The Leukotrienes in Allergy and Inflammation

Research on these potent new biological agents is filling some of the gaps in what is known about allergy and inflammation

The 3 years since the discovery of the leukotrienes have seen a surge of research on these potent biological agents. The interest has been sparked by evidence indicating that the chemicals mediate allergic and inflammatory reactions and may contribute to the development of such common and debilitating diseases as asthma and rheumatoid arthritis. Edward Goetzl of Harvard Medical School says, "They do everything you ever wanted in terms of allergic reactions."

Although the leukotrienes have been known as such for only a few years, their history actually began in Australia in 1938 when researchers in the laboratory of Charles Kellaway discovered slow reacting substances (SRS), a material that causes slow contractions of smooth muscle. The chemical identity of SRS was to remain unknown until 1979, when it was found to be a mixture of three previously unknown substances—leukotrienes—that are chemically related to the prostaglandins and thromboxanes, which are themselves suspected of participating in allergic and inflammatory reactions.

In the 40 years between the discovery of SRS and its identification, investigators produced a great deal of evidence suggesting that SRS is released during allergic reactions and produces many of the effects experienced by afflicted individuals. [Although the material released in allergic reactions was usually designated SRS-A (for slow reacting substances of anaphylaxis) to distinguish it from SRS released by nonimmunologic means, the materials are now known to be the same.]

In particular, investigators were interested in SRS because they thought that it might help trigger asthma attacks by contracting the smooth muscle of the respiratory tract and causing airway obstruction. Other agents, including certain of the prostaglandins and histamine, also contribute to asthma attacks, but SRS appeared to have a unique role. In the early 1970's, studies in the laboratory of K. Frank Austen at Harvard Medical School showed that it has major effects on the smaller, peripheral airways of the lungs, rather than on the larger central passages, which include the trachea and the bronchi. Contractions in both the larger and smaller passages contribute to the breathing difficulties of asthma patients, but histamine and prostaglandin $F_{2\alpha}$ had little effect on the peripheral airways, whereas SRS contracted them effectively. Austen says of the action of SRS, "It seemed to fill a major hole in understanding the problem of bronchial asthma."

Studies such as these helped to keep alive interest in SRS, but the research was greatly handicapped by the inability to isolate the agent, which is extremely potent and made in very small quantities. Only small amounts of impure SRS were available for the biological studies. Moreover, without purified material structural determinations were impossible, although investigators did manage to partially characterize SRS as a sulfur-containing lipid with a molecular weight of about 400. It was polar and nonvolatile, however, which added to the difficulty of purifying the material and determining its structure. Not until the advent of high-performance liquid chromatography in the latter part of the 1970's was the purification problem solved.

Among the laboratories that contributed prominently to the eventual solution of the SRS structure were those of Austen, Charles Parker of the Washington University School of Medicine and the Howard Hughes Medical Institute Laboratory, Priscilla Piper of the Royal College of Surgeons in London, and Bengt Samuelsson of the Karolinska Institutet.

There were a number of milestones and a false lead or two along the way. The latter include a report by the Austen group that SRS was inactivated by treatment with the enzyme arylsulfatase. Because this enzyme attacks sulfate bonds, the investigators concluded that SRS contained sulfur in the form of sulfate.

Austen and his colleagues already had evidence from the chemical analysis of a small amount of partially purified SRS to suggest that it contained sulfur. "But the material was not totally purified," Austen notes. "We wanted a second test with the enzyme. The two together gave me the confidence to say that the sulfur was there."

They were right about the sulfur, but later work by Parker and Samuelsson showed that it is present in a thioether linkage. The apparent inactivation by arylsulfatase was largely caused by a contaminating enzyme that breaks down peptides. Of course at the time the inactivation was reported, no one knew that SRS contained a peptide group.

A major milestone was the discovery that arachidonic acid, an unsaturated fatty acid found in many phospholipids, is a starting point for the synthesis of SRS. Parker and Barbara Jakshik, also of Washington University School of Medicine, came to this conclusion, as did Michael Bach of the Upjohn Company. The investigators showed, for example, that labeled arachidonic acid becomes incorporated into SRS.

