Our observations on human alveolar macrophages differ from the results of other studies that examined the effects of IF- γ on arachidonic acid metabolism in murine macrophages. Resident mouse peritoneal macrophages after preincubation with murine IF- γ and subsequent challenge with either A23187 or Zymosan, released less LTC₄ than peritoneal macrophages not primed with murine IF- γ (56). Furthermore, elicited mouse peritoneal macrophages primed with murine IF- γ released no more LTC₄ after challenge with IgG immune complexes than unprimed cells (57). These differences may reflect any of a number of variables between the studies, including those of species, culture conditions, and the nature of the agonists employed.

The findings of our study have potential relevance to host lung defense mechanisms. One classification divides these mechanisms into two broad groups: surveillance mechanisms and augmenting mechanisms (1). Our data suggest that in conditions where local surveillance mechanisms prove insufficient, augmenting mechanisms are initiated, with the activation of T-lymphocytes to release IF- γ . It is reasonable to hypothesize that in such circumstances AM may become "primed." Since the fluid that lines the epithelial surfaces of the lower respiratory tract is rich in IgG (1), AM under specified conditions then would be stimulated by IgG immune complexes to release LTB₄. Thus, the overall defense mechanisms might be further augmented through the recruitment of neutrophils, eosinophils, and monocytes.

Furthermore, the observations that IF- γ augments the number of IgEFcR on the macrophage-like U-937 cell line (58), that normal rat AM release substantial quantities of LTB₄ when activated by IgE immune complexes (59), and that IF- γ primes human AM for the release of LTB₄ in response to an IgG stimulus, raise the possibility that IF- γ may also increase the density of IgE receptors on human AM and similarly prime these cells for LTB₄ release by IgE-dependent mechanisms.

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References

1. Reynolds, H. Y., and J. Chrétien. 1984. Respiratory tract fluids: analysis of content and contemporary use in understanding lung diseases. *Dis. Month.* 30:1-103.
2. Hocking, W. G., and D. W. Golde. 1979. The pulmonary-alveolar macrophage. *N. Engl. J. Med.* 301:580-517, 639-645.
3. Lewis, R. A., and K. F. Austen. 1984. The biologically active leukotrienes. Biosynthesis, metabolism, receptors, functions, and pharmacology. *J. Clin. Invest.* 73:889-897.
4. Benveniste, J. 1985. Paf-Acether (platelet-activating factor). *Adv. Prostaglandins Thromb. Leuk. Res.* 13:11-18.
5. Fels, A. O., N. A. Pawlowski, E. B. Cramer, T. K. C. King, Z. A. Cohn, and W. A. Scott. 1982. Human alveolar macrophages produce leukotriene B₄. *Proc. Natl. Acad. Sci. USA.* 79:7866-7870.
6. Laviolette, M., R. Coulombe, S. Picard, P. Braquet, and P. Borgeat. 1986. Decreased leukotriene B₄ synthesis in smokers' alveolar macrophages. *J. Clin. Invest.* 77:54-60.
7. Martin, T. R., L. C. Altman, R. K. Albert, and W. R. Henderson. 1983. Leukotriene B₄ production by the human alveolar macrophage: A potential mechanism for amplifying inflammation in the lung. *Am. Rev. Respir. Dis.* 129:106-111.
8. Smith, M. J. H., A. W. Ford-Hutchinson, and M. A. Bray. 1980. Leukotriene B: a potential mediator of inflammation. *J. Pharm. Pharmacol.* 32:517-518.
9. Ford-Hutchinson, A. W., M. A. Bray, M. F. Doig, M. E. Shipley, and M. J. H. Smith. 1980. Leukotriene B: a potent chemotactic and aggregating substance released from polymorphonuclear leukocytes. *Nature (Lond.)*. 286:264-265.
10. Nagy, L., T. H. Lee, E. J. Goetzl, W. C. Pickett, and A. B. Kay. 1982. Complement receptor enhancement and chemotaxis of human neutrophils and eosinophils by leukotrienes and other lipoxygenase products. *Clin. Exp. Immunol.* 47:541-547.
