

International Union of Pharmacology XXXVII. Nomenclature for Leukotriene and Lipoxin Receptors

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† During the preparation of this manuscript, Professor Nicosia passed away following a long illness. Her efforts in the organization of this committee and her scientific work on the leukotriene receptors over the past years will be missed.

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Abstract—The leukotrienes and lipoxins are biologically active metabolites derived from arachidonic acid. Their diverse and potent actions are associated with specific receptors. Recent molecular techniques have established the nucleotide and amino acid sequences and confirmed the evidence that suggested the existence of different G-protein-coupled receptors for these lipid mediators. The nomenclature for these receptors has now been established for the leukotrienes. BLT receptors are activated by leukotriene B₄ and related hydroxyacids and this class of receptors can be subdivided into BLT₁ and BLT₂. The cysteinyl-

leukotrienes (LT) activate another group called CysLT receptors, which are referred to as CysLT₁ and CysLT₂. A provisional nomenclature for the lipoxin receptor has also been proposed. LXA₄ and LXB₄ activate the ALX receptor and LXB₄ may also activate another putative receptor. However this latter receptor has not been cloned. The aim of this review is to provide the molecular evidence as well as the properties and significance of the leukotriene and lipoxin receptors, which has lead to the present nomenclature.

I. Introduction

Feldberg, Kellaway, and coworkers (Feldberg and Kellaway, 1938; Feldberg et al., 1938; Kellaway and Trethewie, 1940) observed that perfusion of guinea pig lungs with antigen induced the release of a material named "slow reaction smooth muscle-stimulating substance (SRS¹)" that caused a contraction of the isolated guinea pig ileum bioassay tissue. These observations were confirmed by several workers (Schild et al., 1951; Brocklehurst, 1960) who demonstrated that SRS (renamed slow-reacting substance of anaphylaxis or SRS-A) was also released from the human lung following antigen challenge. Sweatman and Collier (1968) reported that SRS-A constricted human airways and the compound FPL 55712 (Augstein et al., 1973) was shown to inhibit SRS-A-induced contractions in the guinea pig ileum assay. These observations provoked an intense interest in elucidating the biochemical nature of this entity. Initial attempts to characterize this substance revealed that the factor was a low-molecular weight derivative of arachidonic acid (Orange et al., 1973; Bach et al., 1977; Jakschik et al., 1977) containing sulfur (Orange et al., 1973; Parker et al., 1979). SRS-A was

identified subsequently to be a family of lipid mediators known as leukotrienes, a name derived from their cell source (leukocytes) and their conjugated double bonds (triene) structure (Borgeat et al., 1976; Borgeat and Samuelsson, 1979a,b,c; Murphy et al., 1979; Corey et al., 1980; Lewis et al., 1980; Morris et al., 1980; Rokach et al., 1980). Leukotriene B₄ (LTB₄) was the first of the leukotrienes to be isolated (Borgeat et al., 1976).

The elucidation of the structures and synthetic pathways for the leukotrienes lead to a considerable amount of research on these arachidonic acid metabolites (Fig. 1). This work involved comprehensive assessments of the biological profiles of both the cysteinyl-leukotrienes (cys-LTs: LTC₄, LTD₄, and LTE₄) as well as dihydroxy-leukotriene (LTB₄) and, more recently, the lipoxins. Lipoxins (LX), an acronym for eicosanoids, which are often generated during the transcellular metabolism of arachidonic acid via the sequential actions of the 15- and 5- or 5- and 12-lipoxygenase enzymatic pathways (Serhan et al., 1984; Samuelsson et al., 1987). When the synthetic ligands were made available many studies documented a myriad of actions for these lipid mediators (Table 1) providing pertinent evidence for their possible patho-physiological roles in inflammatory diseases, in particular asthma. During the last 20 years significant efforts involving diverse chemical strategies have been directed toward the identification and development of receptor antagonists. These compounds have facilitated the identification and characterization of distinct receptors, which are activated by either the dihydroxy- or cysteinyl-leukotrienes.

II. General Considerations

A. Biochemical Pathways

The leukotrienes are formed via activation of the 5-lipoxygenase enzyme (5-LO) in collaboration with the "5-

¹Abbreviations: SRS, slow reaction smooth muscle-stimulating substance; SRS-A, slow reacting substance of anaphylaxis; LT, leukotriene; LX, lipoxin; LO, lipoxygenase; HETE, hydroxyeicosatetraenoic acid; PMN, polymorphonuclear leukocyte; cys-LT, cysteinyl-leukotriene; IUPHAR, International Union of Pharmacology; GPCR, G-protein-coupled receptor; kbp, kilobase pair(s); ORF, open reading frame; bp, base pair(s); CHO, Chinese hamster ovary; IL, interleukin; UTR, untranslated region; HPETE, hydroperoxyeicosatetraenoic acid; PAF, platelet-activating factor; HEK, human embryonic kidney; FPR, formyl peptide receptor; fMLP, formyl-methionyl-leucyl-phenylalanine; PTX, pertussis toxin; PKC, protein kinase C; HUVEC, human umbilical vein endothelial cell; GST, glutathione S-transferase; ATL, aspirin-triggered lipoxin; HIV, human immunodeficiency virus.

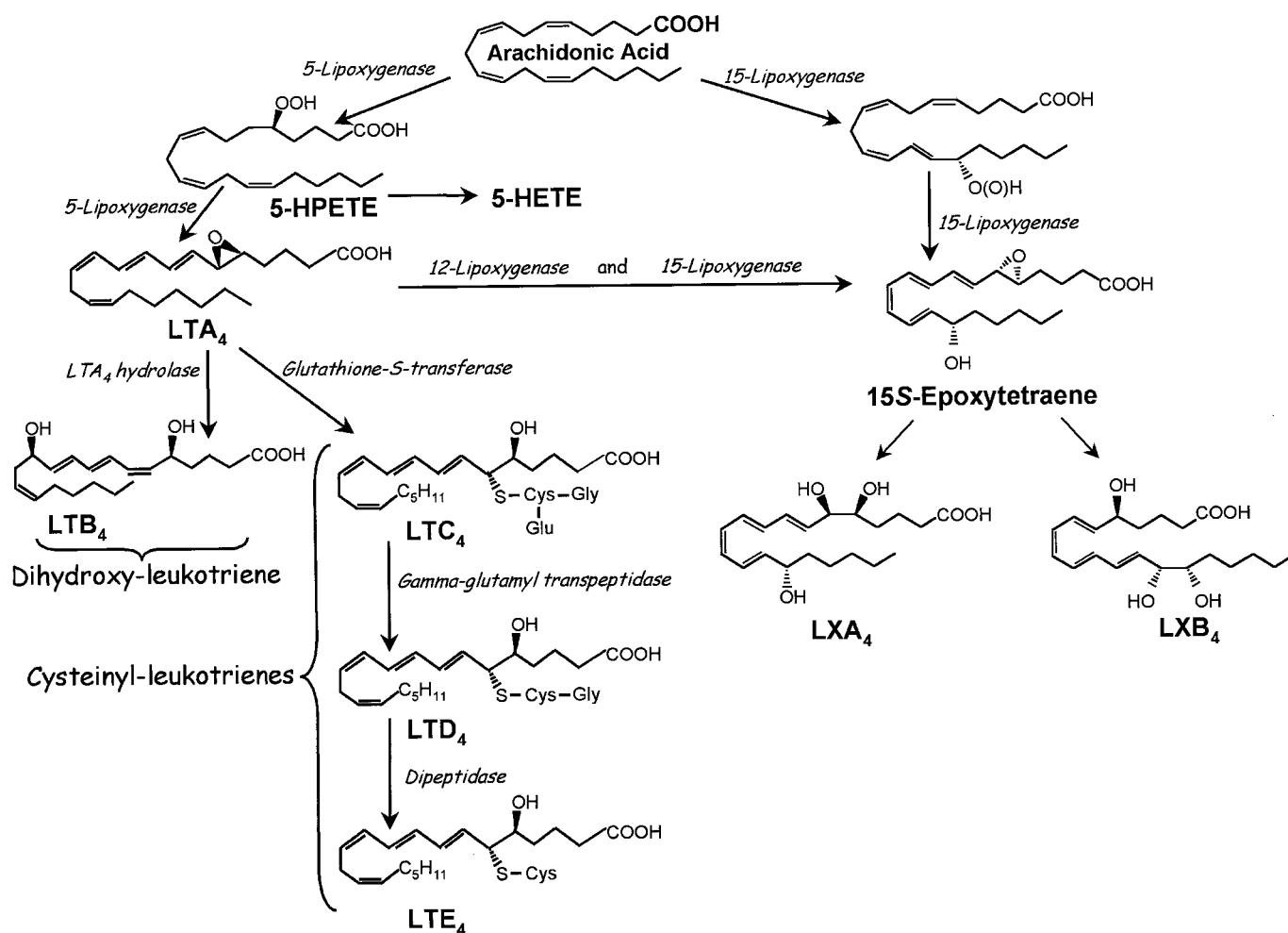


FIG. 1. Major pathways for leukotriene and lipoxin formation. The leukotrienes and lipoxins are lipid mediators derived from arachidonic acid, which is released from cell membrane phospholipids by the action of phospholipase A₂. Leukotriene formation is initiated by 5-lipoxygenase, which catalyzes the dioxygenation of arachidonic acid to 5-HPETE and the subsequent conversion to LTA₄. This latter unstable epoxide is transformed either to LTB₄ or LTC₄, and LTC₄ is further catalyzed to LTD₄ and LTE₄. The tetraene epoxide intermediate can be formed either from LTA₄ or 15-HPETE depending upon the interactions of the different lipoxygenases, and this metabolite is enzymatically hydrolyzed to the lipoxins (LXA₄ and LXB₄).

