Leukotriene B₄ synthesis and metabolism by neutrophils and granule-free cytoplasts

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Leukotriene B₄ [LTB₄, (5*S*,12*R*)-hydroxyeicosa-6,14-*cis*-8,10-*trans*-tetraenoic acid], a potent mediator of inflammation, is released from neutrophils by agonists that provoke degranulation of the cell. To examine whether degranulation is a necessary requirement for synthesis and metabolism of LTB₄ (or of other arachidonate metabolites), we prepared neutrophil-derived cytoplasts (neutroplasts), organelle-depleted vesicles of cytoplasm surrounded by the plasma membrane. In the presence of extracellular Ca²⁺ with or without exogenous arachidonic acid (150 μM), neutroplasts were exposed to the Ca²⁺ ionophore A23187 (10 μM) and the resultant lipoxygenation products of arachidonate were determined. Neutrophils metabolize arachidonic acid to 5-HETE > 15-HETE > LTB₄ > all-*trans*-LTB₄ isomers. Neutroplast products of arachidonate lipoxygenation were 15-HETE > 5-HETE > LTB₄ > all-*trans*-LTB₄ isomers. Neutroplasts, like neutrophils, were capable of converting LTB₄ into its 20-hydroxy and 20-carboxy metabolites. Finally, neutroplasts could utilize intrinsic arachidonate, since the neutroplasts synthesized LTB₄ (30 pmol/mg of protein) in the absence of added arachidonic acid. The data demonstrate that neutrophil degranulation is not required for synthesis or metabolism of LTB₄ by neutrophils.

INTRODUCTION

In response to soluble or particulate stimuli, neutrophils aggregate, move toward a chemoattractant, release toxic oxygen species and produce inflammatory mediators from arachidonate (i.e. LTB₄) (Weissman *et al.*, 1981). It is unclear, however, whether any or all of these processes is linked to degranulation (which is provoked by the same set of stimuli) either as a necessary prerequisite or as a parallel event (Ward & Becker, 1970; Mehta *et al.*, 1981; Boxer *et al.*, 1982; Fletcher *et al.*, 1982). By using neutrophil-derived cytoplasts or neutroplasts, Roos *et al.* (1983) and Korchak *et al.* (1983) have shown that these organelle-depleted cell fragments are capable of aggregating, generating O₂⁻ and ingesting particles, despite their lack of granules.

LTB₄ is one of the most potent endogenous mediators of inflammation that have been described (Weissmann *et al.*, 1981; Samuelsson, 1983; Lewis & Austen, 1984). In neutrophils it serves both as a potent chemotactic agent and a complete secretagogue. LTB₄ is synthesized by neutrophils, as well as by other phagocytic cells, after their stimulation with Ca²⁺ ionophores or after uptake of particles such as opsonized zymosan (Borgeat & Samuelsson, 1979a; Rouzer *et al.*, 1980; Claesson *et al.*, 1981). Whether the synthesis and metabolism of LTB₄ is linked to the degranulation that accompanies exposure of cells to ionophores or particles has not been examined. To determine whether a stimulus provoking degranulation is required for the generation of LTB₄, we have compared neutroplasts with intact neutrophils and found that, when neutroplasts were exposed to the Ca²⁺ ionophore A23187 in the presence of external Ca²⁺, they are as capable

as intact cells of synthesizing and catabolizing LTB₄; stimulus-induced degranulation is therefore unnecessary.

MATERIALS AND METHODS

Buffers and reagents

Hepes buffer comprised 150 mM-Na⁺, 5 mM-K⁺, 1.2 mM-Mg²⁺, 1.3 mM-Ca²⁺, 155 mM-Cl⁻, 10 mM-Hepes and 5 mM-glucose, pH 7.45. Phosphate-buffered saline contained 10 mM-PO₄³⁻, 5 mM-glucose and 138 mM-NaCl, pH 7.42; ACD contained 67 mM-citric acid, 85 mM-sodium citrate and 110 mM-dextrose. Cytochalasin B was from Aldrich, Ficoll 70 was from Sigma, arachidonic acid was from Nu Chek and ionophore A23187 was from Calbiochem. Methanol, hexane and propan-2-ol were h.p.l.c. grade from Burdick and Jackson, and anhydrous diethyl ether (h.p.l.c. grade) was from J. T. Baker. Standard LTB₄, all-*trans*-LTB₄ isomers and mono-HETEs were kindly given by Dr. P. Borgeat, University of Laval, Quebec, Canada, Dr. J. Rokach, Merk Frosst, Canada, and Dr. C. N. Serhan, Karolinska Institute, Stockholm, Sweden.

