Leukotrienes: Mediators of Immediate Hypersensitivity Reactions and Inflammation

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Summary. Arachidonic acid plays a central role in a biological control system where such oxygenated derivatives as prostaglandins, thromboxanes, and leukotrienes are mediators. The leukotrienes are formed by transformation of arachidonic acid into an unstable epoxide intermediate, leukotriene A_4, which can be converted enzymatically by hydration to leukotriene B_4, and by addition of glutathione to leukotriene C_4. This last compound is metabolized to leukotrienes D_4 and E_4 by successive elimination of a γ-glutamyl residue and glycine. Slow-reacting substance of anaphylaxis consists of leukotrienes C_4, D_4, and E_4. The cysteinyl-containing leukotrienes are potent bronchoconstrictors, increase vascular permeability in postcapillary venules, and stimulate mucus secretion. Leukotriene B_4 causes adhesion and chemotactic movement of leukocytes and stimulates aggregation, enzyme release, and generation of superoxide in neutrophils. Leukotrienes C_4, D_4, and E_4, which are released from the lung tissue of asthmatic subjects exposed to specific allergens, seem to play a pathophysiological role in immediate hypersensitivity reactions. These leukotrienes, as well as leukotriene B_4, have pro-inflammatory effects.

In a biological system for the regulation of various cell functions, arachidonic acid plays a unique role as a precursor molecule which is transformed into potent mediators with far-ranging effects. The prostaglandins are one group of biologically active compounds derived from arachidonic acid. Mechanistic studies of the biosynthesis of the prostaglandins provided evidence for the existence of unstable intermediates in the biosynthetic process. This led to the isolation of the endoperoxides, which were found to have pronounced biological effects that could not be explained by their conversion to the known prostaglandins. This finding resulted in the detection of thromboxane A_2, an unstable platelet-aggregating and vasoconstrictor substance. Subsequent research led to the detection and isolation of prostacyclin, a compound with opposite biological effects. Prostacyclin has strong pro-inflammatory effects and, in most systems, is more potent than prostaglandin E_2.

Nonsteroidal anti-inflammatory drugs such as aspirin inhibit the enzyme (cyclooxygenase) responsible for conversion of arachidonic acid into prostaglandins (including prostacyclin) and thromboxanes. However, anti-inflammatory steroids prevent the formation of prostaglandins by a different mechanism, that is, by inhibition of the release of the precursor acid from the phospholipid stores. Since steroids and aspirin-type drugs have significantly different anti-inflammatory effects it seemed conceivable that some of these differences might be due to cyclooxygenase independent formation of additional pro-inflammatory derivatives of arachidonic acid. Studies of the metabolism of arachidonic acid in leukocytes then led to the recognition of the leukotrienes. These compounds seem to play a role in immediate hypersensitivity reactions and also have pronounced pro-inflammatory effects.

Discovery of the Leukotrienes

In an attempt to identify the products generated from arachidonic acid in inflammatory cells, we added ^{14}C-labeled material to polymorphonuclear leukocytes from the peritoneal cavity of rabbits. The major metabolite was a new lipoxygenase product, that is, 5(*S*)-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) (*1*). However, more polar products were also formed (Fig. 1). Structural studies demonstrated that they consisted of 5(*S*),12(*R*)-dihydroxy-6,8,10,14-eicosatetraenoic acid (leukotriene B_4, the major product), two additional 5(*S*),12-dihydroxy-6,8,10-*trans*,14-*cis*-eicosatetraenoic acids, epimeric at C-12, and two isomeric 5,6-dihydroxy-7,9,11,14-eicosatetraenoic acids (Fig. 1) (*2, 3*).

Analysis of the stereochemistry, demonstrating formation of two acids with an all *trans* conjugated triene, epimeric at C-12, and one major isomer (12-*R*) with different configuration of the triene, raised the question of the mechanism of formation (*3*). With the aid of isotopic oxygen it was demonstrated that the oxygen of the alcohol group at C-5 originated in molecular oxygen, whereas the oxygen of the alcohol group at C-12 was derived from water (Fig. 1) (*4*). We therefore developed the hypothesis that leukocytes generated an unstable intermediate that would undergo nucleophilic attack by water, alcohols, and other nucleophiles. When we incubated leukocytes for 30 seconds with arachidonic acid before adding 10 volumes of methanol, 10 volumes of ethanol, or 0.2 volume of *N* HCl, we obtained the products shown in Fig. 2.

The derivatives formed upon trapping with methanol (or ethanol) included two new, less polar compounds present in equal amounts. Their ultraviolet spectra were identical to those of compounds **1** and **2** (Fig. 1), indicating the presence of three conjugated double bonds. Infrared spectrometry indicated that the conjugated double bonds have *trans* geometry. Gas chromatographic–mass spectrometric analyses of several derivatives of the two compounds showed that they were isomeric and carried hydroxyl groups at C-5 and methoxy groups at C-12. Steric analyses demonstrated that the alcohol groups had (*S*) configuration and that the compounds were the C-12 epimers of 5(*S*)-hydroxy,12-methoxy-6,8,10,14-(*E*,*E*,*E*,*Z*)-eicosatetraenoic acid (Fig. 1).

Corresponding derivatives were obtained when ethanol or ethylene glycol were used for trapping. These results showed that a metabolite of arachidonic acid in leukocytes can undergo a facile nucleophilic reaction with alcohols. Analysis of samples obtained from trapping experiments performed under various conditions always indicated inverse

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relationships between the amount of compounds **1** and **2** and their 12-*O*-alkyl derivatives. This finding suggested that compounds **1** and **2** were formed nonenzymatically from the same intermediate that gave rise to the 12-*O*-alkyl derivatives.