11. Czarnetzki, B. M., and T. Rosenbach. 1986. Chemotaxis of human neutrophils and eosinophils towards leukotriene B₄ and its 20- ω -oxidation products in vitro. *Prostaglandins.* 31:851-858.
12. Sigal, C. E., F. H. Valone, M. J. Holtzman, and E. J. Goetzl. 1987. Preferential human eosinophil chemotactic activity of platelet-activating factor (PAF) 1-*o*-hexadecyl-2-acetyl-sn-glycervl-3-phosphocholine (AGEPC). *J. Clin. Immunol.* 7:179-184.
13. Wardlaw, A. J., R. Moqbel, O. Cromwell, and A. B. Kay. 1986. Platelet-activating factor. A potent chemotactic and chemokinetic factor for human eosinophils. *J. Clin. Invest.* 78:1701-1706.
14. Migliorisi, G., E. Folkes, N. Pawlowski, and E. B. Cramer. 1987. In vitro studies of human monocyte migration across endothelium in response to leukotriene B₄ and f-met-leu-phe. *Am. J. Pathol.* 127:157-167.
15. Showell, H. J., P. H. Naccache, P. Borgeat, P. Picard, P. Vallerand, E. L. Becker, and R. I. Sha'afi. 1982. Characteristics of the secretory activity of leukotriene B₄ toward rabbit neutrophils. *J. Immunol.* 128:811-816.
16. Atluru, D., and J. S. Goodwin. 1984. Control of polyclonal immunoglobulin production from human lymphocytes by leukotrienes; leukotriene B₄ induces an OKT8+, radiosensitive suppressor cell from resting: human OKT8-T-cells. *J. Clin. Invest.* 74:1444-1450.
17. Rola-Pleszcynski, M., L. Gagnon, and P. Sirois. 1983. Leukotriene B₄ augments human natural cytotoxic cell activity. *Biochem. Biophys. Res. Commun.* 113:531-537.
18. Payan, D. G., and E. J. Goetzl. 1984. Recognition of leukotriene B₄ by a unique subset of human T-lymphocytes. *J. Allergy Clin. Immunol.* 74:403-406.
19. Martinez-Maza, O., U. Andersson, J. Andersson, S. Brittons, and M. DeLuy. 1984. Spontaneous production of interferon- γ in adult and newborn humans. *J. Immunol.* 132:251-252.
20. Cunningham, A. L., and T. C. Merigan. 1984. Leu3+ T-cells produce γ -interferon in patients with recurrent herpes labialis. *J. Immunol.* 132:197-202.
21. Robinson, B. W. S., T. L. Mclemore, and R. G. Crystal. 1984. Gamma interferon is spontaneously released by alveolar macrophages and lung T lymphocytes in patients with pulmonary sarcoidosis. *J. Clin. Invest.* 75:1488-1495.
22. Friedman, R. M., and S. M. Vogel. 1983. Interferons with special emphasis on the immune system. *Adv. Immunol.* 34:97-141.
23. Celada, A., P. W. Gray, E. Rinderknecht, and R. D. Schneider. 1984. Evidence for a gamma-interferon receptor that regulates tumoricidal activity. *J. Exp. Med.* 160:55-74.
24. Orchansky, P., M. Rubinstein, and D. G. Fischer. 1986. The interferon- γ receptor in human monocytes is different from the one in nonhematopoietic cells. *J. Immunol.* 136:169-173.
25. Zlotnick, A., R. P. Shimonkevitz, M. L. Gafter, J. Kappler, and P. Marrack. 1983. Characterization of the γ -interferon-mediated induction of antigen presenting ability in P388D1 cells. *J. Immunol.* 131:2814-2810.
26. Le, J., W. Prenskey, Y. K. Yip, Z. Chang, T. Hoffman, H. Stevenson, I. Balazs, J. Sadlick, and J. Vilcek. 1983. Activation of human monocyte cytotoxicity by natural and recombinant immune interferon. *J. Immunol.* 131:2821-2816.