lipoxygenase activating protein" (FLAP). A prerequisite for this enzymatic reaction is the hydrolyzation of arachidonic acid from membrane phospholipids by phospholipase A₂. The principal 5-LO products of arachidonic acid metabolism are LTC₄ and LTB₄ as well as 5-hydroxyeicosatetraenoic acid (5-HETE). In addition, eicosanoids that are formed by pathways that involve the dual lipoxygenation of arachidonic acid by either 15- and 5-LO or 5- and 12-LO are referred to as lipoxins (Serhan et al., 1984; Samuelsson et al., 1987). The transcellular metabolism of intermediates such as LTA₄ and 15(S)-HETE is associated with LX formation (Serhan, 1994). LX and their carbon 15-epimer-LXs (aspirin-triggered lipoxins; ASA-15-epi-LX) are bioactive and structurally distinct from other eicosanoids in that they carry a conjugated tetraene system and are present in biological matrix in two main forms that are positional isomers, namely, lipoxin A₄ (5*S*,6*R*,15*S*-trihydroxyeicos-7*E*,9*E*,11*Z*,13*E*-tetraenoic acid) and lipoxin B₄ (5*S*,14*R*,15*S*-trihydroxyeicos-6*E*,8*Z*,10*E*,12*E*-tetrae-

noic acid; Serhan, 1997; Fig. 1). The aspirin-triggered form carry their C15 alcohol in the *R* configuration, which is inserted by COX-2 following aspirin treatment (denoted ASA-15-epi-LX). These metabolites are often produced during cell-to-cell interactions, and the principal targets appear to be platelets and leukocytes. During these cellular interactions, platelets convert neutrophil derived LTA₄ to 5,6-epoxytetraene through the action of platelet 12-LO. However, under these conditions the term 12-LO is a misnomer since this enzymatic activity was originally based on an interaction with arachidonic acid. This enzyme functions as a 15-LO (LX synthase) when the substrate is LTA₄. Thus in an inflammatory condition LTA₄ serves as a pivotal intermediate for both leukotriene and lipoxin formation.

B. Cellular Origins

The leukotrienes are formed in different cell types as well as via transcellular metabolism involving multiple cells such as neutrophil and platelets and vascular cells

TABLE 1
Major biological actions of leukotrienes and lipoxins

Actions	References
Dihydroxy-leukotrienes (LTB₄)	
Leukocyte activation	Bray et al., 1980; Ford-Hutchinson et al., 1980; Goetzl and Pickett, 1980; Serhan et al., 1982
Cytokine secretion	Luscinskas et al., 1990; Rola-Pleszczynski et al., 1993
Nuclear transcription (PPAR α)	Devchand et al., 1996
IgE synthesis	Odlander et al., 1988; Yamaoki et al., 1994
Cysteinyl-leukotrienes (LTC₄, LTD₄, LTE₄)	
Bronchospasm	Drazen et al., 1980; Weiss et al., 1982; Barnes et al., 1984; Jones et al., 1985; Davidson et al., 1987; Smith et al., 1993
Plasma exudation	Woodward et al., 1983; Evans et al., 1985; Obata et al., 1992
Vasoconstriction	Smedegard et al., 1982; Filep et al., 1985; Fiedler and Abram, 1987; Garcia et al., 1987; Evans et al., 1989
Vasodilation	Secrest et al., 1985; Sakuma and Levi, 1988; Lawson et al., 1989; Pawloski and Chapwick, 1993b; Ortiz et al., 1995
Eosinophil recruitment	Foster and Chan, 1991; Laitinen et al., 1993; Smith et al., 1993; Spada et al., 1994; Underwood et al., 1996
Cardiodepression	Levi et al., 1980; Burke et al., 1982; Letts and Piper, 1983; Bittl et al., 1985; Roth et al., 1985
Smooth muscle proliferation	Wang et al., 1993; Panettieri et al., 1998
Mucus secretion	Shelhamer et al., 1980; Coles et al., 1983; Labat et al., 1999
Lipoxins (LX) and Aspirin-Triggered Lipoxin Analogs (ATLA)	
Regulation of cellular function	Fiore et al., 1994; Chiang et al., 2000; Kang et al., 2000; Gronert et al., 2001
Inhibition of PMN-mediated inflammation in skin, lung and kidney	Badr et al., 1989; Takano et al., 1998; Clish et al., 1999; Chiang et al., 2000; Godson and Brady, 2000
Protection in reperfusion injury	Chiang et al., 1999
Enhancement of macrophage phagocytosis of leukocytes	Godson and Brady, 2000
Redirection of chemokine, cytokine expression and gene regulation	Gewirtz et al., 1998; Hachicha et al., 1999; Sodin-Semrl et al., 2000; Qui et al., 2001
Enhancement of clearance and accelerate resolution of pulmonary edema	Bandeira-Melo et al., 2000
Anti-angiogenic properties	Fierro and Serhan, 2001
Reduction of COX-2 traffic in pain responses	Serhan et al., 2001
Inhibition of cell proliferation	Clària et al., 1996

(Feinmark and Cannon, 1986; Maclouf and Murphy, 1988; Sala et al., 1993). Human eosinophils and neutrophils synthesize both LTC₄ and LTB₄, respectively (Bray et al., 1980; Ford-Hutchinson et al., 1980). Monocytes and macrophages also synthesize both LTB₄ and the cys-LTs (Samuelsson, 1983). LTC₄ is metabolized to LTD₄ and LTE₄ by the cells in which this mediator is formed. In addition, the cys-LTs can be transformed into 6-*trans*-LTB₄ by hypochlorous acid, which is generated during the respiratory burst in leukocytes (Henderson et al., 1982; Lee et al., 1983). LTB₄ is also metabolized in the cells which produce this metabolite, by a unique membrane bound cytochrome P450 enzyme. LTB₄ is metabolized to 20-hydroxy-LTB₄ (Hansson et al., 1981; Shak and Goldstein, 1985; Soberman et al., 1985). There is also evidence for a reductase dehydrogenase in polymorphonuclear leukocytes (PMN) that appears to be specific for LTB₄ (Powell et al., 1989).

C. Nomenclature for Leukotriene Receptors

The previous IUPHAR publication (Coleman et al., 1995) introduced two main classes of leukotriene receptors. One based on the biological activities of leukotriene B₄ and related hydroxyacids, referred to as BLT receptors, and a second class identified by the cysteinyl-leu-

kotrienes (cys-LTs). The different profiles of biological activity for these two classes of metabolites were the initial basis for these categories and were supported by structure-activity data obtained in studies with a variety of compounds that selectively antagonized the different ligands. Activation of the BLT receptors initially was shown to produce potent chemotactic activities on leukocytes whereas the latter class (CysLT receptors) stimulated smooth muscle as well as other cells. However, the structures of the leukotriene receptors have recently been deduced from the nucleotide sequences of the cDNAs and the encoding proteins are now known for human, mouse, and rat. These data have permitted the IUPHAR committee to establish the nomenclature for the leukotriene receptors, and this is presented in Table 2. The phylogenetic tree for the different eicosanoid and bioactive lipid G-protein-coupled receptors (GPCR) is illustrated in Fig. 2 and shows the molecular families with the relationship between leukotrienes and lipoxins as well as other proteins with seven transmembrane helices.

The lipoxins, are chemically and functionally different from the leukotrienes (Fig. 1). Although LXA₄ and LXB₄ are similar in structure, these mediators display biological activities that are quite distinct. LXA₄ interactions with neutrophils involves binding sites that are not rec-

TABLE 2

Human cloned leukotriene receptors^a

Data are from the following references: Fiore et al., 1992, 1994; Perez et al., 1992; Maddox et al., 1997; Takano et al., 1997; Yokomizo et al., 1997; Lynch et al., 1999; Sarau et al., 1999; Heise et al., 2000; Nothacker et al., 2000; Takasaki et al., 2000; Figueroa et al., 2001.

IUPHAR name	BLT ₁	BLT ₂	CysLT ₁	CysLT ₂	ALX
IUPHAR code	2.1 :BLT :1 :BLT1	2.1 :BLT :2 :BLT2	2.1 :CLT :1 :CLT1	2.1 :CLT :2 :CLT2	2. :ALX
Alternate names	Leukotriene B ₄	Leukotriene B ₄	Leukotriene D ₄ /E ₄ ^b	Leukotriene C ₄ ^b	Lipoxin A ₄ /FPRL-1
Amino acid composition	352 (h)	358 (h)	337 (h)	346 (h)	351
Selective ligand	None	None	None	None	None
Non-selective ligand	LTB ₄	LTB ₄	LTC ₄ /LTD ₄ /LTE ₄	LTC ₄ /LTD ₄ /LTE ₄	LXA ₄ /fMLP
Gene/chromosome	14q11.2-q12	14q11.2-q12	Xq13-q21	13q14.2	19q13.3
Primary coupling ^c	G ₁₆ , G _{i2}	G _q -like, G _i -like, G _z -like	G _{αq}	G _{αq}	G _i , G _q
Primary expression	Leukocytes, thymus, spleen	Ovary, liver, leukocytes, ubiquitous	Smooth muscle (lung, intestine), spleen, peripheral blood leukocytes	Heart, spleen, peripheral blood leukocytes, adrenal medulla, brain	Lung, peripheral blood leukocytes, spleen
Accession number (Swiss-Prot)	Q15722 (h)	Q9NPC1 (h)	Q9Y271 (h)	Q9NS75 (h)	P25090 (h)

^a The reader is referred to the text for the mouse cloned receptors.

^b No longer acceptable since all ligands activate the same receptor in isolated human bronchi.

^c These observations should be considered with some reserve since there is marked variation between the types of G-proteins present in cells (see text). (h) indicates human.