The stability of the intermediate was determined by incubating rabbit polymorphonuclear leukocytes (PMNL) with arachidonic acid for 45 seconds before adding 1 volume of acetone (to stop enzymatic activity). Portions of the mixture were transferred at different time intervals to flasks containing 15 volumes of methanol. The relative amounts of the metabolites were determined by reversed phase–high-pressure liquid chromatography (RP-HPLC). The half-time for production of the intermediate, measured as the 12-*O*-methyl derivative at *p*H 7.4 and 37°C, was 3 to 4 minutes. Simultaneously with the decrease in the concentration of the intermediate, the concentrations of compounds **1**, **2**, **4**, and **5** increased, whereas the concentrations of compounds **3** and 5-hydroxy-6,8,11,14-eicosatetraenoic acid remained constant. This suggested that the epimeric 5,6- and 5,12-dihydroxy acids (compounds **1**, **2**, **4**, and **5**) are formed nonenzymatically by hydrolysis of a common unstable intermediate, whereas compound **3** arises by enzymatic hydrolysis of the same intermediate (Fig. 1). Similar experiments performed at acid and alkaline *p*H showed that the intermediate was acid labile and stabilized by alkaline *p*H.

On the basis of these data, the structure 5,6-oxido-7,9,11,14-eicosatetraenoic acid (Fig. 1) was proposed for the intermediate (*4*). The hydrolysis of epoxides is acid-catalyzed, and opening of allylic epoxides is favored at allylic positions (C-6 in this case). The findings that two 5,6-dihydroxy derivatives (**4** and **5**) were formed nonenzymatically from the same intermediate as the enzymatic product, 5*S*,12*R*-dihydroxy-eicosatetraenoic acid, and that ^{18}O from molecular oxygen was exclusively retained at the C-5 of these derivatives whereas ^{18}O from water was introduced at C-6 or C-12, were of crucial importance in assigning the 5,6-oxido structure to the unstable intermediate (Fig. 1). The formation of compounds **1** to **5** from the epoxide intermediate is shown in Fig. 1. Except for compound **3** these are formed by chemical hydrolysis of the epoxide through a mechanism involving a carbonium ion. The latter added a hydroxyl anion preferentially at C-6 and C-12 to yield four isomeric products that contain the stable conjugated triene structure. Compound **3** is formed enzymatically from the intermediate since it is not racemic at C-12 and because it is only formed by nondenatured cell preparations.

The structure 5,6-oxido-7,9,11,14-eicosatetraenoic acid (leukotriene A_4) (*4*) that was proposed for the intermediate was confirmed by chemical synthesis, and its stereochemistry has been elucidated (*5*). The enzymatically formed 5*S*,12*R*-dihydroxy acid was previously shown to contain one *cis* and two *trans* double bonds in the conjugated triene. The location of the *cis* double bond at the Δ^6-position was recently determined by means of a synthetic approach (*6*). The allylic epoxide intermediate has been isolated from human polymorphonuclear leukocytes (*7*). It can thus exist in free form in cells and tissues.

The suggested mechanism for the biosynthesis of the epoxide from arachidonic acid (Fig. 1) involves initial formation of 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HPETE). The epoxide is formed from 5-HPETE by abstraction of a hydrogen at C-10 and elimination of a hydroxyl anion from the hydroperoxy group. This reaction is catalyzed by a soluble enzyme that was recently isolated from leukocytes (*8*).

Slow-Reacting Substance of Anaphylaxis

The name slow-reacting substance (SRS) was introduced in 1938 by Feldberg and Kellaway (*9*) for a smooth muscle contracting factor that appeared in the perfusate of guinea pig lung treated with cobra venom. Subsequent studies indicated that SRS is important as a mediator in asthma and other types of immediate hypersensitivity reactions (*10*). Immunologically generated SRS is usually referred to as SRS-A (slow-reacting substance of anaphylaxis). SRS-A is considered to be released together with other mediators (for example, histamine and chemotactic factors) after interaction between immunoglobulin E (IgE) molecules bound to membrane receptors and antigens such as pollen.

Structural work on SRS was previously limited by the lack of pure preparations of SRS. However, it has been characterized as a sulfur-containing polar lipid with ultraviolet absorption (*11–15*). Previous studies indicated that labeled arachidonic acid was incorporated into SRS (*16*).

We found that treatment of human neutrophils with the calcium ionophore A23187 stimulated the synthesis of the

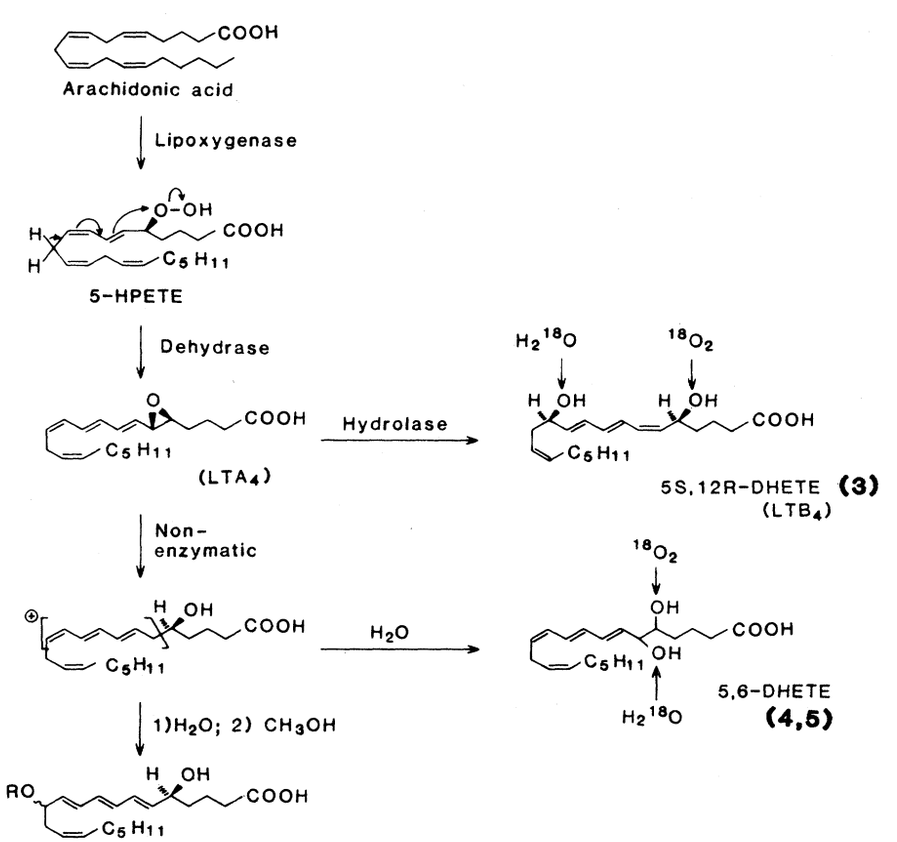

Fig. 1. Formation of dihydroxy derivatives by way of unstable intermediates. The origin of oxygen is shown as well as the points at which trapping experiments are conducted.