Arachidonic acid was already known to be the precursor for the synthesis of prostaglandins and thromboxanes, through the action of an enzyme called cyclooxygenase. SRS synthesis does not require this enzyme, however. Parker and Jakshik found that indomethacin, which inhibits cyclooxygenase, did not prevent SRS formation.

Meanwhile, Samuelsson and his colleagues were coming to similar conclusions by a different route. While studying the metabolism of arachidonic acid in white blood cells, they identified an unstable epoxide that was a key intermediate in the formation of a number of hydroxy derivatives of arachidonic acid. The production of certain of these acids was greatly stimulated by the ionophore A23187, which also stimulates the production of SRS. (Ionophores are compounds that carry ions across membranes; A23187 is a calcium ionophore.) Moreover, their ultraviolet spectra resembled that of SRS, which had been reported by Piper and her co-workers. This finding indicated that the materials have similar structures.

The agents were all linear compounds, unlike the prostaglandin and thromboxane products of the cyclooxygenase pathway, which contain ring structures. The formation of the epoxide intermediate (now known as leukotriene A_4) is catalyzed by other enzymes, including a lipoxygenase.

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Samuelsson concluded that the intermediate might also be an SRS precursor, and this turned out to be a key idea. The structure of the intermediate was known and could serve as a guide to the structure of the lipid portion of SRS, which proved to be a 5-hydroxy-7,9,11,14-eicosatetraenoic acid. Further work in the various laboratories showed that the SRS components carry thioether substituents on carbon 6 of this acid.

Samuelsson's group published the first complete structure of an SRS component, leukotriene C_4 (LTC_4), which has a glutathionyl residue as its thioether substituent. Glutathione is a tripeptide consisting of linked residues of three amino acids, cysteine, glycine, and glutamic acid. The cysteine residue contains a free sulfhydryl group, which forms the thioether link when glutathione reacts with LTA_4 to produce LTC_4.

The final proof of the structure of LTC_4 was the complete synthesis of the molecule by E. J. Corey of Harvard University and the demonstration that the properties of the synthetic material were identical to those of the natural one.

In addition to LTC_4, SRS contains large amounts of an even more active muscle contractor, LTD_4, which differs from LTC_4 by the loss of the glutamic acid residue from the peptide part of the molecule. Finally, there may also be small amounts of LTE_4, which has lost both the glutamic acid and glycine residues, leaving just the cysteine residue attached to the fatty acid moiety.

Samuelsson suggested that the compounds be called leukotrienes because they are made by leukocytes and have three conjugated double bonds. The numbered subscripts indicate the total number of double bonds in the molecules.