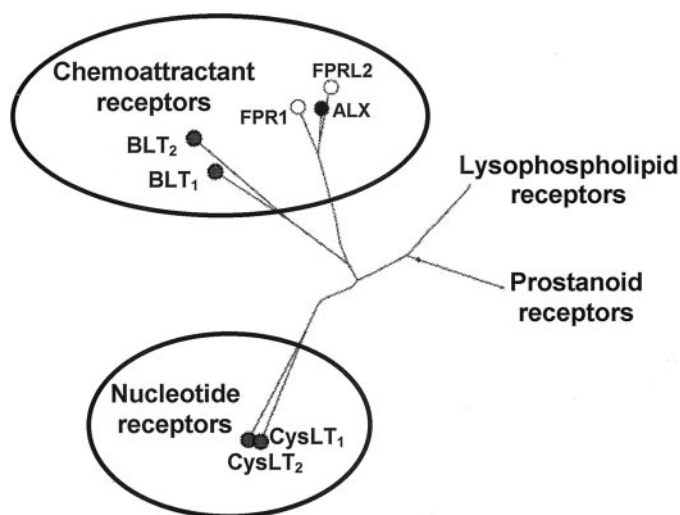


FIG. 2. Dendrogram of several GPCRs. The major receptor families for lipid mediators are indicated in this phylogenetic tree. There are four known cell surface GPCRs for the leukotrienes, which are classified as either chemoattractants (BLT₁ and BLT₂) or nucleotide receptors (CysLT₁ and CysLT₂). The lipoxin receptor (ALX) is also included in the chemoattractant receptor class along with formyl peptide receptors (FPL). This evolutionary tree was constructed using the sequences from the receptors. Construction was performed by using the "All All Program" at the Computational Biochemistry Server at ETHZ (<http://cbg.inf.ethz.ch/ServerBooklet/chapter2-3.html>).

ognized by LXB₄ (Nigam et al., 1990; Fiore et al., 1992). LXB₄ is a potent agonist for stimulating proliferation and differentiation of granulocyte-monocyte colonies from human mononuclear cells (Popov et al., 1989), increasing the S-phase in the cell cycle and enhancing nuclear protein kinase C activity (Beckman et al., 1992) actions, which have not been reported for LXA₄. However, LXB₄ has also been shown to share actions with LXA₄, such as, both selectively stimulate human peripheral blood monocytes (Maddox and Serhan, 1996) and

enhance growth of myeloid progenitor cells (Stenke et al., 1991). Furthermore, LXA₄ does not activate BLT (Fiore et al., 1992) but activates FPRL-1 receptors (Chiang et al., 2000; Resnati et al., 2002; Perretti et al., 2002). These investigators have shown that ALX and FPRL-1 are the same receptor and that LXA₄ is the natural and most potent ligand. In addition, Takano et al. (1997) have identified the amino acid sequence for the receptor associated with the LXA₄ responses. In line with the IUPHAR nomenclature directives, this committee recommends that ALX be used to designate the receptor that has been cloned and is activated by the native ligand LXA₄ (Table 2). LXB₄-induced responses, although different from those of LXA₄, have not to date provided sufficient evidence to specify another receptor. Since this receptor has not been cloned, the LXB₄ response is associated with activation of a putative receptor.

The aim of this review is to present the evidence that led to the leukotriene nomenclature. To this end, information not only from the molecular database but also derived from the properties and significance of leukotriene receptors will be presented. Furthermore, the above nomenclature for the LX receptors is recommended as the framework for this evolving area of receptor research.

III. Molecular Database for Leukotriene Receptors

A. Molecular and Structural Aspects of Dihydro-Leukotriene Receptors

1. *BLT₁*. The cloning and characterization of the BLT₁ receptor was achieved by cDNA subtraction using human leukemic cells HL-60, which were differentiated

into granulocyte-like cells (Yokomizo et al., 1997). The BLT₁ receptor was identified as a putative seven transmembrane domain receptor with 352 amino acids. This receptor had been initially misidentified as a purinergic receptor, P2Y7 (Akbar et al., 1996). BLT₁ shares low homology to P2Y receptors and belongs to a family of receptors for chemoattractants including complement receptors and a recently identified novel prostaglandin D₂ receptor, CRTH2 (Hirai et al., 2001). The homology between the BLT₁ receptor for mouse and humans is presented in Fig. 3.

Kato et al. (2000) reported that the BLT₁ gene consists of three exons located in a region of 5 kbp on chromosome 14. Whereas the open reading frame (ORF) for BLT₁ is in the last exon (exon 3), the basal promoter activity is found 100-bp upstream from the transcriptional initiation site. This report and the data of Yokomizo et al. (2000) demonstrated that there was no TATA or CAT element near the transcription site. However, they did observe the presence of an Sp-1 site at -50 bp, which played a major role in the basal transcription of BLT₁. Since the promoter region of BLT₁ is rich in GC sequences and methylated in nonleukocyte cells but nonmethylated in leukocyte cells expressing BLT₁, Kato et al. (2000) have suggested that the methylation state may, in part, be responsible for transcription of the BLT₁ gene.

COS-7 membranes transfected with human BLT₁ exhibited [³H]LTB₄ binding with a *K_d* of 0.15 nM, comparable to the *K_d* of 0.14 nM for the differentiated HL-60 cells (Fiore et al., 1993; Yokomizo et al., 1997; Table 3).

Various eicosanoids also competed for the [³H]LTB₄ binding to COS-7 membranes expressing human BLT₁ and the potency ranking (*K_i* values) were: LTB₄ (0.38 nM) > 20-hydroxy-LTB₄ (7.6 nM) = 12-oxo-LTB₄ (7.6 nM) > 12R-HETE (30 nM) > 20-COOH-LTB₄ (190 nM). This profile agrees with the previously characterized LTB₄ binding sites in human granulocytes. The structures and potencies of several antagonist for these receptors in Chinese hamster ovary (CHO) cells expressing human BLT (CHO-BLT) are presented in Table 4.

BLT₁ mRNA is predominantly expressed in leukocytes, granulocytes, macrophages, and eosinophils with high amounts of BLT₁ in human and mouse cells (Yokomizo et al., 1997; Huang et al., 1998). In addition, BLT₁ expression is inducible in activated macrophages (Toda et al., 1999) and eosinophils treated with IL-5 (Huang et al., 1998), suggesting an association of BLT₁ with various inflammatory diseases. BLT₁-transgenic mice showed enhanced PMN responses in the lung following ischemia-reperfusion and increased expression of 5-lipoxygenase in peritonitis (Chiang et al., 1999). Recently, two lines of BLT₁-deficient mice have been reported, and a reduction in leukocytes migration was demonstrated (Haribabu et al., 2000; Tager et al., 2000).

2. BLT₂. During the analysis of transcriptional regulation of human BLT₁ gene (Kato et al., 2000), a putative ORF for a novel GPCR with structural similarity to BLT₁ was identified (Yokomizo et al., 2000). This novel receptor was also found in a human genome sequence database, reported to act as a low-affinity receptor acti-

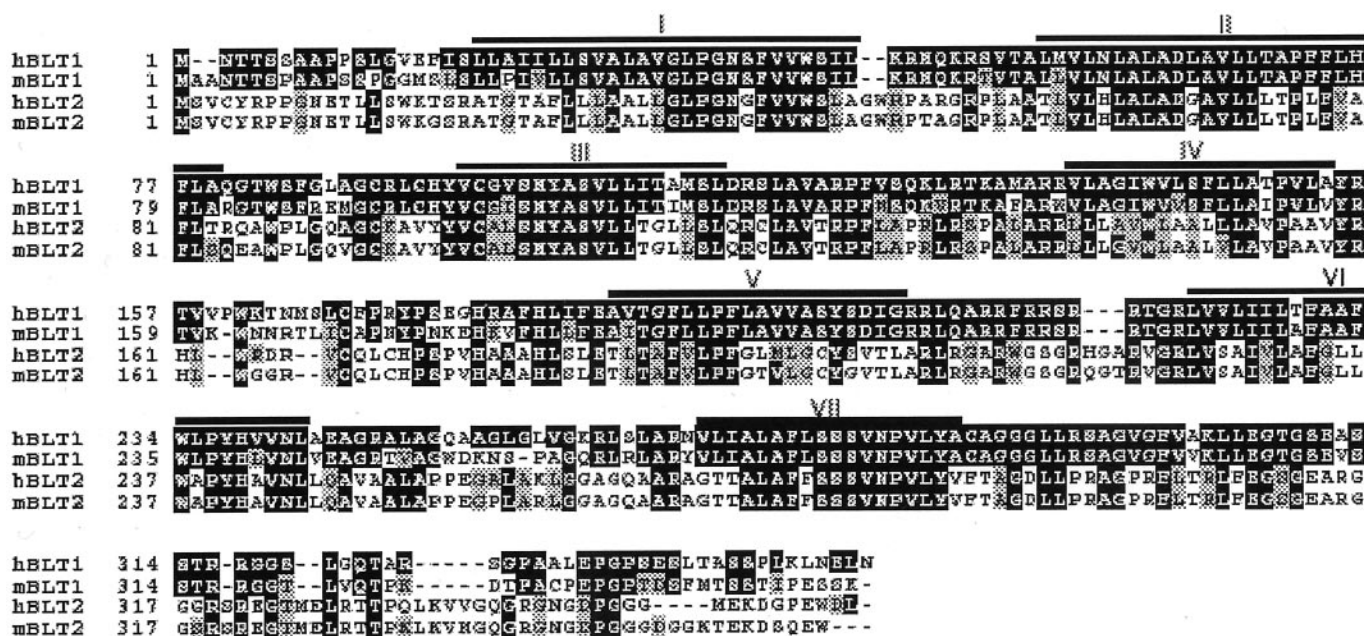


FIG. 3. The sequence alignment of BLT₁ and BLT₂ from human and mouse receptors. The amino acid sequences were aligned using ClustalW and converted using Boxshade 3.21. The putative transmembrane domains of hBLT₁ predicted by Kyte-Doolittle hydrophobicity analysis are overlined and labeled as I-VII. Consensus matches are boxed and shaded with darker shading for identities and light shading for conservative substitutions. The amino acid identity between human and mouse BLT₁ was 78.6% whereas BLT₂ was 92.7%. The mouse sequence data are available from Swiss-Prot under accession numbers (mBLT₁: no entry presently available) and (mBLT₂: Q9JJL9).