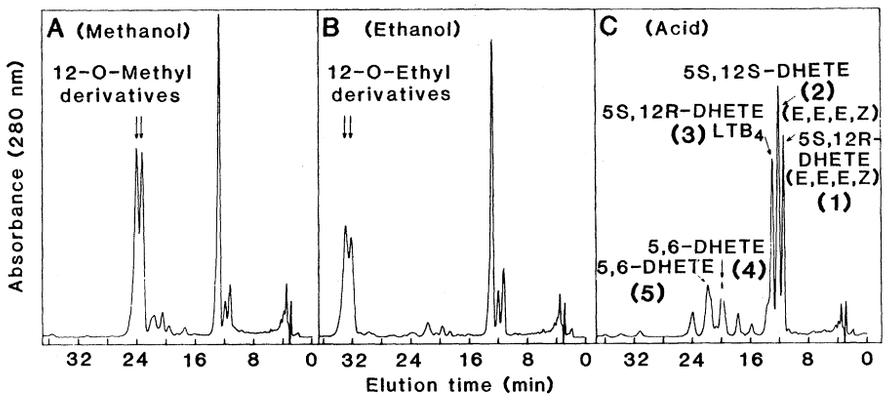

Fig. 2. Reversed-phase–HPLC chromatograms of the products obtained upon addition of (A) 10 volumes of methanol; (B) 10 volumes of ethanol; and (C) 0.2 volume of *N* HCl to suspensions of PMNL incubated for 30 seconds with arachidonic acid. The boldface numerals refer to the structures shown in Fig. 1.

5,12-dihydroxy acid (LTB_4) (*17*). The stimulation was not only due to increased release of the precursor acid. These findings were of considerable interest since previous studies had shown that the ionophore also stimulates release of SRS from leukocytes (*18*). In addition, the ultraviolet (UV) absorbance of purified SRS was similar to that of the dihydroxy acids of leukocyte origin (*3, 12, 14*). On the basis of the effects of the ionophore, the UV absorbance data, and other considerations we developed the hypothesis that there was a biogenetic relation between the unstable allylic epoxide intermediate in neutrophils and the SRS generated in a variety of systems.

A survey of the procedures that were used for generating SRS (*19*) led to the development of a new system (murine mastocytoma cells) for producing better yields of the substance. The mouse mast cell tumors were propagated in the peritoneal cavities of syngeneic mice (*20*), and cells were removed and incubated with labeled arachidonic acid or labeled cysteine. Then, SRS was generated by challenging the cells with ionophore A23187.

This method proved superior to previous systems with respect to the formation of spasmogenic material antagonized by the SRS antagonist FPL55712 and the incorporation of isotopically labeled precursors (*20*). The isolation procedure involved precipitation of protein with ethanol, alkaline hydrolysis, separation on Amberlite XAD-8 and silicic acid, and two steps of RP-HPLC. The material thus obtained was essentially pure, showed an absorbance maximum at 280 nanometers, and caused a typical contraction of guinea pig ileum that was reversed by FPL55712 (*20*). The UV spectrum resembled the spectra of the dihydroxy acids described above; however, the maximum wavelength was shifted 10 nm higher. This was consistent with a sulfur substituent in the α position. Experiments with labeled precursors showed that arachidonic acid and cysteine were incorporated into the products.

When the isolated SRS was degraded by Raney nickel desulfurization it produced 5-hydroxyarachidic acid, which indicated that the arachidonic acid derivative and cysteine were linked by a thioether bond. The presence of an alcohol group at C-5 in the fatty acid reinforced the hypothesis of a biogenetic relation between the arachidonic acid metabolites we had found in leukocytes and SRS.

The positions of the double bonds in SRS were determined by subjecting material biosynthesized from tritiated arachidonic acid to reductive ozonolysis. The isolation of labeled 1-hexanol among the products indicated that the Δ^{14}-double bond of arachidonic acid had been retained. The method used for locating the conjugated triene was based on previous studies in our laboratory. These studies had shown that arachidonic acid and related fatty acids containing two methylene-interrupted *cis* double bonds at the ω6 and ω9 positions are oxygenated to give derivatives with isomerization of the ω6 double bond to ω7. Incubations of the isolated SRS with lipoxygenase resulted in isomerization of the Δ^{14}-double bond with the conjugated triene (forming a tetraene), since there was a bathochromic shift of 30 nm. This result indicated the presence of a Δ^{11}-*cis* double bond and additional double bonds at Δ^7 and Δ^9 in SRS.

The structural work at this stage showed that the SRS was a derivative of 5-hydroxy-7,9,11,14-eicosatetraenoic acid with a cysteine-containing substituent in thioether linkage at C-6. Derivatization of cysteine was suggested by the failure to isolate alanine after desulfurization. The cysteine-containing substituent was therefore referred to as RSH in the reports of this work (*20–22*). Further studies demonstrated that in addition to cysteine, 1 mole of glycine and 1 mole of glutamic acid were present per mole of SRS. End-group (dansyl method and hydrazinolysis) and sequence analyses (dansyl-Edman procedure) of the peptide showed that it was γ-glutamylcysteinylglycine (glutathione). The structure of the SRS from murine mastocytoma cells was therefore 5-hydroxy-6-*S*-glutathionyl-7,9,11,14-eicosatetraenoic acid, LTC_4 (Fig. 3) (*23*).