Cell separation

Pools of human polymorphonuclear leucocytes from normal adult volunteers were isolated by centrifugation over a Ficoll/Hypaque cushion and subsequent dextran sedimentation by the method of Boyum (1968). The remaining erythrocytes were removed by hypo-osmotic lysis. Neutrophils were kept in Hepes buffer at room temperature during the period of neutroplast preparation. Platelets were prepared from blood taken from normal

Abbreviations used: LTB₄, leukotriene B₄ [(5*S*,12*R*)-hydroxyeicosa-6,14-*cis*-8,10-*trans*-tetraenoic acid]; (5*S*,12*S*)-di-HETE, (5*S*,12*S*)-dihydroxyeicosa-6-*trans*, 14-*cis*-8-*cis*, 10-*trans*-tetraenoic acid; HETE, hydroxyeicosatetraenoic acid; all-*trans*-LTB₄, (5*S*,12*R*)- and (5*S*,12*S*)-hydroxyeicosa-6,8,10-*trans*-14-*cis*-tetraenoic acids; ACD, acid/citrate/dextrose; s.p.- or r.p.-h.p.l.c., straight- or reverse-phase h.p.l.c.; PGB₂, prostaglandin B₂.

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volunteers; aliquots (6 ml) were collected in ACD (1 ml) and were spun at 200 *g* in a table-top centrifuge and the platelet-rich plasma was removed. The platelets were washed three times with Hepes buffer/ACD (99:1, v/v, pH 6.5) warmed to 37 °C. They were resuspended in Hepes buffer immediately before use. Platelet activity was verified by aggregation and release of 12-HETE when stimulated with 10 μ M-A23187.

Neutroplast preparation

Neutroplasts were prepared by the method of Roos *et al.* (1983). Briefly, 1×10^8 neutrophils were mixed with 12.5% (w/v) Ficoll 70 containing 5 μ g of cytochalasin B/ml and layered over a discontinuous gradient of 16% and 25% Ficoll 70 containing 5 μ g of cytochalasin B/ml that had been prewarmed to 37 °C. A portion of intact neutrophils was removed beforehand to serve as a positive control. The cells were spun at 82000 *g* in a Beckman L-2 ultracentrifuge (SW27 rotor; 35 °C; 30 min). The layer that formed at the 12.5%/16%-Ficoll interface was removed, washed three times in Hepes buffer and resuspended to a concentration of 9×10^7 neutroplasts/ml, assuming one neutroplast is formed per neutrophil, as had previously been shown (Korchak *et al.*, 1983). A portion was removed for determination of protein concentration, which was done by the method of Lowry *et al.* (1951), with lysozyme as the standard.

Neutroplasts and neutrophils were incubated for 15 min at 37 °C with or without 150 μ M-arachidonic acid in 0.1% ethanol and then stimulated with 10 μ M-A23187 for 5 min. The reaction was stopped by the addition of 2 vol. of methanol and kept at -20 °C overnight.