TABLE 3

Recombinant BLT receptors in transfected cells and [3 H]LTB $_4$ binding[3 H]LTB $_4$ binding in transfected cells. K_d and B_{max} indicate affinity and receptor density, respectively. Values are mean data from the references indicated.

Receptor	Cells	K_d	B_{max}	References
		<i>nM</i>	<i>fmol/mg protein</i>	
Human BLT $_1$	COS-7	0.154	430	Yokomizo et al., 1997
Human BLT $_1$	HEK293	1.1	3.8 pmol/mg protein	Yokomizo et al., 2001c
Human BLT $_1$	COS-7	2.1	17 pmol/mg protein	Owman et al., 1997
Mouse BLT $_1$	CHO	0.64	NR	Huang et al., 1998
Guinea pig BLT $_1$	HEK293	0.27	425	Masuda et al., 1999
	COS-7	17	556	
Guinea pig BLT $_1$	EBNA293	0.4	9–12 pmol/mg protein	Boie et al., 1999
Rat BLT $_1$	HEK293	0.68	1245	Toda et al., 1999
Human BLT $_2$	HEK293	22.7	925	Yokomizo et al., 2000
Human BLT $_2$	COS-7	0.17	70	Wang et al., 2000

NR, not reported.

vated by LTB $_4$ (Kamohara et al., 2000; Tryselius et al., 2000; Wang et al., 2000) and subsequently referred to as the BLT $_2$ receptor (Yokomizo et al., 2001b). The gene structure for BLT $_2$ has also been established (Yokomizo et al., 2001b). Of considerable interest is that the promoter region (Fig. 4) of human BLT $_1$ overlaps BLT $_2$ ORF (Kato et al., 2000). This represents the “promoter in ORF”, as has been reported in prokaryotes but the biological significance of this rare gene structure is presently not clear. However, there is sufficient evidence that BLT $_1$ and BLT $_2$ form a gene cluster both in human (chromosome 14 q11.2-q12;) and mouse (Yokomizo et al., 2000) chromosomes suggesting that these receptors may be generated by gene duplication.

The K_d values for LTB $_4$ derived from transfection assays were 23 nM in human embryonic kidney (HEK)-293 cells (Yokomizo et al., 2000), and a K_d value of 0.17 nM in COS-7 cells (Wang et al., 2000). Although there are differences in the reported K_d values (Table 3 and Table 4), generally BLT $_2$ is considered as a low-affinity receptor since the pEC $_{50}$ value of LTB $_4$ required for adenylyl cyclase inhibition and the concentrations required for LTB $_4$ -dependent chemotaxis were higher than those for BLT $_1$ receptor activation (Kamohara et al., 2000; Yokomizo et al., 2000). The tissue distribution of BLT $_2$ assessed by different groups using Northern blots are varied. Using the ORF probe of BLT $_2$ (Kamohara et al., 2000; Yokomizo et al., 2000) the highest expression was found in spleen, followed by leukocytes and ovary. Other groups reported that BLT $_2$ is expressed highest in liver and intestine (Tryselius et al., 2000; Wang et al., 2000) using the 5'-UTR probes. The abundance of low-affinity binding sites for LTB $_4$ in spleen membrane supports the data showing BLT $_2$ expression in spleen (Showell et al., 1998). Of interest, the rank order of potency of several eicosanoids for BLT $_2$ activation was LTB $_4$ > 12-epi-LTB $_4$ > 12S-HETE > 12S-HPETE > 12R-HETE > 20-hydroxy-LTB $_4$, which is quite different from BLT $_1$ (Yokomizo et al., 2001c). Currently, there are no data on specific in vivo functions for BLT $_2$ receptors, however, lymphocytes have been suggested to be the

TABLE 4
Several BLT antagonists and structures

CP-195543 IC$_{50}$ BLT $_1$ (570 nM) BLT $_2$ (1.5 μ M)	
CP-105696 IC$_{50}$ BLT $_1$ (58 nM) BLT $_2$ (>10 μ M)	
LY-223982 IC$_{50}$ BLT $_1$ (>10 μ M) BLT $_2$ (>10 μ M)	
LY-255283 IC$_{50}$ BLT $_1$ (>10 μ M) BLT $_2$ (917 nM)	
ONO-4057 (No Data)	
U-75302 IC$_{50}$ BLT $_1$ (1 μ M) BLT $_2$ (>10 μ M)	
ZK-158252 IC$_{50}$ BLT $_1$ (260 nM) BLT $_2$ (1.5 μ M)	

[3 H]LTB $_4$ (5 nM) radioligand binding to membrane fractions of CHO cells expressing BLT receptors. IC $_{50}$ values are indicated (see references: Yokomizo et al., 1997, 2001c).

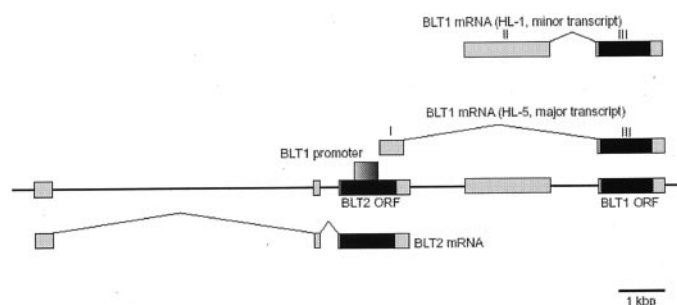


FIG. 4. Structure of the human genomic DNA containing BLT₁ and BLT₂ located on human chromosome 14q11.2-q12. Chromosome 14 is indicated by the line; the transcribed segments are indicated by open boxes; putative ORFs are shown as filled boxes. Note that the promoter region for BLT₁ is located in the ORF of BLT₂. This is the first mammalian example of "promotor in ORF" (Yokomizo et al., 2001b).

target, since BLT₂ is highly expressed in splenic T-cells (Yokomizo et al., 2001a).

3. Phenotypes Involving BLT Receptors. Investigations with transgenic mice expressing the human BLT₁ receptor on leukocytes (Chiang et al., 1999) as well as targeted gene disruption of the BLT₁ receptor in knock-out mice (BLT^{-/-}) indicate that an apparent phenotypic difference (Haribabu et al., 2000; Tager et al., 2000) from wild type littermates is not observed unless the animals are subject to experimental disease or injury, which are known to stress the effector immune system (vide infra).

In BLT₁^{-/-} mice (Haribabu et al., 2000; Tager et al., 2000) leukocytes exhibited a normal response to C5a and platelet-activating factor (PAF), however, there was a selective loss of responsiveness to exogenous LTB₄. Tager et al. (2000) also reported that adhesion to the endothelium in response to LTB₄ was diminished in leukocytes from BLT₁^{-/-} animals. These findings are in line with earlier observations with LTB₄ in the hamster cheek pouch (Raud et al., 1991) and suggest that the BLT receptor is pivotal for the LTB₄-induced leukocyte activation.

Unfortunately, there are only a limited number of investigations examining the relative input of LTB₄ in chemotaxis during inflammation in vivo in genetically modified animals. Two groups (Haribabu et al., 2000; Tager et al., 2000) have provided evidence that peritoneal inflammation is suppressed in these deficient mice. Interestingly, Haribabu et al. (2000) described a loss of this protection at 72 h between ^{-/-} and ^{+/+} animals, whereas Tager and colleagues (2000) observed an increase in protection between ^{-/-} and ^{+/+} with time intervals greater than 50 h. These latter investigators also reported a marked diminution in the number of eosinophils, which accounted for virtually all of the changes in cellular influx. In contrast, Haribabu et al. (2000) noted reductions in both PMNs and macrophage populations. The basis for these differences is presently not known, but may be related to the different stimuli (zymosan versus thioglycollate) used to induce the inflammatory cellular infiltration in vivo.

Expression of the human BLT₁ receptor in leukocytes has also been carried out using the CD11b promoter (Chiang et al., 1999). BLT receptor-transgenic mice displayed enhanced leukocyte responsiveness in acute dermal inflammation, with leukocyte trafficking to remote organs (as in secondary organ reperfusion injury), or in leukocyte recruitment following a peritoneal challenge. These results are consistent with the interpretation that LTB₄ is associated with an excessive activation of leukocytes and the 5-LO pathway provoking leukocyte-mediated injury. Expression of the human BLT₁ receptor in vivo lead to an up-regulation of 5-LO expression and leukotriene biosynthesis (Chiang et al., 1999). These results suggest that receptor expression may amplify proinflammatory circuits in vivo.

B. Molecular and Structural Aspects of Cysteinyl-Leukotriene Receptors

1. CysLT₁. The cloning and characterization of the human CysLT₁ receptor (hCysLT₁) was achieved by two groups under the general program of identifying cognate ligands for orphan GPCRs, a process which has been termed "ligand fishing" (Lynch et al., 1999; Sarau et al., 1999). The hCysLT₁ receptor was identified as a 337-amino acid putative seven transmembrane domain receptor, termed either HG55 (Lynch et al., 1999) or HMTMF81 (Sarau et al., 1999) (Fig. 5). The former investigators demonstrated that LTD₄ produced activation of a calcium-activated chloride channel in *Xenopus laevis* oocytes expressing the cRNA for HG55 but not in control cells or oocytes expressing other GPCRs. This LTD₄-induced stimulation of oocytes was blocked by the selective CysLT₁ receptor antagonist MK-571 (Lynch et al., 1999) (Table 5). Similar results were obtained using the *X. laevis* melanophore signaling assay and in mammalian monkey kidney COS-7 cells expressing the HG55 (hCysLT₁) receptor (Lynch et al., 1999).