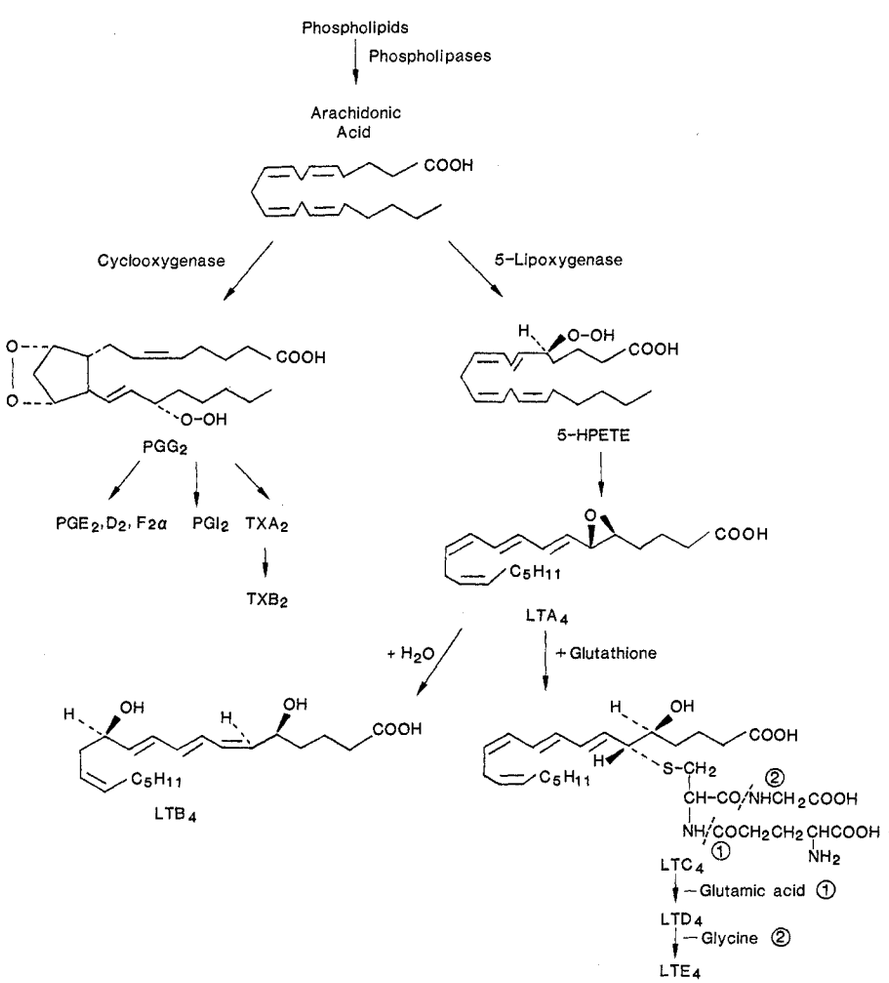
The leukotrienes and their analogs are now being synthesized by Corey and Joshua Rokach of Merck Frosst Research Laboratory in Pointe-Claire/Dorval, Quebec, among others. As a result of the availability of large quantities of pure materials, says Robert Murphy of the University of Colorado Health Sciences Center in Denver and a collaborator with Samuelsson on some of the structural work, "The field is exploding."

The work with the pure synthetic materials is showing that the agents are extremely potent compared to other agents thought to be involved in mediating allergic and inflammatory responses. For example, investigators generally find that LTC_4, LTD_4, and LTE_4 are 100 to 1000 times more potent, on a molar basis, than histamine or the prostaglandins in their effects on the pulmonary airways.

The Austen group found this with isolated lung preparations, especially with parenchymal strips, which represent the peripheral airways. Samuelsson and his colleagues found LTC_4, administered in aerosols, to be about 100 times more potent than histamine in decreasing pulmonary airflow in monkeys. And, according to Austen, LTC_4 was 3800 times as active as histamine in decreasing the airflow in lungs of normal human volunteers who inhaled the agents. Austen says, "The activity of this group of molecules is absolutely phenomenal."

The same assessment applies to leukotriene effects in inflammatory reactions. Edema, one of the hallmarks of inflammation, may result from the action of the agents on small blood vessels. For example, the Austen and Samuelsson groups have shown that when LTC_4 and LTD_4 are injected into the skin of experimental animals, it causes fluid leaks from the blood vessels. They first injected the animals intravenously with a blue dye that does not normally pass through blood vessel walls. The animals' skin turned blue around the sites of the leukotriene injections, indicating that the dye was escaping.

By directly observing the effects of leukotrienes on the small blood vessels of the hamster cheek pouch, Samuelsson and his colleagues showed that the agents produce a contraction of the vessels, especially the terminal arterioles. The contraction is short-lived—less than 5 minutes—and is followed by leakage of fluid from the vessels. According to Samuelsson, LTC_4, LTD_4, and LTE_4 all increase vascular permeability at much lower concentrations than does histamine.