This represented the first determination of the structure of an SRS-A (*23*). The preparation and some properties of the corresponding cysteinylglycine derivative (LTD_4) and cysteinyl derivative (LTE_4) were reported at the same time (*23*). These compounds were isolated later from natural sources. After our collaboration with Corey *et al.* (*24, 25*), the structure of LTC_4 was confirmed by comparison with the synthetic material and preparation of stereoisomers of LTC_4 (*25*). Leukotriene C_4 is thus 5(*S*)-hydroxy,6(*R*)-*S*-glutathionyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid.

Subsequent studies with rat basophilic leukemia cells demonstrated that the major SRS was less polar than LTC_4 (*25*). That the fatty acid part of this compound and LTC_4 were identical was indicated by their UV spectra, the product obtained after Raney nickel desulfurization, and the spectral change observed after treatment with soybean lipoxygenase. Amino acid analyses, however, showed that the less polar product lacked glutamic acid. Edman-degradation indicated that glycine was COOH-terminal. Incubation of LTC_4 with γ-glutamyl transpeptidase yielded additional proof of the structure. The product, 5(*S*)-hydroxy,6(*R*)-*S*-cysteinyl-glycine-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid (LTD_4), was identical with the less polar product from RBL-1 cells (Fig. 3). LTD_4 is more potent than LTC_4 in the guinea pig ileum bioassay and the contraction is faster (*26*).

After the structure of SRS from mastocytoma cells was determined (*20, 23*) and after LTC_4, LTD_4, and LTE_4 were synthesized (*23*), all of these cysteine-containing leukotrienes (Fig. 3) were found in a variety of biological systems (Table 1). Thus SRS-A is a mixture of the cysteine-containing leukotrienes, that is, the parent compound LTC_4 and the metabolites LTD_4 and LTE_4. The relative proportion of these leukotrienes de-

pends on the procedure used to prepare the SRS-A.

The original premise (*20*) that the unstable epoxide intermediate (LTA_4) is the precursor of SRS (LTC_4) has been confirmed. When mastocytoma or basophilic leukemia cells were incubated in the presence of arachidonic acid and ionophore A23187, the addition of acidic methanol terminated the reactions so that the diastereoisomeric 5-hydroxy-12-methoxy-6,8,10-*trans*-14-*cis*-eicosatetraenoic acids were trapped as products of the epoxide LTA_4 (*4, 27*). Time-course analyses demonstrated an early transient accumulation of LTA_4 (maximum after about 30 seconds of incubation) which preceded the formation of LTC_4 by mastocytoma cells and the formation of LTD_4 in the leukemia cells. The direct conversion of LTA_4 into LTC_4 has been demonstrated in both mastocytoma cells and human leukocytes treated with the inhibitor of arachidonic acid metabolism, BW755C (*28*). These experiments thus confirm the originally proposed pathway (*20, 23*) for the biosynthesis of SRS (Fig. 3), that is, formation of LTA_4 from arachidonic acid via 5-HPETE followed by glutathione conjugation of LTA_4 with opening of the epoxide at the allylic position C-6.

The significance of the biosynthetic pathways described and the cumbersome systematic names of the compounds involved suggested the introduction of a trivial name for these entities (*21*). The term "leukotriene" was chosen because the compounds were discovered in leukocytes and the common structural feature is a conjugated triene. Various members of the group have been designated alphabetically: leukotrienes A are 5,6-oxido-7,9-*trans*-11-*cis*; leukotrienes B, 5(*S*),12(*R*)-dihydroxy-6-*cis*-8,10-*trans*; leukotrienes C, 5(*S*)-hydroxy-6(*R*)-*S*-γ-glutamyl-cysteinyl-glycyl-7,9-*trans*-11-*cis*; leukotrienes D, 5(*S*)-hydroxy-6(*R*)-*S*-cysteinylglycyl-7,9,-*trans*-11-*cis*; and leukotrienes E, 5(*S*)-hydroxy-6(*R*)-*S*-cysteinyl-7,9-*trans*-11-*cis* eicosapolyenoic acids. Since precursor acids containing the $\Delta^{5,8,11}$ double bond system (that is, 5,8,11-eicosatrienoic acid, arachidonic acid, and 5,8,11,14,17-eicosapentaenoic acid) can be converted to leukotrienes containing three to five double bonds, a subscript denoting this number is used (*29*). Leukotriene A_4 is thus the epoxy derivative of arachidonic acid which can be further transformed to leukotrienes B_4, C_4, and E_4. The polyunsaturated fatty acid 8,11,14-eicosatrienoic acid was earlier found to be oxygenated at C-8 to give 8-hydroxy-eicosatrienoic acid (*1*). In accordance with this result a leukotriene C compound with the alcohol group at C-8 and the thioether linkage with glutathione at C-9 has been identified (*30*). This compound is referred to as 8,9-LTC_3.

Leukotriene C_4 can be metabolized to LTD_4 by enzymatic elimination of glutamic acid by γ-glutamyl transpeptidase (*29*). The remaining peptide bond in leukotriene D_4 is hydrolyzed by a renal dipeptidase to give leukotriene E_4 (*31*). It has recently been found that LTE can also function as acceptor of γ-glutamic acid forming a γ-glutamyl, cysteinyl derivative, named LTF (*32*) (Fig. 3).

The metabolism of LTC_3 was investigated by using tritiated material of high specific activity and with the label in the

Table 1. Identification of leukotrienes from different sources.