Researchers at SmithKline Beecham identified a GPCR (HMTMF81) that, when expressed in HEK-293 cells, responded selectively with calcium mobilization to LTC₄, LTD₄, or LTE₄ (0.1–10 μM) but not to more than 900 other ligands, including greater than 200 ligands for GPCRs.

The genomic organization of the hCysLT₁ receptor has not been reported but consists of at least three exons with all of the open reading frame of the receptor in one exon (M. Metzker, Merck unpublished results). Three single nucleotide polymorphisms have been identified in the hCysLT₁ receptor but none relate to the asthmatic phenotype (J. Drazen, unpublished data). The hCysLT₁ receptor is located to chromosome Xq13-q21 (Lynch et al., 1999) and has 31% amino acid identity to the P2Y purinoceptor (Sarau et al., 1999).

Full Scatchard analyses of [³H]LTD₄ binding to the HG55 (hCysLT₁) COS-7 membranes demonstrated a K_d of 0.3 nM and B_{max} of 50 fmol/mg of protein (Lynch et al., 1999). In the structure-affinity relationships, the IC₅₀

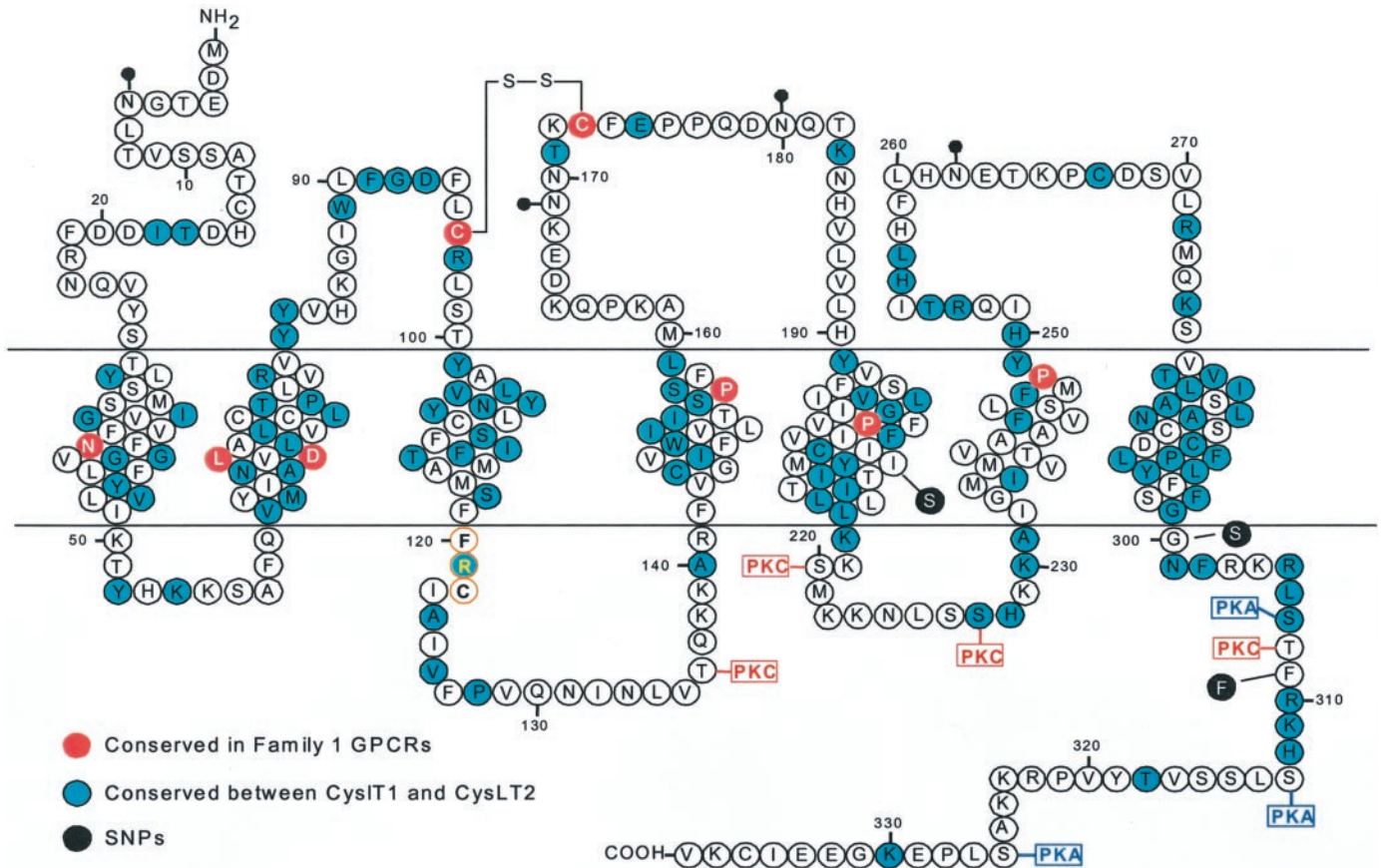


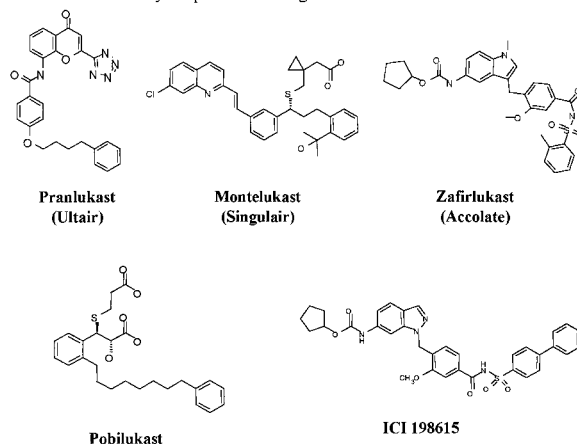
FIG. 5. Comparison of amino acid sequences of the human CysLT₁ and CysLT₂ receptors. A G-protein-coupled receptor snake diagram depiction of the amino acid sequences of the human CysLT₁ and CysLT₂ receptors. The amino acid identities between the hCysLT₁ and hCysLT₂ receptors is 37.3%.

for the agonists were LTD₄ (0.9 nM), LTC₄ (350 nM), and LTE₄ (200 nM). The affinities of antagonists (Table 5) were similar to that demonstrated functionally in other systems for the CysLT₁ receptor as well as in binding experiments on human lung or U937 cells (Frey et al., 1993; Lynch et al., 1999). Likewise the HMTMF81 (hCysLT₁) receptor expressed in HEK-293 cells responded selectively to the cys-LTs with rank order of potency (pEC₅₀ value) were LTD₄ (2.5 nM) > LTC₄ (24 nM) > LTE₄ (240 nM) (Sarau et al., 1999). In these functional studies LTE₄ was shown to be a partial agonist of the HMTMF18 (hCysLT₁) receptor expressed in HEK-293 cells (Sarau et al., 1999). This is similar to evidence derived from the human bronchus (Labat et al., 1992), sheep trachea (Mong et al., 1988), and U937 cells (Saussy et al., 1989). [³H]LTD₄ binding and LTD₄-induced calcium mobilization in HEK-293 cells expressing the HMTMF18 (hCysLT₁) receptor were potently inhibited by the structurally distinct CysLT receptor antagonists and the rank order of potency in this system was pranlukast = zafirlukast > montelukast > pobilukast (Sarau et al., 1999). LTD₄-induced calcium mobilization in HEK-293 cells expressing the HMTMF18 (hCysLT₁) receptor was not affected by pertussis toxin and appeared to result from the release of intracellular calcium

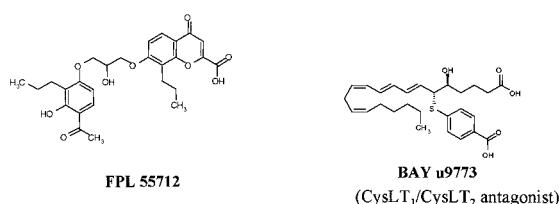
stores (Sarau et al., 1999). In *X. laevis* melanophore signaling system, the HG55 (hCysLT₁) receptor was shown not to couple via Gα_i (Lynch et al., 1999).

Northern analyses of human tissues showed that the hCysLT₁ mRNA was detected as an approximately 2.8-kb species and was expressed in spleen, peripheral blood leukocytes, less strongly in lung, bronchus small intestine, colon, skeletal muscle, pancreas, placenta, and weakly in the prostate, heart, brain, liver, kidney, and adipose tissues (Lynch et al., 1999; Sarau et al., 1999). Further work is necessary to confirm whether expression in some of these tissues may be attributable to expression of the receptor on interstitial myeloid cells. The receptor was also detected in U937 cells and HL-60 cells, with an increase observed in dimethyl sulfoxide-differentiated HL-60 cells (Sarau et al., 1999). In HL-60 cells differentiated with sodium butyrate to eosinophilic-like cells, interleukin-5 treatment resulted in a 5-fold increase in the expression of the hCysLT₁ (Thivierge et al., 2000). Northern analysis in normal and asthmatic lungs suggested no total differences in hCysLT₁ mRNA expression, although in situ studies in asthmatic tissues have not been reported (Sarau et al., 1999). Using in situ hybridization, the hCysLT₁ mRNA was shown in lung smooth muscle cells and interstitial macrophages, with