Extraction and separation procedures

Lipoxygenase products were isolated by diethyl ether extraction by the method of Borgeat & Samuelsson (1979a, b). Briefly, the cell mixture was spun at 1600 *g* at 4 °C in a Sorvall RC3 centrifuge, the supernatant was decanted, and the pH adjusted to 3.0. The supernatant was then extracted with diethyl ether by using diethyl ether, water and supernatant in the proportions 2:1:1 (by vol.). The resulting water phase was discarded, and the diethyl ether phase was washed twice more with water. The ether phase was evaporated to dryness under a stream of N₂ and the products were separated on an Altex Ultrasphere ODS (5 μ m particle size; 4.6 mm $\times$ 25 cm) r.p.-h.p.l.c. column (Beckman Instruments), a methanol/water/acetic acid (7500:2500:1, by vol.) solvent system being used. The eluate was monitored at 270 nm for di-HETEs and 235 nm for mono-HETEs. Fractions were collected, dried under a stream of N₂ and resuspended in methanol until further use. Concentrations of products were determined by measuring u.v. absorbance at 270 nm for di-HETEs (absorption coefficient 45000 litre $\cdot$ mol⁻¹ $\cdot$ cm⁻¹) and at 235 nm for mono-HETEs (absorption coefficient 30500 litre $\cdot$ mol⁻¹ $\cdot$ cm⁻¹). The average recovery of arachidonic acid metabolites as determined by comparing the area under the peak of the r.p.-h.p.l.c. elution profile of 100 ng of PGB₂ with that of the same quantity of PGB₂ that had been added in six experiments, was 105 $\pm$ 30%. Samples for s.p.-h.p.l.c. were resuspended in 100 μ l of acid methanol and 800 μ l of ethereal diazomethane was added. Samples were vortex-mixed and incubated for 15 min at room temperature. They were evaporated to dryness under N₂ and the esterification procedure was repeated. Samples

were resuspended in the appropriate h.p.l.c. buffer and run on an Altex Si column (4.6 mm $\times$ 25 cm). Di-HETEs were separated by using a solvent system comprising hexane/propan-2-ol/acetic acid (9500:500:1, by vol.) and monitored at 270 nm. ω -Oxidized compounds were separated by using a hexane/propan-2-ol/acetic acid (9000:1000:1, by vol.) solvent system and monitored at 270 nm. These compounds possessed the triplet absorption maxima of a conjugated triene and were identified by comparison of elution times with published standards. The ratios of the compounds were determined and were used to calculate the molar quantities obtained by the r.p. procedure.

Neutrophil aggregation

Aggregation was measured as previously described (Craddock *et al.*, 1977). Briefly, 1.25×10^6 cells were stirred in a 100 μ l silicone-treated cuvette in Hepes buffer containing 5 μ g of cytochalasin B/ml. They were stimulated with various concentrations of LTB₄ derived from either neutrophils or neutroplasts. Aggregation was measured as an increase in light transmission with respect to time.

RESULTS

Synthesis of LTB₄ by neutrophils and neutroplasts

In order to test the hypothesis that intact neutrophils containing granules are required for LTB₄ generation, we prepared neutrophil-derived cytoplasts or neutroplasts by the method of Roos *et al.* (1983). Neutrophils and neutroplasts were incubated with or without A23187 (10 μ M) for 5 min after preincubation with arachidonic acid (150 μ M) in 0.1% ethanol. Neither unstimulated neutrophils nor neutroplasts made di-HETE products, but both generated mono-HETEs in the presence of added arachidonate (see Table 1). Neutrophils and neutroplasts pretreated with arachidonate and stimulated with A23187 generated both mono- and di-HETES (Table 1; Fig. 1). Fig. 1(a) shows the r.p.-h.p.l.c. chromatogram of a diethyl ether extract of stimulated neutrophils with the mono- and di-HETE fractions identified. Fig. 1(b) shows a representative chromatogram from the paired sample of stimulated neutroplasts. A comparison of Figs. 1(a) and 1(b) reveals no qualitative difference between the two curves. All peaks generated by neutrophils were also found in the neutroplast preparation. However, the proportions of the various compounds generated by neutrophils and neutroplasts were not identical. Peak d, which was co-eluted with reference LTB₄ in both the neutrophil and neutroplast preparations, displayed the characteristic u.v. spectrum of a conjugated triene: triple absorbance maxima at 279, 269 and 259 nm. This peak also contains the compound (5S,12S)-di-HETE as reported by Borgeat *et al.* (1982) and Marcus *et al.* (1982), which is formed in neutrophil suspensions by a double lipoxygenation reaction. The 5-HETE derived from neutrophils can be acted upon by the 12-lipoxygenase enzyme contributed by any contaminating platelets remaining in the preparation. LTB₄ and (5S,12S)-di-HETE can, however, be resolved by methyl esterification of the mixture and subsequent chromatography on s.p.-h.p.l.c. We found that the 'LTB₄ peak' from neutroplast preparations contained an average of 14% (5S,12S)-di-HETE, as measured by s.p.-h.p.l.c.