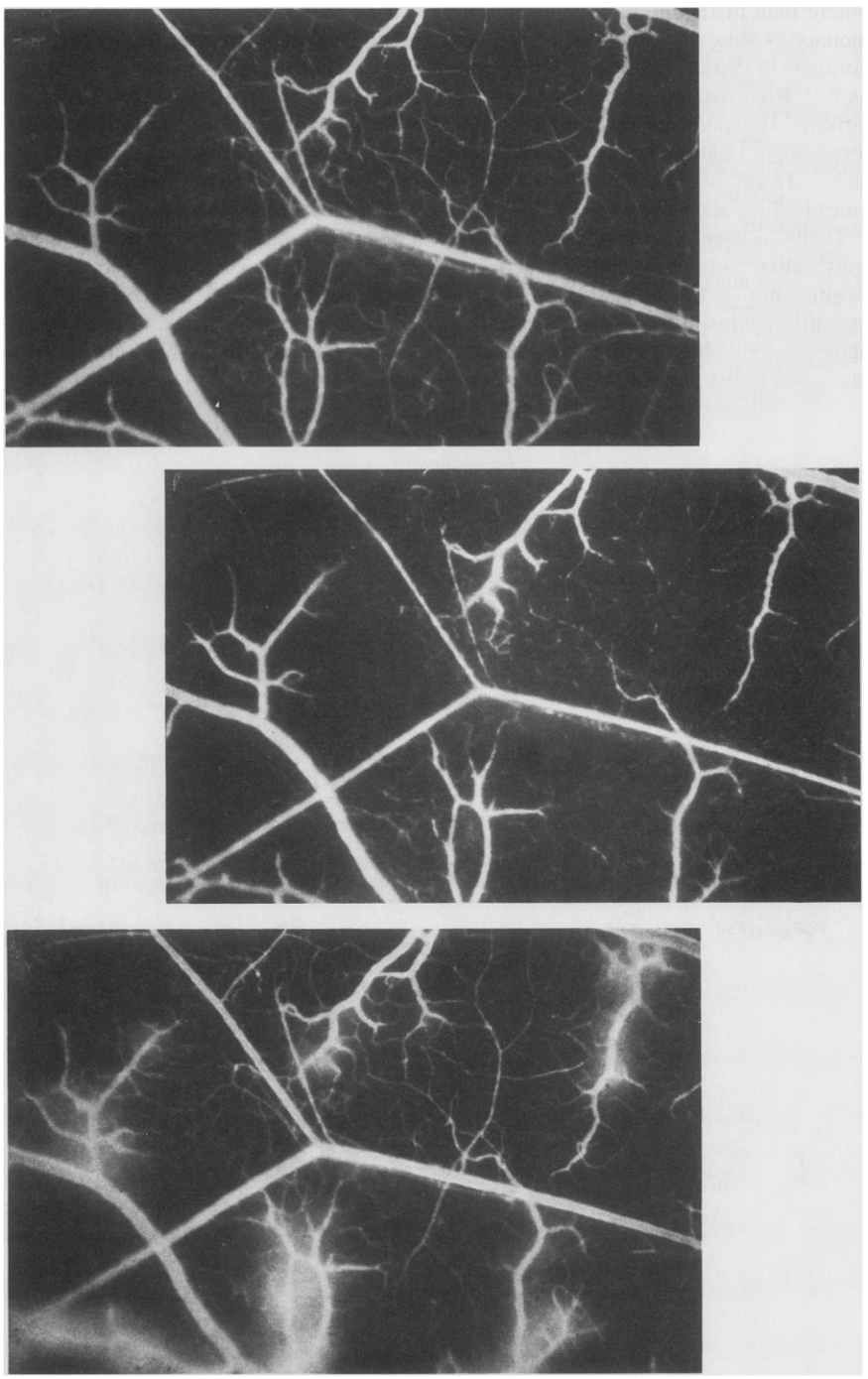


Leukotriene synthesis

Arachidonic acid, which is released from phospholipids by the action of phospholipase enzymes, serves as the starting point for the synthesis of the leukotrienes (LT's) and the prostaglandins (PG's) and thromboxanes (TX's). The latter two groups of compounds are formed by the cyclooxygenase pathway. To form the leukotrienes, the enzyme 5-lipoxygenase first converts arachidonic acid to 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HPETE). 5-HPETE forms the epoxy acid LTA_4, which is converted to LTB_4 by the addition of water or to LTC_4 by the addition of glutathione. LTD_4 is formed by the loss of glutamic acid (step 1) and LTD_4 is converted to LTE_4 by the loss of the glycine residue (step 2).

Other blood vessels that may be affected by the leukotrienes are the coronary arteries, which deliver blood to the heart. Piper and her colleagues find that LTC_4 is a very potent constrictor of these arteries. "We are wondering," she says, "if they [the leukotrienes] are involved in coronary insufficiency and angina." Coronary artery spasms have been implicated as a cause of both angina and heart attacks.