TABLE 5
CysLT₁-selective and nonselective antagonists and structures

CysLT₁ selective antagonists and structures

Non-selective antagonists and structures



BAYu9773		Glossary of chemical names and codes of several cysteinyl-leukotriene antagonists:
BAYx7195		6-(4'-Carboxyphenylthio)-5(<i>S</i>)-hydroxy-7(<i>E</i>), 9(<i>E</i>), 11(<i>Z</i>), 14(<i>Z</i>)-eicosatetraenoic acid
CGS23131		(4 <i>S</i>)-(4-Carboxyphenylthio)-7-[4-(4-phenoxybutoxy)-phenyl]-hept-5-(<i>Z</i>)-enoic acid
CGS25019C		5-(3-Carboxybenzoyl)-2-((6-(4-methoxyphenyl)-5-hexenyl)oxy)benzenepropanoic acid
CP195543		4-(5-[4-{Aminoiminomethyl} phenoxy]-pentoxy)-3-methoxy- <i>N,N</i> -bis(1-methylethyl)-benzamide-(<i>Z</i>)-2-butenedioate
FPL55712		(+)-2-(3-Benzyl-4-hydroxychroman-7-yl)-4-trifluoromethylbenzoic acid
ICI198615		Sodium 7-(3-(4-acetyl-3-hydroxy-2-propyl-phenoxy)-2-hydroxypropoxy)-4-oxo-8-propyl-4 <i>H</i> -1-benzopyran-2-carboxylate
ICI204219	Zafirlukast (Accolate TM)	(1-[2-Methoxy-4-((phenylsulfonylamino)carbonyl)phenyl] methyl]-1 <i>H</i> -indazol-6-yl) carbamic acid cyclopentyl ester
LY170680	Sulukast	4-(5-Cyclopentyloxy-carbonyl-amino-1-methyl-indol-3-ylmethyl)-3-methoxy- <i>N-O</i> -tolylsulfonylbzamide
LY171883	Tomelukast	5-(3-[2(<i>R</i>)-Carboxyethylthio]-1(<i>S</i>)-hydroxypentadeca-3(<i>E</i>),5(<i>Z</i>)-diethyl]phenyl)-1 <i>H</i> -tetrazole
LY223982		1-[2-Hydroxy-3-propyl-4-[4-(1 <i>H</i> -tetrazol-5-yl)butoxy]phenyl]ethanone
LY293111		(<i>E</i>)-5-(3-Carboxybenzoyl)-2-([6-(4-methoxyphenyl)-5-hexenyl]oxy)benzenepropanoic acid
MK476	Montelukast (Singulair TM)	(2-[2-Propyl-3-{2-ethyl-4-(4-fluorophenyl)-5-hydroxyphenyl}propoxy]phenoxy)benzoic acid
MK571		(1-[[<i>(R)</i>]-(-[2-[7-Chloro-2-quinolinyl]-(<i>E</i>)-ethenyl]phenyl)(3-2-[1-hydroxy-1-methylethyl]phenyl)propyl]thio)methyl)cyclopropane acetic acid sodium
SB209247		[3-[2-(7-Chloro-2-quinolinyl)ethenyl] phenyl][3-(dimethylamino-3-oxopropyl)thio)methyl]thio propanoic acid
ONO1078	Pranlukast (Onon TM)	(<i>E</i>)-3-(6-[[2,6-Dichlorophenyl]-thio)methyl]-3-[2-phenylethoxy]-2-pyridinyl)-2-propenoic acid
SB205312		4-Oxo-8-[<i>p</i> -(4-phenylbutoxy)benzoylamino]-2-(tetrazol-5-yl)-4 <i>H</i> -1-benzopyran hemihydrate
SC53228		4-Oxo-8-[<i>p</i> -(4-phenylbutoxy)benzoylamino]-2-(tetrazol-5-yl)-4 <i>H</i> -1-benzopyran hemihydrate
SKF104353	Pobilukast	((+)-(<i>S</i>)-7-[3-[2-Cyclopropylmethyl]-3-methoxy-4-[(methylamino)carbonyl]phenoxy]propoxy)-3,4-dihydro-8-propyl-2 <i>H</i> -1-benzopyran-2-propanoic acid
SR2640		2 <i>S</i> -Hydroxy-3 <i>R</i> -(2-carboxyethylthio)-3-(2-[8-phenyloctyl]phenyl)-propanoate
WY48252		2-(3-[2-Quinolylmethoxy]phenylamino)benzoic acid
		<i>N</i> -(3-[2-Quinolylmethoxy]phenyl)-trifluoromethanesulphonamide

little or no expression in epithelial cells (Lynch et al., 1999). These in situ findings were confirmed with immunohistochemical localization of the receptor protein using a specific hCysLT₁ antiserum (Figueroa et al., 2001). In addition, the hCysLT₁ mRNA and protein have been detected recently in normal peripheral blood eosinophils, subsets of monocytes and macrophages and in pregranulocytic CD34+ cells (Figueroa et al., 2001). The hCysLT₁ protein was not observed on CD4+ or CD8+ peripheral T cell populations (Figueroa et al., 2001). In situ and immunohistochemical studies of the hCysLT₁ in diseased lung and other tissues are needed to determine whether the expression of the hCysLT₁ changes in asthma, allergy, or other pulmonary and inflammatory diseases.

Two isoforms of the mouse CysLT₁ receptor (mCysLT₁) have been cloned from a mouse lung cDNA library (Maekawa et al., 2001; Martin et al., 2001; Mollerup et al., 2001). A short isoform cDNA containing two exons encodes a polypeptide of 339 amino acids with 87.3% amino acid identity to the hCysLT₁ (Lynch et al., 1999; Sarau et al., 1999; Maekawa et al., 2001). A long isoform has two additional exons and an in-frame upstream start codon resulting in a 13-amino acid extension at the N terminus of the receptor (Maekawa et al., 2001). Northern blot analysis of the mCysLT₁ detecting both isoforms of the receptor showed expression of a 3.5-kb transcript in the lung and skin, whereas reverse transcriptase polymerase chain reaction showed wide expression of the long isoform, with strongest expression in the lung and skin. Unlike the hCysLT₁, neither form of the mouse receptor is expressed in the spleen. In addition, the long rather than the short isoform of the mCysLT₁ receptor is the closer counterpart to the human receptor, and that is expressed more abundantly in the mouse tissues. The mCysLT₁ receptor maps to the X chromosome at band XD. LTD₄-induced intracellular calcium mobilization in CHO cells stably expressing either isoform of mCysLT₁ was blocked by the CysLT₁ receptor antagonist MK-571 (Maekawa et al., 2001). The rank order agonist functional potency for calcium mobilization or the potency for competition of LTD₄ binding to the recombinant mouse receptor was similar to that described above for the human receptor, except that LTC₄ was relatively less potent for the mouse than for hCysLT₁ (Lynch et al., 1999; Sarau et al., 1999; Maekawa et al., 2001).

2. CysLT₂. The cloning and characterization of the CysLT₂ receptor (hCysLT₂) was initially reported by Heise et al. (2000) (Fig. 5). This publication confirmed the previous pharmacological characterization of a human CysLT₂ receptor in different tissues, based upon the relative potencies of the cys-LT agonists and the lack of sensitivity of the responses to classical CysLT₁ receptor antagonists, and the antagonist activity of the partial agonist BAY u9773 (Labat et al., 1992; Tudhope et al., 1994; Heise et al., 2000). Subsequent to this initial

publication, the Takeda group published an article confirming the identification of the hCysLT₂ (Takasaki et al., 2000), and then a third report by the Nothacker et al. (2000), on the characteristics of the hCysLT₂ was published, which revealed similar distribution and functional data to the previous publications but with more details on the partial agonist activity of BAY u9773 (Nothacker et al., 2000). Recently, the cloned mCysLT₂ has also been reported (Hui et al., 2001).

The initial CysLT₂ receptor identification was made through homology to a rat CysLT receptor homolog, namely, RSPBT32, followed by cloning of a human homolog of RSPBT32 (Heise et al., 2000). Using FASTA, the hCysLT₂ was shown to have 37.5% amino acid identity with the hCysLT₁ over a 315-amino acid overlap and the extreme carboxyl termini of these proteins have little homology (Heise et al., 2000). The hCysLT₂ receptor was also identified from the Helix Research Institute database (Takasaki et al., 2000). The human chromosomal localization of the hCysLT₂ in 13q14, a region that has been identified as a polygenic atopic linkage (Kimura et al., 1999).

The cRNA for the hCysLT₂ was expressed in *X. laevis* oocytes and either LTD₄ or LTC₄ produced a calcium-dependent chloride flux in these cells that was not blocked by the selective CysLT₁ receptor antagonist, MK-571, but was inhibited by the dual CysLT₁/CysLT₂ receptor antagonist BAY u9773 (Heise et al., 2000). In addition, LTD₄ and LTC₄ elicited a concentration-dependent activation of calcium flux in HEK-293 cells coexpressing the aequorin bioluminescent protein and the recombinant hCysLT₂ (Heise et al., 2000). This activation was blocked by BAY u9773 but not significantly by the CysLT₁ antagonists MK-571, montelukast, zafirlukast, or pranlukast (Heise et al., 2000). Experiments involving radiolabeled LTD₄ binding to COS-7 cell membranes expressing the hCysLT₂ demonstrated high-affinity competition by LTD₄ and LTC₄, with weaker competition by LTE₄ and no effect by LTB₄ (Heise et al., 2000). Similar studies using radiolabeled LTD₄ binding to COS-7 cell membranes expressing the hCysLT₂, showed that the dual antagonist BAY u9773 fully competed for the binding, whereas CysLT₁ antagonists showed no or minimal competition (Heise et al., 2000). Furthermore, Kamohara et al. (2001) have shown by in situ hybridization the presence of hCysLT₂ mRNA in human atrium, ventricle, and at intermediate coronary arteries with little detection of hCysLT₁. These investigators also reported that human coronary smooth muscle cells stimulated with LTC₄ caused an increase in calcium mobilization. Thus the existence of a CysLT₂ functional receptor in the human heart has been documented.