Table 1. Lipoxygenase products generated by neutrophils and neutroplasts with exogenous arachidonic acid

Neutrophils and neutroplasts were incubated for 15 min at 37 °C in the presence of 150 μ M-arachidonic acid. They were stimulated for 5 min with or without 10 μ M-A23187 and reaction products were isolated as described in the Materials and methods section. Values are means $\pm$ S.E.M.

	Content (pmol/mg of protein)							
	20-Hydroxy-LTB ₄	20-Carboxy-LTB ₄	All- <i>trans</i> -LTB ₄	LTB ₄	(5 <i>S</i> ,12 <i>S</i>)-Di-HETE	15-HETE	12-HETE	5-HETE
Neutrophils								
Unstimulated (n = 3)	0	0	0	0	0	160 $\pm$ 100	65 $\pm$ 25	58 $\pm$ 12
+10 μ M-A23187 (n = 3)	70 $\pm$ 20	12 $\pm$ 7	180 $\pm$ 20	300 $\pm$ 20	20 $\pm$ 16	490 $\pm$ 270	230 $\pm$ 70	890 $\pm$ 80
Neutroplasts								
Unstimulated (n = 4)	0	0	0	0	0	1020 $\pm$ 660	271 $\pm$ 660	130 $\pm$ 70
+10 μ M-A23187 (n = 4)	67 $\pm$ 41	22 $\pm$ 19	135 $\pm$ 50	370 $\pm$ 190	51 $\pm$ 26	410 $\pm$ 2370	716 $\pm$ 370	810 $\pm$ 420

Fig. 1. R.p.-h.p.l.c. of a diethyl ether extract of neutrophils and neutroplasts

Neutrophils or neutroplasts were stimulated with A23187 (10 μ M) in the presence of arachidonic acid (150 μ M). The reaction was stopped by the addition of 2 vol. of methanol and the supernatant was extracted with diethyl ether (see the Materials and methods section). (a) R.p.-h.p.l.c. of diethyl ether extract of neutrophil preparation. Peaks are as follows: a, void volume containing ω -oxidation products; b, (5*S*,12*R*)-all-*trans*-LTB₄; c, (5*S*,12*S*)-all-*trans*-LTB₄; d, LTB₄; e, 15-HETE; f, 12-HETE; g, 5-HETE. The inset is the u.v.-absorption scan of the LTB₄ peak. Elution was monitored at 270 nm for 25 min and then changed to 235 nm. (b) R.p.-h.p.l.c. of diethyl ether extract of neutroplast preparation. Peaks are labelled as in (a). The inset shows the u.v.-absorption scan of the LTB₄ peak. Elution was monitored at 279 nm for 25 min and then changed to 235 nm.

separation. Neutrophil preparations contained 7% of this product (Table 1).

To confirm that neutroplast-generated LTB₄ possessed biological activity, we tested it for its ability to elicit

neutrophil aggregation. Although it was not possible to perform a complete dose-response analysis, owing to the small quantities of LTB₄ produced by each preparation of neutroplasts, equal concentrations of neutrophil and

Fig. 2. Aggregation of neutrophils by LTB_4

Aggregation was monitored continuously as changes in light transmission (ΔT) of a stirred suspension of neutrophils that had been incubated for 5 min at 37 °C with cytochalasin B at 5 μ g/ml before addition of stimulus. Curves are 'stacked' for comparison. A, neutrophil-derived LTB_4 (0.1 μ M); B, neutroplast-derived LTB_4 (0.1 μ M); C, neutroplast-derived LTB_4 (50 nM); D, all-*trans*- LTB_4 (0.1 μ M).

neutroplast LTB_4 consistently produced the same degree of neutrophil aggregation (Fig. 2). All-*trans*- LTB_4 , here derived from neutroplasts, caused no neutrophil aggregation at the highest concentration used (Fig. 2).