Sources	LTA_4	LTB_4	LTC_4	LTD_4	LTE_4	References
Rabbit peritoneal leukocytes	+	+				*2, 4*
Human peripheral leukocytes	+	+	+			*7, 35, 74*
Mouse mastocytoma cells	+		+			*20, 23, 27*
Rat basophilic leukemia cells				+	+	*26, 75*
Rat peritoneal monocytes			+	+		*76*
Rat peritoneal cells (anaphylactic)			+	+	+	*77*
Rat peripheral leukocytes		+				*78*
Rat pleural neutrophils		+				*79*
Rat macrophages		+				*17*
Mouse macrophages			+			*80*
Human lung			+	+		*81*
Guinea pig lung				+		*82*
Cat paws				+	+	*83*

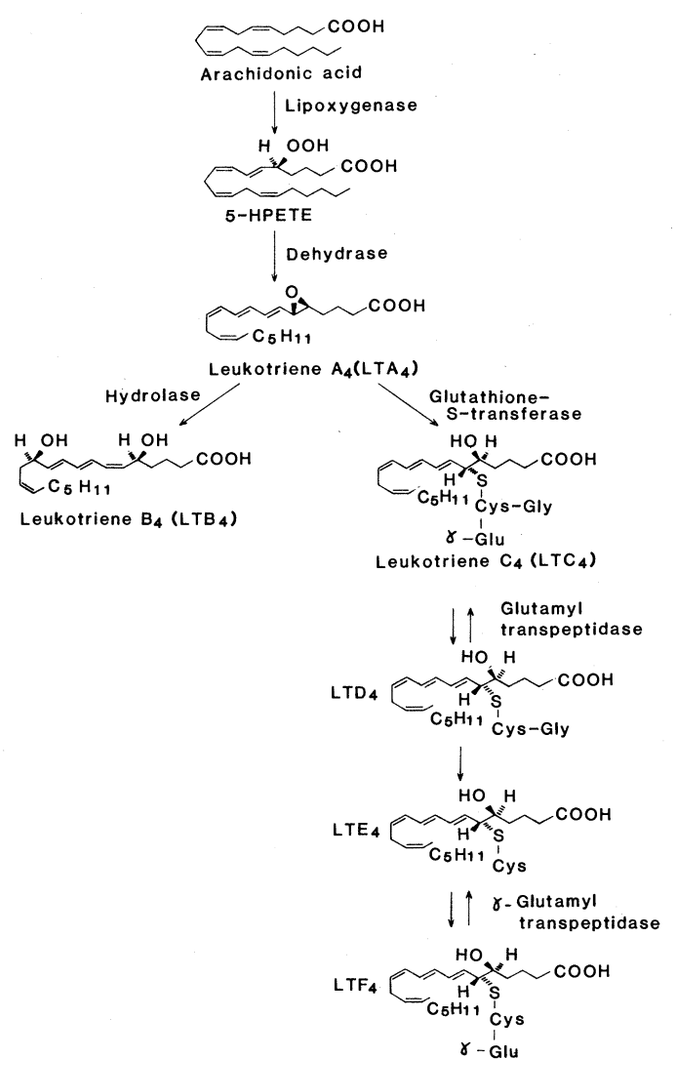

Fig. 3. The formation of leukotrienes by way of the 5-lipoxygenase pathway.

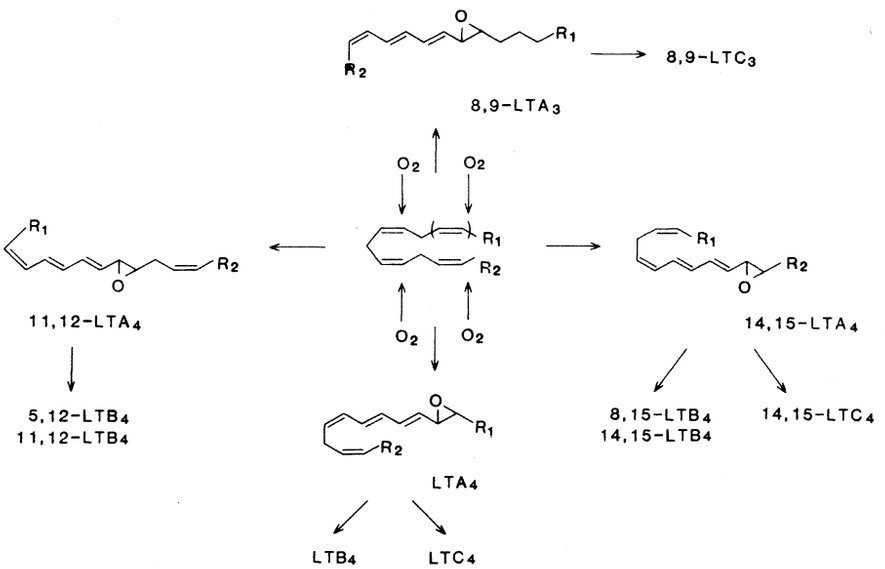

Fig. 4. Novel pathways for leukotriene formation.

fatty acid part of the molecule (*33*). The results showed that homogenates of guinea pig lung rapidly converted LTC_3 to LTD_3. Liver and kidney homogenates did not catabolize LTC_3 appreciably. This was apparently due to high tissue concentrations of glutathione which prevented LTD_3 formation, because LTD_3 was rapidly metabolized by liver and kidney homogenates through hydrolysis of the peptide bond to give 5-hydroxy-6-*S*-cysteinyl-7,9,11-eicotrienoic acid (LTE_3). In accordance with these findings, labeled LTC_3 administered into the right atrium of male monkey was rapidly transformed into LTD_3 and LTE_3 (*34*).

Novel Pathways for Leukotriene Formation

Human leukocytes contain, in addition to the 5-lipoxygenase, enzymes catalyzing the introduction of oxygen at C-12 and C-15 (*35*). We have recently obtained evidence for leukotriene formation after initial oxygenation at either position (*36–38*) (Fig. 4).

Because the diene structures are located symmetrically next to the methylene group (C-10) that is subject to hydrogen abstraction in leukotriene synthesis, our initial attempts to study alternative pathways for leukotriene formation involved characterization of products derived from 15-hydroperoxy-eicosatetraenoic acid (15-HPETE) (*15, 38*). When the 10,000*g* supernatant from a homogenate of human leukocytes was incubated with 15-HPETE, several compounds exhibiting a leukotriene-like UV spectrum were produced. These compounds were also obtained from incubations of intact leukocytes, and were identified as two isomers of 14,15-dihydroxy-5,8,10,12-eicosatetraenoic acid (14,15-LTB_4) and two 8,15-dihydroxy-isomers, that is, 8(*R*),15(*S*)-dihydroxy-5-*cis*, 9,11,13-*trans*-eicosatetraenoic acid and 8(*S*),15(*S*)-dihydroxy-5-*cis*, 9,11,13-*trans*-eicosatetraenoic acid. The same compounds were formed from arachidonic acid.