Northern blot analyses of hCysLT₂ expression showed high expression in the human heart, adrenals, peripheral blood leukocytes, placenta, spleen, and lymph nodes with weaker expression in the brain (Heise et al., 2000;

Nothacker et al., 2000; Takasaki et al., 2000). A reverse transcription polymerase chain reaction comparison of the CysLT receptors showed negligible hCysLT₁ expression but high expression of hCysLT₂, in the heart and eosinophils (Mita et al., 2001), whereas only hCysLT₁ was expressed in tracheal tissue (Takasaki et al., 2000). Both receptors were highly expressed in spleen (Heise et al., 2000; Nothacker et al., 2000; Takasaki et al., 2000). In situ hybridization analyses in the human lung demonstrated hCysLT₂ mRNA strongly expressed on interstitial macrophages and weak expression on smooth muscle cells (Heise et al., 2000). In human peripheral blood monocytes, about 30% of cells expressed the hCysLT₂, whereas greater than 90% of purified human eosinophils expressed the receptor (Heise et al., 2000). In the adrenal gland, in situ hybridization for the hCysLT₂ demonstrated localization to the chromaffin-containing adrenal medulla cells (Heise et al., 2000). In the heart, the hCysLT₂ was expressed in Purkinje fiber conducting cells (Heise et al., 2000).

The recent molecular cloning, expression and characterization of the hCysLT₁ and hCysLT₂ receptors (Table 2) represents a significant milestone in the history of CysLT research and is predicted to lead to a renaissance in interest in this area of biology and therapeutics. The findings have confirmed much of the earlier pharmacological characterization of the two receptors, which were based solely on the functional profiling of the effects of agonists and antagonists in different cellular and tissue systems. However, there are some unexpected findings with regard to genomic structure and tissue expression, as well as the relatively low homology (38%) between the two receptors. The cloning of the receptors allows the generation of tools to investigate in more detail the regulation of CysLT₁ and CysLT₂ receptor expression, the roles of these CysLT receptors in normal and diseased states, and their potential as new therapeutic targets. The ultimate goals will be the identification of potent and selective CysLT₂ receptor antagonists and combined CysLT₁/CysLT₂ receptor antagonists and their clinical evaluation in the diverse diseases in which the cys-LTs have been implicated as significant pathophysiological mediators.

C. Lipoxin Receptors

Of the nonprostanoid eicosanoid GPCRs, the LXA₄ receptor (ALX) was the first recognized at the molecular level (Fiore et al., 1993, 1994). In addition, ALX was initially identified as the only inhibitory or anti-inflammatory receptor that acts via an agonist role as a "stop signal" (Fiore et al., 1994; Serhan, 1994, 1997; Takano et al., 1997). This action appears to be a unique flexibility of GPCR that functions within the immune system. Since LXA₄ shares some structural features with LTC₄ and LTD₄ as well as prostaglandins, LXA₄ competed for CysLT₁ receptors identified on isolated human vascular endothelial cells (Gronert et al., 2001) and mesangial

cells (McMahon et al., 2000) and antagonized either LTC₄- or LTD₄-induced bronchoconstriction in humans (Christie et al., 1992) and animals (Badr et al., 1989; Gronert et al., 2001). In addition, lipoxin B₄ has also been reported to activate another receptor. The present nomenclature for the lipoxin receptors is therefore based on the cloned receptor sequence as well as the observation that LXA₄ is the natural and most potent ligand. In contrast, the putative receptor activated by LXB₄ has not been cloned. ALX activation has been reported to generate intracellular stop signals (Serhan et al., 1994; Levy et al., 1997, 1999) and thereby promote resolution of inflammation.

1. Molecular and Structural Aspects of Lipoxin Receptors. Based on the finding that functional ALX are inducible in promyelocytic lineages (HL-60 cells) (Fiore et al., 1993), several putative receptor cDNAs cloned earlier from myeloid lineages and designated orphans were screened for their ability to bind and signal in response to LXA₄ (Fiore et al., 1994). When transfected into CHO cells, one of the orphans (previously denoted as pINF114 or a formyl peptide receptor-like-1 (FPRL-1), displayed both specific [³H]LXA₄ binding with high affinity (*K_d* of 1.7 nM) and demonstrated ligand selectivity when compared with LXB₄, LTB₄, LTD₄, and prostaglandin E₂ (Fiore et al., 1994). LXB₄ did not act via the ALX receptor and interacted with a specific receptor present on human leukocytes (Maddox and Serhan, 1996). In transfected CHO cells, LXA₄ activated both GTPase and released arachidonic acid from membrane phospholipids, indicating that this cDNA encodes a functional receptor for ALX in myeloid cells. A mouse ALX receptor cDNA was also identified and cloned from a spleen cDNA library. This receptor expressed in CHO cells displayed specific [³H]LXA₄ binding, and LXA₄ initiated GTPase activity (Takano et al., 1997).

The ALX cDNA was originally cloned by several groups who were evaluating fMLP receptor (FPR) using the FPR cDNA as a probe under low-stringency hybridization conditions. ALX has high sequence homology (~70%) to FPR. In light of this homology, the term FPRL1 (FPR-like-1) (Murphy et al., 1992) or FPRH1 (Bao et al., 1992) was designated by different research groups. Similarly this receptor was also known as FPR2 (Ye et al., 1992) or RFP (receptor related to FPR) (Perez et al., 1992). ALX was also cloned by Nomura et al. (1993) from human monocyte cDNA library and was designated as an orphan receptor (HM63). Both human and mouse ALX cDNA contain an open reading frame of 1051 nucleotides that encode a protein of 351 amino acids. Northern blot analysis demonstrated that ALX mRNA is ~1.4 kb in both human and mouse (Takano et al., 1997). Deduced amino acid sequences indicated that ALX belongs to the GPCR superfamily characterized by seven putative transmembrane segments with N termini on the extracellular side of the membrane and C terminus on the intracellular side.

The overall homology between human and mouse ALX is 76% in nucleotide sequence and 73% in deduced amino acid (Takano et al., 1997). A high homology was present in the sixth transmembrane segment and the second intracellular loop, a finding that suggests an essential role for these regions in ligand recognition and signaling. The molecular evolution, as assessed from computer-based sequence analyses indicates that ALX (Fig. 2) is not related to prostanoid receptors and belongs to a rapidly growing cluster of chemoattractive peptide and chemokine receptors, exemplified by fMLP, C5a, and IL-8 receptors (Toh et al., 1995). The BLT₁ receptor cloned from human HL-60 cells (Yokomizo et al., 1997) and mouse eosinophils (Huang et al., 1998) shares an overall homology of ~30% with ALX in deduced amino acid sequences.

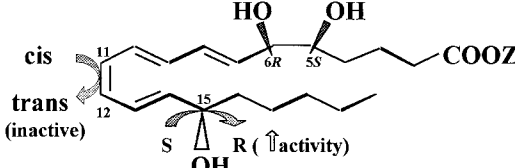
Human and mouse ALX cDNA, when transfected into CHO cells, displayed specific binding to [³H]LXA₄, with a K_d of 1.7 nM (Fiore et al., 1994) and 1.5 nM (Takano et al., 1997), respectively. Human ALX-transfected CHO cells were also tested for binding with other eicosanoids, including LXB₄, LTD₄, LTB₄, and prostaglandin E₂. Only LTD₄ shows competition with [³H]LXA₄ binding, with a K_i value of 80 nM (Fiore et al., 1994). Moreover, LXA₄ does not compete with radiolabeled LTB₄ binding to either recombinant human BLT₁ or isolated human peripheral blood neutrophils (Fiore et al., 1992; Chiang et al., 2000). Hence, the ability of LXA₄ and 15-epi-LXA₄ to block LTB₄-induced responses in vivo and in isolated PMNs may not be via direct interactions at either BLT₁ or BLT₂. Although ALX shares ~70% homology with

FPR, ALX binds [³H]fMLP with only low affinity (K_d ~5 μM) and therefore is selective for LXA₄ by 3 orders of magnitude (Fiore and Serhan, 1995).

Preparation of radiolabeled [11,12-³H]LXA₄ (Brezinski and Serhan, 1991) enabled the direct characterization of specific LXA₄ binding sites present on PMNs (Fiore et al., 1992). Intact PMN demonstrated specific and reversible [11,12-³H]LXA₄ binding (K_d ~0.5 nM and B_{max} ~1,830 sites/PMN) that are modulated by guanosine stable analogs. These LXA₄-specific binding sites are inducible in HL-60 cells exposed to differentiating agents (e.g., retinoic acid, dimethyl sulfoxide, and phorbol 12-myristate 13-acetate) and confer LXA₄-induced phospholipase D activation in these cells (Fiore et al., 1993). The various ligands that activate the ALX receptor are presented in Table 6. These results provided further evidence that LXA₄ interacts with specific membrane-associated receptors on human leukocytes. These binding data have corroborated with the functional results for LXA₄. In contrast, although functional studies (Maddox et al., 1998) have indicated the existence of a receptor activated by LXB₄, this receptor has not been cloned and is presently referred to as a putative receptor. In addition, the receptor binding investigations with LXB₄ must await the synthesis of a suitable LXB₄ radiolabel with high specificity. Such an analog has proven to be quite difficult to achieve.

Northern blot analysis of multiple murine tissues demonstrated that, in the absence of challenge to the host, ALX mRNA is most abundant in neutrophils, spleen, and lung with lesser amounts in heart and liver

TABLE 6
Ligand specificity for human ALX

	
Compounds compete with [³ H]-LXA ₄ :	DO NOT compete:
• LXA ₄ (Z = H) and methyl ester (Z = CH ₃)	• 6(S)-LXA ₄
• 15-epi-LXA ₄	• 11-trans-LXA ₄
• 15(R/S)-methyl-LXA ₄	• 15-deoxy-LXA ₄
• 16-phenoxy-LXA ₄	• LXB ₄
• 15-epi-16-(para-fluoro)-phenoxy-LXA ₄	• LTB ₄
	• PGE ₂

(Takano et al., 1997). In humans, ALX mRNA is also abundant in PMNs, as well as in spleen, lung, placenta, and liver (Fiore et al., 1994; Takano et al., 1997). In human enterocytes, ALX is inducible by IL-13 and interferon present in crypt and brush-border colonic epithelial cells (Gronert et al., 1998). The location of this receptor provides additional evidence that ALX is not associated with bacterial chemotaxis as observed for *N*-formyl peptide FPR-signaling since the gastrointestinal tract always has a very high level of bacteria present.