Metabolism of LTB_4 by neutrophils and neutroplasts

During cell activation, LTB_4 is converted into its more polar metabolites, the 20-hydroxy and 20-carboxy compounds, which possess less activity with respect to neutrophil activation than LTB_4 (Hansson *et al.*, 1981; Lindgren *et al.*, 1981, 1982; Serhan *et al.*, 1982; Ford-Hutchinson *et al.*, 1983). To determine whether neutroplasts also retain the ability to convert LTB_4 into

its terminal oxidation products, the initial fractions from the r.p. separation were pooled, evaporated to dryness, methyl-esterified and separated by s.p.-h.p.l.c. Both 20-hydroxy- and 20-carboxy- LTB_4 were isolated from the neutroplast preparation in the same proportion as from the neutrophils (Table 1).

Utilization of intrinsic arachidonate stores

The experiments above were all performed in the presence of added arachidonic acid. Having shown that neutroplasts can utilize free arachidonate to generate LTB_4 , we next examined whether neutroplasts were able to mobilize intrinsic arachidonic acid and to utilize it in forming lipoxygenase products. Neutroplasts exposed to A23187 (10 μ M) proved capable of forming LTB_4 , albeit in much smaller quantities than those generated in the presence of exogenously added arachidonate (30 pmol/mg of protein as against 300 pmol/mg of protein; Tables 1 and 2).

Neutrophil and neutroplast preparations are always contaminated by a small, but detectable, number of platelets, as demonstrated by the constant finding of 12-HETE among the lipoxygenase products. Previous work in this laboratory has shown that, after activation, platelets release free arachidonate, which can be used as a precursor by intact neutrophils for the generation of LTB_4 (Marcus *et al.*, 1982). In order to determine whether the LTB_4 found in the neutroplast preparations was generated from the intrinsic arachidonic acid of the neutroplast or from arachidonate released by the contaminating platelet population, we added large numbers of platelets to a neutroplast preparation, stimulated the mixture with the ionophore A23187, and isolated the lipoxygenase products. Neutroplasts did not utilize platelet arachidonate in significant quantities, as shown in Table 2. Neutroplasts generated the same amount of LTB_4 with and without added platelets. The quantity of 12-HETE produced increased by more than 60-fold, showing that the platelets were active. In addition, the amount of (5*S*, 12*S*)-di-HETE (the double-lipoxygenation product) increased over 10-fold.

DISCUSSION

Many neutrophil functions have been linked to degranulation; these include aggregation, O_2^- generation and the mobilization of receptors for chemotactic factors (Ward & Becker, 1970; Mehta *et al.*, 1981; Oseas *et al.*, 1981; Fletcher *et al.*, 1982). Alternatively, Roos *et al.*

Table 2. Lipoxygenase products generated by neutroplasts and platelets (without exogenous arachidonic acid)

Neutroplasts were incubated for 5 min at 37 °C with 10 μ M-A23187 with or without platelets from 4 ml of platelet-rich plasma. Reaction products were isolated as described in the Materials and methods section. All values are means $\pm$ S.E.M. ($n = 3$).

	Content (pmol/mg of protein)			
	LTB_4	(5 <i>S</i> ,12 <i>S</i>)-di-HETE	12-HETE	5-HETE
Neutroplasts + 10 μ M-A23187	30 $\pm$ 12	0.4 $\pm$ 0.4	5 $\pm$ 2	45.0 $\pm$ 28
Neutroplasts + platelets + 10 μ M-A23187	32 $\pm$ 20	90 $\pm$ 40	587 $\pm$ 330	35.3 $\pm$ 28
Platelets + 10 μ M-A23187	0	0	830 $\pm$ 75	0

(1983) and Korchak *et al.* (1983), by means of neutroplast preparations, have shown that aggregation, release of toxic oxygen products, phagocytosis and changes in membrane potential in the neutrophil can proceed in the absence of degranulation. Preparations of cytosol from various cells and cell lines have been shown to generate various products of the lipoxygenase pathways. Interpretations of these experiments are fraught with difficulty, since different methods of preparing cytosol can lead to opposite conclusions (Shak & Goldstein, 1985; Soberman *et al.*, 1985), possibly owing to contamination with cell membranes or organelle fragments and contents. To examine whether granules and/or an intact mechanism of degranulation are required for leukotriene synthesis and catabolism, we utilized neutroplasts. We have found that neutroplasts synthesize 5-lipoxygenase products, including LTB₄. Neutroplasts also contain the enzymes necessary to metabolize LTB₄ to the more-polar, less-active products: 20-hydroxy- and 20-carboxy-LTB₄. We do not consider it appropriate to quantitatively compare neutroplast and neutrophil synthesis of these compounds. In addition to the donor-to-donor variation in the quantity of lipoxygenase products produced by neutrophils, there is also significant donor-related variation in the size and protein content of their neutroplasts; hence the variability of lipoxygenase products produced by neutroplasts is large. However, it is important to note that neutroplasts produce a significant amount of 5-lipoxygenase products in ratios similar to those generated by neutrophils.