Since it seemed likely that biosynthesis of the dihydroxy derivatives involved intermediate formation of the allylic epoxide 14,15-oxido,5,8,10,12-eicosatetraenoic acid (14,15-LTA_4), experiments were designed to trap the epoxide by addition of acidic methanol as previously described for the 5,6-epoxide (*32*). Mass spectrometric analysis of the trapping products demonstrated that they consisted of isomeric 8-methoxy-15-hydroxy-5,9,11,13-eicosatetraenoic acids, epimeric at C-8. The formation of these derivatives is analogous to the reaction of the 5,6-epoxide and provided strong evidence for the intermediary formation of 14,15-LTA_4. Concerning the stereochemistry of the epoxide, it is assumed that the transformations involve retention of configuration at C-15 and that in analogy with the structure of 5,6-LTA_4 the epoxide has a *trans* configuration and 5,8-*cis*,11,12-*trans* double bonds (*33*).

Incubation of intact cells with arachidonic acid in an atmosphere containing $^{18}O_2$ showed that molecular oxygen was incorporated at C-14 and C-15 in the two isomeric 14,15-LTB_4 compounds, whereas when 15-HPETE was used as the substrate $^{18}O_2$ was only incorporated at C-14. The major part of 14,15-LTB_4 therefore seems to be formed by reaction of 14,15-LTA_4 with an active oxygen species, the nature of which has not been established (*39, 40*). Similar results have been obtained by Maas *et al.* (*41*). Recently, synthetic 14,15-LTA_4 was incubated with mastocytoma cells in the presence of glutathione; this resulted in addition of the thiol to C-14 to form a 14,15-LTC_4 in analogy with the formation of the glutathione adduct 5,6-LTC_4 (*42*).

The conversion of arachidonic acid by the lipoxygenase-catalyzed introduction of oxygen at C-12, to form 12-hydroperoxy-eicosatetraenoic acid (12-HPETE) and the reduction product 12-HETE, was discovered in human platelets and has subsequently been observed in several tissues, including human leukocyte preparations (*39, 40*). We have now obtained evidence that leukotrienes are also formed by the 12-lipoxygenase pathway (*37*). Human neutrophils were incubated with arachidonic acid and the ionophore A23187, and the products extracted from the medium were separated by RP-HPLC. The "LTB_4" fraction was converted to methyl esters and treated with phenylboronic acid in order to prepare boronates of the vicinal diols. This product was separated by straight-phase HPLC, and the boronate fraction, after hydrolysis of the boronate and conversion to trimethyl silyl ether derivatives, was separated by gas chromatography and analyzed by mass spectrometry. In addition to 14,15-LTB_4 isomers, a component with a mass spectrum consistent with a dihydroxy-eicosatetraenoic acid (mass-to-charge ratio, 494) with hydroxyl groups at C-11 and C-12 (*m/e* 213 and 281) and double bonds at 5, 7, 9, and 14 was detected. A corresponding hydrogenated derivative showed the expected major ions at *m/e* 215 and 287. These results therefore indicated the formation of 11,12-dihydroxy-5,7,9,14-eicosatetraenoic acid (11,12-LTB_4) (*37*). To obtain evidence for the intermediate generation of the corresponding 11,12-epoxide, we trapped the products formed from arachidonic acid with acidic methanol. Mass spectrometric analysis of one of these products demonstrated that the methoxy group was located at C-12 and the hydroxyl group at C-5 (*m/e* 203 and 325), indicating formation of 11,12-oxido,5,7,9,14-eicosatetraenoic acid (11,12-LTA_4).

Biological Effects of Leukotrienes

Recent studies with pure leukotrienes, prepared biosynthetically or synthetically, have provided detailed information about the effects of this group of compounds in different biological systems. Thus, the cysteine-containing leukotrienes (LTC_4, LTD_4, and LTE_4) are potent bronchoconstrictors in several

species, including humans, with specific effects on the peripheral airways (*43–48*). They are also potent vasoconstrictors and show negative inotropic effects on the cardiac contractions (*49*). Recent studies involving intravital microscopy of the cheek pouch of the hamster (*Mesocricetus auratus*) have demonstrated specific effects of these leukotrienes on the permeability of the postcapillary venules (*43*). This preparation for microcirculatory studies also revealed specific effects of LTB_4 on the adhesion of neutrophils to endothelial cells and extravasation of white cells (*43*). Other studies have shown that LTB_4 is a potent chemotactic agent toward polymorphonuclear cells, eosinophils, and monocytes (*50, 51*). Recent work suggests that LTB_4 can stimulate enzyme release and superoxide generation in human neutrophils (*52*) and may have ionophoric activity (*53*).

Pulmonary effects of leukotrienes. Intravenous administration of either LTC_4 or histamine increases the tracheal insufflation pressure in anesthetized and artificially ventilated guinea pigs (*44, 54*). LTC_4 was at least 100 times as potent as histamine and caused a long-lasting increase in insufflation pressure, whereas histamine gave a more transient effect. When given as an aerosol, LTC_4 was at least 1000-fold more potent than histamine, the effects of LTD_4 and LTE_4 being practically identical to those of LTC_4.

The bronchoconstrictor activity of LTC_4 has also been studied in artificially ventilated monkeys (Fig. 5) (*48*). When injected into the right atrium, LTC_4 and histamine were practically equipotent in increasing the transpulmonary pressure, although the time course of their actions differed in the same way as in the guinea pig. However, when given as an aerosol, LTC_4 was at least 100 times as potent as histamine. A short-lasting increase in transpulmonary pressure was observed with histamine (1000 to 5000 nanomoles), whereas LTC_4 (20 nmole) caused a severe bronchoconstriction, as evidenced by a marked rise in transpulmonary pressure and a simultaneous fall in the arterial partial pressure of oxygen (PO_2). The bronchoconstriction and the decrease in PO_2 were long-lasting, and they normalized only after 45 to 50 minutes. The increase in transpulmonary pressure induced by LTC_4 was accompanied by a marked decrease in dynamic compliance, whereas airway resistance was little affected. These findings indicate that cysteinyl-containing leukotrienes preferentially affect the peripheral airways. Similar results have been obtained in man (*55*).