27. Murray, H. W., B. Y. Rubin, and C. D. Rothermel. 1983. Killing of intracellular *Leishmania donovani* by lymphokine-stimulated human mononuclear phagocytes. Evidence that interferon- γ is the activating lymphokine. *J. Clin. Invest.* 72:1506-151.
28. Guyre, P. M., P. M. Morganelli, and R. Miller. 1983. Recombinant immune interferon increases immunoglobulin G Fc receptors on cultured human mononuclear phagocytes. *J. Clin. Invest.* 72:393-397.
29. Rankin, J. A., G. P. Naegel, C. E. Schrader, R. A. Matthey, and H. Y. Reynolds. 1983. Air-space immunoglobulin production and levels in bronchoalveolar lavage fluid of normal subjects and patients with sarcoidosis. *Am. Rev. Respir. Dis.* 127:442-448.
30. Henson, P. M., H. B. Johnson, and H. L. Spiegelberg. 1972. The release of granule enzymes from human neutrophils stimulated by aggregated immunoglobulins of different classes and subclasses. *J. Immunol.* 109:1182-1192.
31. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.
32. Levin, J., and F. B. Bang. 1964. The role of endotoxin in the extracellular coagulation of limulus blood. 1951. *Bull. Johns Hopkins Hosp.* 115:264.
33. Rokach, J., E. C. Hayes, Y. Girard, D. L. Lombardo, A. L. Maycock, A. S. Rosenthal, R. N. Young, R. Zamboni, and H. J. Zweerink. 1984. The development of sensitive and specific radioimmunoassays for leukotrienes. *Prostaglandins Leukotrienes Med.* 13:21-25.
34. Hayes, E. C., D. L. Lombardo, Y. Girard, A. L. Maycock, J. Rokach, A. S. Rosenthal, R. N. Young, R. W. Egan, and H. J. Zweerink. 1983. Measuring leukotrienes of slow reacting substance of anaphylaxis: Development of a specific radioimmunoassay. *J. Immunol.* 131:429-433.
35. Williams, J. D., T. H. Lee, R. A. Lewis, and K. F. Austen. 1985. Intracellular retention of the 5-lipoxygenase pathway product, leukotriene B₄, by human neutrophils activated with unopsonized zymosan. *J. Immunol.* 134:2624-2630.
36. Metz, S. A., M. E. Hall, T. W. Harper, and R. C. Murphy. 1982. Rapid extraction of leukotrienes from biological fluids and quantitation by high-performance liquid chromatography. *J. Chromatogr.* 233:193-201.
37. Gonzalez-Molina, A., and H. L. Spiegelberg. 1977. A subpopulation of normal human peripheral B lymphocytes that bind IgE. *J. Clin. Invest.* 59:616-624.
38. Naegel, G. P., K. R. Young, Jr., and H. Y. Reynolds. 1984. Receptors for human IgG subclasses on human alveolar macrophages. *Am. Rev. Respir. Dis.* 129:413-418.
39. Kurlander, R. J., A. F. Haney, and J. Gartrell. 1984. Human

- peritoneal macrophages possess two populations of IgG Fc receptors. *Cell. Immunol.* 86:479-490.
40. Scatchard, G. 1949. The attraction of proteins for small molecules and ions. *Ann. NY Acad. Sci.* 51:660-663.
41. Darnowski, J. W., and R. E. Handschumacher. 1985. Tissue uridine pools: evidence in vivo of a concentrative mechanism for uridine uptake. *Cancer. Res.* 45:3155-3160.
42. Rankin, J. A., M. Hitchcock, W. W. Merrill, S. S. Huang, J. R. Brashler, M. K. Bach, and P. W. Askenase. 1984. IgE immune complexes induce immediate and prolonged release of leukotriene C4 (LTC4) from rat alveolar macrophages. *J. Immunol.* 132:1993-1999.