Finally we have demonstrated not only that neutroplasts retain the ability to synthesize lipoxygenase products after the initial step of arachidonic acid liberation, but that these cell fragments can form the same products in the absence of added arachidonate. Previous work has demonstrated that neutrophils can utilize arachidonic acid released from stimulated platelets to synthesize LTB₄ (Marcus *et al.*, 1982). When suspensions were enriched in platelets (over 60-fold as measured by the increment in 12-HETE synthesis), no increase in the synthesis of LTB₄ was found. Failure of neutroplasts to form LTB₄ was not simply due to saturation of the lipoxygenase enzymes, since ionophore provoked a concomitant increase in the double-lipoxygenase product, (5*S*,12*S*)-di-HETE. Moreover, adding free arachidonic acid (cf. Tables 1 and 2) resulted in enhanced synthesis of both LTB₄ and (5*S*,12*S*)-di-HETE.

Stimulated neutroplasts, in the presence of added arachidonate, generate larger quantities of 15-HETE than do neutrophils. In the absence of added arachidonate, the quantity of 15-HETE is almost undetectable. It is possible that, in contrast with added arachidonate, *intrinsic* arachidonic acid metabolized during stimulation may not be available to the 15-lipoxygenase enzyme. We have not, however, determined whether neutroplast 15-HETE is enzymically produced by examining the chirality of the -OH grouping either by oxidative ozonolysis or application to a chiral column. An alternative explanation is that the free arachidonate spontaneously reacts with active oxygen products produced by neutroplasts to form 15-HETE. Borgeat & Samuelsson (1979a), in their initial observations on neutrophil-derived 15-HETE, noted that the stereospecificity of this compound was less than would be expected if the 15-HETE was synthesized solely by means of an enzymic reaction and suggested that some 15-HETE may

have been generated by non-specific oxidation. At present we cannot rule out either of these two possibilities.

We have demonstrated that neutroplasts can generate the more polar metabolites of LTB₄, the 20-hydroxy- and 20-carboxy-leukotrienes. These products are less active than LTB₄ in neutrophil aggregation, O₂⁻ and enzyme-release assays, and LTB₄ may be inactivated *in vivo* by ω -oxidation (Hansson *et al.*, 1981; Lindgren *et al.*, 1981, 1982; Serhan *et al.*, 1982; Ford-Hutchinson *et al.*, 1983). Chacos *et al.* (1982) and Oliw *et al.* (1982) have shown that liver microsomes can oxidize arachidonate, and it has been suggested by Shak & Goldstein (1984, 1985) that cytochrome P-450 is the site of the terminal oxidation of LTB₄ and (5*S*,12*S*)-di-HETE. At present, we have no information as to whether neutroplasts contain what would constitute a 'microsomal fraction' isolated from neutrophils. Since neutroplasts do, however, form the ω -oxidation products of leukotrienes, they contain the enzymes required for further metabolism of di-HETEs.

In summary, neutroplasts respond to the Ca²⁺ ionophore by mobilizing intrinsic arachidonate, activating the lipoxygenase pathway of arachidonate metabolism to synthesize mono- and di-HETE products, and metabolizing di-HETEs to their ω -oxidation products. LTB₄ synthesis and metabolism, therefore, proceeds unimpaired in the absence of organized intracellular structures.