Some of the leukotrienes can activate certain responses of white blood cells. In particular, Goetzl finds that LTB_4 (which has a hydroxyl group instead of a thioether on carbon 6 of the lipid chain and is also called 5,12-DHETE for 5,12-dihydroxyeicosatetraenoic acid) attracts neutrophils and eosinophils, two types of white blood cells that are present in high numbers at inflammatory sites. They can contribute to tissue damage by directly attacking cells or by releasing enzymes, such as lysozyme, that digest cell constituents. Goetzl suggests that LTB_4, by attracting these cells, could contribute to the pathology of inflammation. The leukotriene has this effect at concentrations comparable to those at which known chemotactic agents work. In addition, at higher concentrations LTB_4 may stimulate release of lysozyme from the granules of white blood cells.



Leukotriene effects

When LTD_4 is applied to the hamster cheek pouch, it causes constriction of the small blood vessels, especially the arterioles. The top micrograph shows the normal vasculature. One minute after addition of LTD_4 (middle micrograph), the straight Y-shaped arteriole has narrowed and the terminal arteriole running vertically through the center of the field appears to have completely closed. Four minutes later (bottom micrograph) vasoconstriction has ceased, but fluid is leaking from the blood vessels as indicated by the movement of a fluorescent dye into the surrounding tissues. [From the laboratory of Bengt Samuelsson at the Karolinska Institutet; originally published in Proc. Natl. Acad. Sci. U.S.A. *78, 3887 (1981)]*

That LTB_4 is involved in human inflammatory disease is suggested by Goetzl's finding that the concentration of the leukotriene is higher in joint fluid from patients with rheumatoid arthritis or spondyloarthritis than in fluid from individuals with noninflammatory joint problems. The LTB_4 concentrations in the former case were higher than concentrations that were chemotactic in vitro. Moreover, injection of an anti-inflammatory steroid drug into the joints of six of the patients brought about a reduction in the number of white blood cells in the joint fluid. Other workers have suggested that steroids combat inflammation by inhibiting the release of arachidonic acid from phospholipids, an effect that would increase the formation of arachidonic acid products such as LTB_4.

How the leukotrienes work is not yet known, nor is the relation between leukotriene actions and those of the thromboxanes and prostaglandins. In some cases these agents may antagonize each other's effects. For example, prostaglandins may cause vasodilation, whereas leukotrienes usually constrict blood vessels. In other circumstances they may act together. Thromboxane A_2, LTC_4, and LTD_4 all constrict airway passages. Moreover, according to workers in Samuelsson's laboratory, the leukotrienes may enhance the release of the thromboxane.

A development that would be helpful is the identification of specific inhibitors of leukotriene formation. This would be useful for basic studies of leukotriene action and possibly for the treatment of asthma, rheumatoid arthritis, and related conditions. "It is conceivable," writes Samuelsson, in what may be a masterpiece of understatement, "that compounds interfering with the formation or action of leukotrienes could be of therapeutic value." Drug companies, includ-

ing Upjohn, are interested in identifying such inhibitors.

A final intriguing finding suggests that the leukotrienes may have a role in the nervous system. Murphy and Barry Hoffer, who is also in Denver, find that very low concentrations of LTB_4, LTC_4, and LTD_4 cause prolonged excitation of some brain neurons. Murphy says, "They increased almost irreversibly the firing rate of Purkinje cells and cells in the basal ganglia. . . . We were surprised that they were that active."

Murphy and Hoffer do not yet know what, if anything, this means; the leukotrienes have not yet been detected in brain. But they note that the agents stimulate contraction of guinea pig ileum (intestinal smooth muscle), a point of resemblance between them and many known or suspected neurotransmitters, including acetylcholine and the endogenous brain opiates. They suggest that the leukotrienes may serve as long-term regulators of nerve excitability during allergic reactions and possibly under normal conditions. More work will be needed to test this idea, but with pure preparations of leukotrienes now available, this and the other lines of research should progress far more rapidly than did SRS research during the first 40 years of its history.—JEAN L. MARX

New Theory of Hormones Proposed

Hormones appear to be far more universal than anyone imagined, occurring in flies, worms, and even bacteria

A group of endocrinologists at the National Institutes of Health (NIH) have proposed a substantially new theory of what hormones are and how they work. The theory, as it is currently formulated, explains a number of biological mysteries, ranging from the question of why many cancer patients act as though their cancers are overproducing hormones even though no excess hormones can be detected in their bodies to the question of why some plants make substances that bind more specifically to animal hormone receptors than do animal hormones.