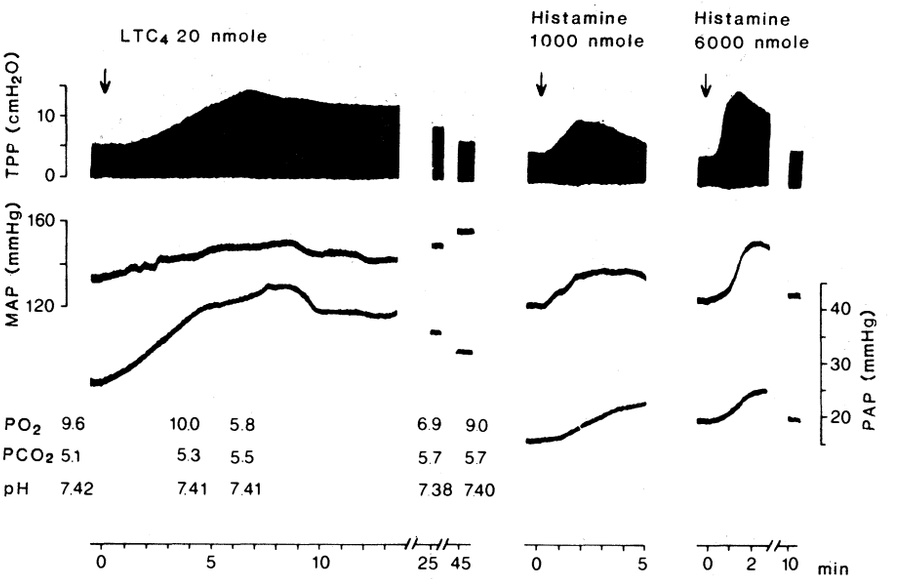

Fig. 5. Effects of LTC_4 and histamine aerosols (exposure for 2 minutes) on transpulmonary pressure (*TPP*), mean systemic arterial pressure (*MAP*), and pulmonary arterial pressure (*PAP*) in artificially ventilated monkey (*Macaca irus*). Blood samples were withdrawn for determination of the partial pressures of O_2 and CO_2 (PO_2, PCO_2) and *p*H as indicated in the figure.

The difference in bronchoconstriction following administration of leukotrienes as aerosols and by the intravenous route might be due to activation of different receptors. Thus, aerosol administration of LTC_4 elicits an apparently normal response in guinea pigs desensitized to LTC_4 injected intravenously (*56, 57*). Furthermore, indomethacin, a cyclooxygenase inhibitor, blocks the bronchoconstrictor effects of intravenously administrated LTC_4 (*56–59*), indicating that this response is mainly due to release of thromboxane A_2, the bronchoconstrictor and cyclooxygenase product released by leukotrienes in lung preparations (*60*). In the form of an aerosol, however, LTC_4 elicits a bronchoconstrictor response that is enhanced by indomethacin (*56, 59*). Leukotriene B_4 is approximately 100 times less potent than LTC_4 (*61*) and induces its effects essentially via release of thromboxane A_2 (*62*).

Studies with bronchi from atopic patients sensitive to birch pollen (*63*) demonstrate the relative importance of leukotrienes as mediators of anaphylaxis. Combined treatment of the preparation with a histamine antagonist, mepyramine, and with indomethacin did not reduce the contraction response to the specific allergen. However, benoxaprofen and a prostacyclin derivative (U-60257), both of which block leukotriene formation (*64, 65*), inhibited the anaphylactic contraction in bronchi from asthmatics induced by birch pollen. Furthermore, incubation of the atopic lung tissue with antigen resulted in a release of LTC_4, LTD_4, and LTE_4 which could be inhibited by U-60257 (*63*).

Leukotriene C_4 and especially LTD_4 have recently been shown to induce mucus secretion in tracheal submucosal glands in dogs (*66*).

The studies described above indicate that the cysteinyl-containing leukotrienes (LTC_4, LTD_4, and LTE_4) are major mediators of airway anaphylaxis, whereas LTB_4, histamine, and thromboxane A_4 are less important in this respect. The recent finding that asthma induced in monkeys by extracts of the U-60257 intestinal nematode *Ascaris* can be blocked by U-60257 supports this contention and indicates that leukotriene antagonists, or inhibitors of leukotriene formation, could be of therapeutic value in the treatment of bronchial asthma (*64*).

Microvascular effects of leukotrienes. Leukotriene C_4 and LTD_4 mimic the effects of crude SRS-A by causing extravasation of Evan's blue when injected intradermally into guinea pigs (*43, 47, 54*). We have recently studied the microvascular actions of leukotrienes in more detail using the hamster cheek pouch preparation in vivo (*43*).

Leukotrienes C_4 and D_4, when applied topically to the cheek pouch for a 3-minute period in 0.3 to 20 nanomolar concentrations, elicited an intense dose-dependent contraction of arterioles, in particular the terminal arterioles. The vasoconstriction was short-lived, and was consistently followed by a dose-dependent and reversible leakage of macromolecules, as indicated by extravasation of fluorescein-conjugated dextran. This leakage appeared at postcapillary venules, in acordance with previous

observations that this vessel segment is the target for action of substances that cause reversible changes in vascular permeability. According to dose-response curves assessed noncumulatively, LTC_4, LTD_4, and LTE_4 all induced a significant increase of vascular permeability at much lower concentrations than histamine (LTC_4 was approximately 5000 times more potent than histamine).

The increased vascular permeability induced by the cysteinyl-containing leukotrienes seems to be caused by a direct action on the vessel wall, since it occurs rapidly and does not require release of histamine or prostaglandins or the participation of PMNL's. Leukotriene B_4 also elicits extravasation of plasma, although at higher concentrations. The reaction occurs with some latency and requires adhering leukocytes. Administration of a vasodilator together with leukotrienes potentiates the increase in plasma leakage caused by a submaximal dose of leukotrienes, as has been reported in the guinea pig for PGE_2 and LTD_4 (*67*) and in the guinea pig, rabbit, and rat for PGE_2 and LTB_4 (*68*).