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REFERENCES

- Borgeat, P. & Samuelsson, B. (1979a) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2148–2152
- Borgeat, P. & Samuelsson, B. (1979b) *J. Biol. Chem.* **254**, 2643–2646
- Borgeat, P., Fruteau de Lacroix, B., Picard, S., Vallerand, P. & Sirois, P. (1982) in *Leukotrienes and other Lipoxygenase Products* (Samuelsson, B. & Paoletti, R. eds.), pp. 45–51, Raven Press, New York
- Boxer, L. A., Coates, T. D., Haak, R. A., Wolach, J. B., Hoffstein, S. & Baihner, R. L. (1982) *N. Engl. J. Med.* **307**, 404–410
- Boyum, A. (1968) *Scand. J. Clin. Lab. Invest. Suppl.* **97**, 21, 77–89
- Chacos, N., Falck, J. R., Wixtrom, C. & Capdevila, J. (1982) *Biochem. Biophys. Res. Commun.* **104**, 916–922
- Claesson, H.-E., Lundberg, U. & Malmsten, C. (1981) *Biochem. Biophys. Res. Commun.* **99**, 1230–1237
- Craddock, P. R., Hammerschmidt, D. E., Dalmasso, A. P., White, J. G. & Jacob, H. S. (1977) *J. Clin. Invest.* **60**, 260–264
- Fletcher, M. P., Seligmann, B. E. & Gallin, J. I. (1982) *J. Immunol.* **105**, 1057–1067
- Ford-Hutchinson, A. W., Rackham, A., Zamboni, R., Rokach, J. & Roy, S. (1983) *Prostaglandins* **25**, 29–37
- Hansson, G., Lindgren, J. A., Dahlen, S.-E., Hedqvist, P. & Samuelsson, B. (1981) *FEBS Lett.* **130**, 107–112
- Korchak, H. M., Roos, D., Giedd, K. N., Wynkoop, E. M., Vienne, K., Rutherford, L. E., Buyon, J. P., Rich, A. M. & Weissmann, G. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4968–4972
- Lewis, R. A. & Austen, K. F. (1984) *J. Clin. Invest.* **73**, 889–897
- Lindgren, J. A., Hansson, G. & Samuelsson, B. (1981) *FEBS Lett.* **128**, 329–335
- Lindgren, J. A., Hansson, G., Claesson, H.-E. & Samuelsson, B. (1982) in *Leukotrienes and other Lipoxygenase Products* (Samuelsson, B. & Paoletti, R. eds.), pp. 53–60, Raven Press, New York

- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Marcus, A. J., Broekman, M. J., Safier, L. B., Ullman, H. L., Islam, N., Serhan, C. N., Rutherford, L. E., Korchak, H. M. & Weissman, G. (1982) *Biochem. Biophys. Res. Commun.* **109**, 130–137
- Mehta, J., Mandell, B., Kleyman, T. & Spilberg, I. (1981) *Inflammation (N.Y.)* **5**, 89–101
- Oliw, E. H., Guengerich, F. P. & Oates, J. A. (1982) *J. Biol. Chem.* **257**, 3771–3781
- Oseas, R. S., Allen, J., Yang, H.-H., Baehner, R. L. & Boxer, L. A. (1981) *Infect. Immun.* **33**, 523–526
- Rouzer, C. A., Scott, W. A., Cohn, Z. A., Blackburn, R. & Manning, J. M. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4928–4932
- Roos, D., Voetman, A. A. & Meerhof, L. J. (1983) *J. Cell Biol.* **97**, 368–377
- Samuelsson, B. (1983) *Science* **220**, 568–575
- Serhan, C. N., Radin, A., Smolen, J. E., Korchak, H. M., Samuelsson, B. & Weissman, G. (1982) *Biochem. Biophys. Res. Commun.* **107**, 1006–1012
- Shak, S. & Goldstein, I. M. (1984) *J. Biol. Chem.* **259**, 10181–10187
- Shak, S. & Goldstein, I. M., (1985) *J. Clin. Invest.* **76**, 1218–1228
- Soberman, R. J., Harper, T. W., Murphy, R. C. & Austen, K. F. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 2292–2295
- Ward, P. A. & Becker, E. L. (1970) *J. Immunol.* **105**, 1057–1067
- Weissmann, G., Korchak, H. M., Perez, H. D., Smolen, J. E., Goldstein, I. M. & Hoffstein, S. T. (1981) in *Membranes, Molecules, Toxins and Cells* (Bloch, K., Bolis, L. & Tosteson, D. C., eds.), pp. 175–191, PSG Publishing Co., Boston, Bristol and London

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