43. Ferreri, N. R., W. C. Howland, and H. L. Spiegelberg. 1986. Release of leukotrienes C4 and B4 and prostaglandin E2 from human monocytes stimulated with aggregated IgG, IgA and IgE. *J. Immunol.* 136:4186-4193.
44. Ham, E. A., D. D. Soderman, M. E. Zanetti, H. W. Dougherty, E. McCauley, and F. A. Kuehl, Jr. 1983. Inhibition by prostaglandins of leukotriene B4 release from activated neutrophils. *Proc. Natl. Acad. Sci. USA.* 80:4349-4353.
45. Bray, M. A. 1985. The pharmacology and pathophysiology of leukotriene B4. *Br. Med. Bull.* 39:249-254.
46. Goetzl, E. J. 1980. Mediators of immediate hypersensitivity derived from arachidonic acid. *N. Engl. J. Med.* 303:822-825.
47. Goodwin, J. S., D. Atluru, S. Sierakowski, and E. A. Lianos. 1986. Mechanism of action of glucocorticoids. Inhibition of T cell proliferation and interleukin 2 production by hydrocortisone is reversed by leukotriene B4. *J. Clin. Invest.* 77:1244-1250.
48. Williams, J. D., J. K. Czop, and K. F. Austen. 1984. Release of leukotrienes by human monocytes on stimulation of their phagocytic receptor for particulate activators. *J. Immunol.* 132:3034-3040.
49. Bigby, T. A., and M. J. Holtzman. 1987. Enhanced 5-lipoxygenase activity in lung macrophages compared to monocytes from normal subjects. *J. Immunol.* 138:1546-1550.
50. Bremm, K. D., W. Konig, B. Spur, A. Crea, and C. Galanos. 1984. Generation of slow-reacting substance (leukotrienes) by endotoxin and lipid A from human polymorphonuclear granulocytes. *Immunology.* 53:299-305.
51. Luderitz, T., U. Schade, and E. Th. Rietschel. 1986. Formation and metabolism of leukotriene C4 in macrophages exposed to bacterial lipopolysaccharide. *Eur. J. Biochem.* 155:377-382.
52. Aderem, A. A., D. S. Cohen, S. D. Wright, and Z. A. Cohn. 1986. Bacterial lipopolysaccharides prime macrophages for enhanced release of arachidonic acid metabolites. *J. Exp. Med.* 164:165-179.
53. Jesaitis, A. J., J. O. Tolley, L. A. Sklar, R. A. Allen, C. A. Parkos, and C. G. Cochrane. 1985. Chemoattractant receptor-cytoskeletal interaction: Evidence consistent with a role in regulation of activation of human granulocytes. *Fed. Proc.* 44:781A.
54. Forti, R. L., W. M. Mitchell, W. C. Hubbard, R. J. Workman, and J. T. Forbes. 1984. Pleiotropic activities of human interferons are mediated by multiple response pathways. *Proc. Natl. Acad. Sci. USA.* 81:170-174.
55. Michel, F. B., P. H. Godard, M. Damon, C. Chavis, and A. Crastes De Paulet. 1986. Chemical mediators of anaphylaxis released by alveolar macrophages in bronchial asthma. *Eur. J. Respir. Dis.* 69(Suppl. 146):189-194.
56. Boraschi, D., S. Censini, M. Bartalini, and A. Tagliabue. 1985. Regulation of arachidonic acid metabolism in macrophages by immune and nonimmune interferons. *J. Immunol.* 135:502-505.
57. Hamilton, T. A., J. E. Rigsbee, W. A. Scott, and D. O. Adams. 1985. γ -interferon enhances the secretion of arachidonic acid metabolites from murine peritoneal macrophages stimulated with phorbol diesters. *J. Immunol.* 134:2631-2636.
58. Naray-fejes-toth, A., and P. M. Guyre. 1984. Recombinant human immune interferon induces increased IgE receptor expression on the human monocyte cell line U-937. *J. Immunol.* 133:1914-1919.
59. Rankin, J. A. 1986. IgE immune complexes induce LTB4 release from rat alveolar macrophages. *Ann. Instit. Pasteur.* 137c:364-367.