When the NIH group first began to work out the implications of their data on hormones they had to overcome considerable resistance within themselves. "As endocrinologists," explains Jesse Roth of NIH, "we were shackled by outmoded concepts, many of them deriving from experiments done more than 50 years ago. There is a philosophical problem. We have a general concept of how we understand hormones but a lot of the original data are buried in the literature. Only the conclusions remain fresh in our minds."

The tradition in endocrinology had been to think that only highly specialized glands make hormones. Then, about 10 years ago, evidence began accumulating that cancer cells, and even nerve cells, can make hormone molecules. But, says Roth, endocrinologists, rather than questioning their entire dogma, modified it so these findings fit in. They tended to believe that hormones are made only by glands, cancers, and nerves, and did not ask whether hormone synthesis might be far more widespread.

Roth, with Jana Harran Kova and other NIH colleagues, in the meantime, was looking for insulin receptors in the brain. "We're basically insulin receptorologists," he remarks. "We found insulin receptors all over the brain and they were absolutely the classical insulin receptors. We were struck by several things. First, the receptors were present in lots of areas of the brain and they had different distributions in different areas. Second, the receptor levels didn't change under conditions of hyper- and hypoinsulinemia. [In the rest of the body, more receptors show up when there is too little insulin around and fewer appear when there is too much insulin.] Third, insulin has little access to the brain. If you inject an animal with insulin, little of it gets to the brain." These observations led Roth to believe that the brain might be making insulin and that it might be using the hormone in very different ways than the rest of the body.

After looking for, and finding, insulin in the brain, Roth and his colleagues decided to see where else in the body it might be. Roth, with James Rosenzweig and other colleagues, found insulin in the testes and liver.

"When we found insulin in these other cell types, we began to think that perhaps other cells besides those of the pancreas and the brain can make insulin. We began to break the monopoly of the gland," says Maxine A. Lesniak, one of Roth's associates. A few years ago, William Odell of the University of Utah had come to similar conclusions in his work with another hormone, human chorionic gonadotropin. Says Roth, "We asked how far back in evolution does the ability to make hormones go? And we decided to try to reach bottom. We got flies, worms, and unicellular organisms and looked for insulin production."

"We started with flies and worms and we looked at the heads and bodies of the flies and the internal organs and skins of the worms," says Derek LeRoith, a member of Roth's research team. "We found material similar to insulin. Then we jumped to protozoa and once again we found material that looked like insulin. We tried fungi and *Escherichia coli* and came up with the same result."

LeRoith, Roth, and their associates characterized the material from fruit flies, earthworms, protozoa, and *E. coli* by determining that it reacted with insulin antibodies, it was the same size as insulin, it promoted glucose oxidation in isolated fat cells (this is a standard insulin assay), and its action on fat cells was abolished if it was first allowed to react with insulin antibody. In short, the material appeared to be insulin or more like insulin than any known substance.

Next, LeRoith and Roth in collaboration with colleagues in Bethesda, New York, Cincinnati, Dallas, Los Angeles, and La Jolla began looking for other peptide hormones in primitive organisms and found evidence that a long list of hormones, including ACTH, β-endorphin, somatostatin, cholecystokinin, calcitonin, glucagon, and arginine vasotocin, may be there.

Working with Dorothy Krieger of Mt. Sinai School of Medicine and Candace Pert of NIH, Roth and his associates characterized β-endorphin and ACTH in protozoa. The evidence for endorphin is its size, its reactivity with anti-endorphin antibodies and opioid receptors, and its physical-chemical characteristics in high-performance liquid chromatography. The evidence for ACTH is its size, immunoreactivity, and biological activity.

In addition, Krieger found some high

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