Effects of leukotrienes on leukocyte functions. When LTB_4 was administered to the hamster cheek pouch in the same dose-range as LTC_4, it did not elicit vasoconstriction and had no effect on plasma leakage. However, LTB_4 caused a dramatic increase in leukocyte adhesion to endothelium in small venules (*43*). Upon addition of LTB_4 to the medium superfusing the cheek pouch (final concentration, 500 picomolar), marginating leukocytes immediately started to roll slower, and, although blood flow was unchanged, the number of leukocytes adhering to the endothelium increased significantly. This effect was dose-dependent, reached its maximum after 6 to 8 minutes, and remained at this level until LTB_4 was withdrawn, when it gradually subsided to the control value in 5 to 15 minutes. Increased adherence of human leukocytes caused by LTB_4 has also been demonstrated in vitro with a column of nylon fibers (*52*).

Even during a short-lasting superfusion with LTB_4 (6 to 10 minutes) the number of interstitial white cells increased. This finding is consistent with the chemotactic stimulant property of LTB_4, which has been demonstrated in vitro by the Boyden chamber technique and by migration under agarose (*51, 69*). In vivo, this effect has been monitored by determining white cell accumulation in the peritoneal cavity of guinea pigs injected intraperitoneally with LTB_4 (*70*).

The results indicate that LTB_4 might be a mediator in the migration of leukocytes from the blood to areas of inflammation. In this context it is of interest that the chemotactic peptide, formyl-methionyl-leucyl-phenylalanine stimulates the formation of LTB_4 and especially of ω-oxidized leukotrienes in human neutrophils (*71*).

Leukotriene B_4 also activates neutrophils. Within seconds after addition to the cells, nanomolar concentrations of LTB_4 cause aggregation, degranulation, superoxide generation, and mobilization of membrane-associated calcium. The ionophoric effect can also be demonstrated by means of liposomes (*53, 72*).

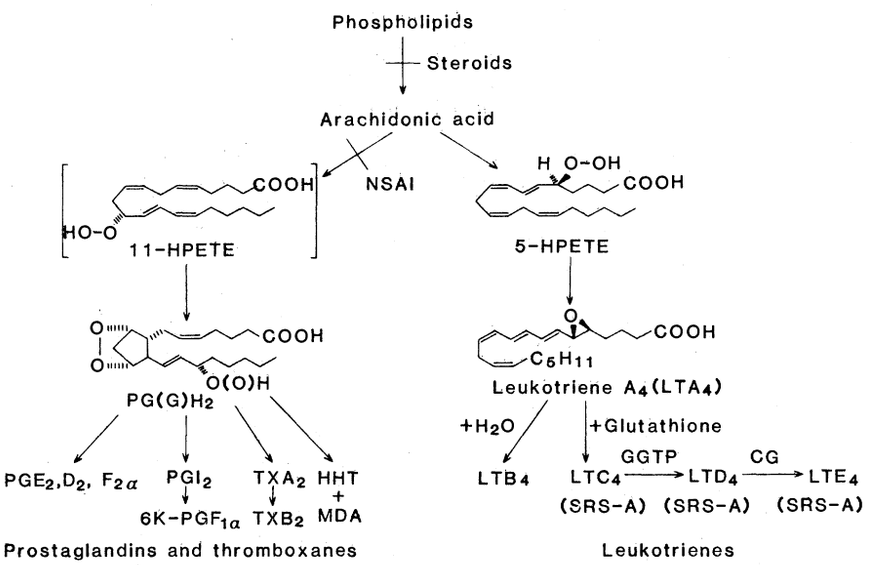

Fig. 6. Formation of prostaglandins, thromboxanes, and leukotrienes. The intermediate 11-HPETE is bracketed because there is only partial evidence of its occurrence; NSAI, nonsteroidal anti-inflammatory compounds.

Conclusions

Biological studies indicate that the cysteinyl-containing leukotrienes are important in such immediate hypersensitivity reactions as human asthma through their potent effects as bronchoconstrictors and stimulators of vascular leakage.

The dihydroxy derivative, LTB_4, has potent effects on neutrophils related to their adhesion to postcapillary venules and extravasation as well as chemotaxis-stimulated migration to areas of inflammation and degranulation (*43, 50–52*). In combination with the effects of the cysteine-containing leukotrienes on the increase in vascular permeability, these effects are reminiscent of acute inflammatory reactions. A synergistic effect between the leukotrienes and the vasodilator prostaglandins PGE_2 and PGI_2 might also be important in the generation of inflammatory edema.

Steroids with anti-inflammatory activity prevent the release of the precursor acid, arachidonic acid, whereas cyclooxygenase inhibitors, such as aspirin, inhibit the transformation of this acid into prostaglandins and thromboxanes. The steroid-induced inhibition of arachidonic acid release, proposed to be due to formation of peptide inhibitors of phospholipase A_2, prevent formation of not only prostaglandins and thromboxanes but also leukotrienes and other oxygenated derivatives (*73*). It is therefore conceivable that some of the therapeutic effects of steroids which are not shared by aspirin-type drugs are due to inhibition of leukotriene formation.

The leukotrienes are constituents of a more general biological control system based on arachidonic acid as the precursor molecule (Fig. 6). This compound is normally stored in biological membrane structures and can be released through activation of a hydrolytic system by a variety of stimuli. Depending on the availability of active enzymes in the stimulated cell, arachidonic acid is converted into one or several biologically active compounds. A variety of stimuli can thus be converted into a multitude of compounds that can regulate or mediate various cell functions. The new knowledge about this system suggests new possibilities for the development of novel and more specific therapeutic agents, particularly in diseases related to immediate hypersensitivity reactions and inflammation. Such drugs might be based on antagonism of end products or inhibition of enzymes involved in the generation and further transformation of the key intermediate, leukotriene A_4. Com-

pounds that have inhibitory effects in both the cyclooxygenase pathway and the leukotriene pathway might also be of importance.

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