D. Receptors and Cellular Signals

Whereas the cascades of cellular events subsequent to GPCR activation have been the subject of many investigations, the exact signal transduction mechanisms for either the leukotrienes or the lipoxins have not been completely elucidated. Generally, agonist interactions with GPCRs involve activation of heterotrimeric G-proteins associated with a group of conventional cellular events. However, effectors for GPCRs that are independent of G-proteins are also known to exist (Hall et al., 1999). G-proteins, composed of α -, β -, and γ -subunits each encoded by a different gene, appear often to be cell specific. Upon ligand-receptor activation, the $G\alpha$ - and $G\beta\gamma$ -subunits stimulate a variety of intracellular molecular systems. Furthermore, G-protein activation leads to increases in intracellular Ca^{2+} and modifications in a number of membrane ion channels.

The cellular responses to ligand activation of GPCRs can also be up-regulated through priming of cells and down-regulated by desensitization. Two types of desensitization have been described, one that results from phosphorylation of the agonist-occupied receptor by G-protein-coupled receptor kinases. These phosphorylated receptors are associated with the arrestin family of proteins. A second type of rapid desensitization (loss of response) following phosphorylation by either second messenger-activated kinases (protein kinase A, protein kinase C) or inhibition of phospholipase C, which are activated by different receptors or signaling processes. Generally, this second type of desensitization does not require agonist-receptor occupancy. In addition, Didsbury et al. (1991) also demonstrated "cross-receptor desensitization", a phenomenon that has been reported for the chemoattractant family receptors. Presently, an exploration of these latter mechanisms associated with the actions of leukotrienes and lipoxins at the molecular level has received little attention.

1. BLT. Investigations involving the intracellular signaling of BLT receptor activation have been performed in peripheral leukocytes specifically granulocytes. One of the problems involved in such studies is that these cells have a limited life span (24 h) making drug and transfection studies difficult. These limitations have caused several investigators to use either CHO cells expressing human BLT receptors (Yokomizo et al.,

1997) or to perform reconstitucional studies with the heterotrimeric GTP-binding proteins (Miki et al., 1990; Igarashi et al., 1999). Although high-affinity binding of LTB_4 (BLT₁ receptor) is found essentially in leukocytes and macrophages, the G-proteins associated with the functions in these cells has not been clearly established. Furthermore, the intracellular signaling pathways for BLT may depend on the G-proteins expressed in the different cells. For example, most of the LTB_4 -dependent signals in granulocytes appear to be mediated by G_i -like G-proteins, (granulocytes express abundant $G\alpha_i$ proteins, mainly $G\alpha_{i2}$), whereas in the nervous system G_{i1} and G_o are mainly present (Simon et al., 1991). In several cell types, LTB_4 signals via G_i -proteins are inhibited by pretreatment of pertussis toxin (PTX). However, LTB_4 -induced calcium mobilization in CHO-BLT₁ was not affected by PTX, suggesting the coupling with G_q -like molecules in these latter cells. Chemotaxis and inhibition of adenylyl cyclase by LTB_4 were completely PTX-sensitive in CHO-BLT₁ cells. The coupling of BLT₁ with various $G\alpha$ -subunits was examined by cotransfection studies using COS-7 cells, and BLT₁-mediated phospholipase C activation was shown to be mediated by $G\alpha_{i6}$ - and $G\beta\gamma$ -subunits released from $G\alpha_i$ (Gaudreau et al., 1998). When expressed heterologously in CHO, HeLa, and COS-7 cells, BLT₂ activation led to the inhibition of adenylyl cyclase and an increase in calcium. However, BLT₂ activation was less potent in mobilizing calcium than BLT₁ receptor activation (Yokomizo et al., 2000). BLT₂ was also shown to mediate LTB_4 -dependent chemotaxis through G_i -like G-proteins (Kamohara et al., 2000; Yokomizo et al., 2000). Recently, Woo et al. (2002) have suggested that LTB_4 stimulation of the Rac-extra-cellular signal-regulated kinase cascade associated with the generation of reactive oxygen species-mediated chemotaxis in Rat-2 cells was via activation of the BLT₂ receptor. This suggestion, although not conclusive, was supported by the observations that BLT₁ expression has not been detected in Rat-2 fibroblasts whereas BLT₂ was expressed. Furthermore, the LTB_4 stimulation of reactive oxygen species was observed at high concentrations (0.3–1 μ M), which are within the range for BLT₂ activation and are 2 orders of magnitude higher than that observed for activation of BLT₁. In addition, this LTB_4 stimulation was blocked by ZK 158252. In an attempt to understand the mechanisms involved in BLT receptor desensitization, Gaudreau et al. (2002) have reported some initial molecular evidence. These investigators showed that the cytoplasmic tail of BLT₁ receptor was intimately involved in the regulation of desensitization and that the amino acid threonine (Thr³⁰⁸) was implicated in the GPCR-specific kinase phosphorylation associated with this phenomenon. This study therefore provides pertinent leads for understanding those structural elements associated with BLT₁ receptor regulation.

The exact role of calcium activation subsequent to the interaction of LTB_4 with a specific receptor exhibits similar complexity. Chemotaxis of human PMN or monocytes to LTB_4 does not absolutely require the mobilization of intracellular Ca^{2+} (Romano et al., 1996; Kuhns et al., 1998). While activation of the LTB_4 receptor does stimulate intracellular Ca^{2+} mobilization (Powell et al., 1996), this Ca^{2+} appears not to be essential for chemotaxis and plays a role only in degranulation and other responses of human neutrophils (Serhan et al., 1982; Luscinskas et al., 1990; Rola-Pleszczynski et al., 1993). Such studies indicate that the unraveling of the intracellular events associated with the BLT receptor activation with the second messenger cascades are only beginning to be understood.

2. *CysLT*. Unfortunately, most studies concerning the CysLT receptors have involved only LTD_4 activation of CysLT₁ receptors. There is little information available concerning G-protein and Ca^{2+} mobilization when the CysLT₂ receptor is activated. Initial studies (Kuehl et al., 1984; Crooke et al., 1989, 1990; Watanabe et al., 1990) demonstrated that LTD_4 activation of the CysLT₁ receptor lead to G-protein activation and the release of several second intracellular messengers, namely, diacylglycerol, inositol phosphates, and Ca^{2+} , events which were followed by activation of protein kinase C (PKC) and accompanied by the mobilization of Ca^{2+} derived from both intracellular and extracellular stores. Clark et al. (1985) demonstrated that LTD_4 activation of CysLT₁ receptors also led to the release of arachidonic acid via stimulation of phospholipase A_2 , which was associated with an enhanced transcription of phospholipase A_2 activating protein. Expression of this latter protein was controlled by activation of topoisomerase I, which in turn was regulated by PKC (Mattern et al., 1991).

LTD_4 is known to induce either Ca^{2+} influx through the plasma membrane without any Ca^{2+} release from intracellular stores (Baud et al., 1987a; Oliva et al., 1994), or Ca^{2+} release without influx (Bouchelouche et al., 1990) and in many cell types can induce both Ca^{2+} influx and release (Mong et al., 1984b, 1987a, 1988; Sjolander et al., 1990). In human airways, Gorenne et al. (1998) demonstrated that LTD_4 -induced contractions may mobilize Ca^{2+} via a receptor-operated channel. Recently, Accomazzo et al. (2001) provided evidence that LTD_4 contractions in human airways did not elicit a marked elevation in $[\text{Ca}^{2+}]_i$ but rather activated Ca^{2+} via a Ca^{2+} -independent isoform of protein kinase C (PKC ϵ). These observations supported an initial report that PKC activation may be associated with increased force development at constant $[\text{Ca}^{2+}]_i$ (Masuo et al., 1994). In addition, PKC ϵ was shown to be necessary for the generation of the LTD_4 -induced Ca^{2+} signal in intestinal epithelial cells. Together these results suggest that the Ca^{2+} signaling for LTD_4 contractions in human airways may involve several intracellular pathways. Unfortunately, the other ligands (LTC_4 and LTE_4) have not been examined in these studies. Interest-

ingly, Sjolander et al. (1990) proposed that LTD_4 and LTE_4 activated different receptors associated with different intracellular signals. However, these observations have not been pursued. The initial observations of Gronroos et al. (1995, 1996, 1998) have recently received support from several investigators (Hoshino et al., 1998; Thodeti et al., 2000; Massoumi and Sjolander, 2001; Paruchuri et al., 2002) and provided evidence that LTD_4 activation of an epithelial cell line (THP-1) led to an association of a protein other than the heterotrimeric G-proteins and this mobilization was necessary for the mobilization of calcium. Although these investigators observed that LTD_4 activation of THP-1 cells altered mitogen-activated protein kinase via protein kinase C and Raf-1, an effect which was PTX-insensitive, they also reported that the chemotactic response of these cells was PTX-sensitive. These observations suggest that diverse pathways at the G-protein level may also exist when LTD_4 interacts with the receptor. However, these investigators did not evaluate different receptor antagonists to determine which receptor was associated with these different pathways.

3. *Lipoxins*. The cytoplasmic signaling cascade of the ALX receptor is also highly specific and selective for different cell types. In human PMNs, LXA_4 stimulates rapid lipid remodeling and release of arachidonic acid via a PTX-sensitive G-protein (Nigam et al., 1990) and blocked intracellular generation of inositol 1,4,5-trisphosphate (Grandordy et al., 1990) as well as Ca^{2+} mobilization (Lee et al., 1989). In contrast, in human monocytes and THP-1 cells, LXA_4 triggers intracellular calcium release (Romano et al., 1996; Maddox et al., 1997), suggesting a different intracellular signaling pathway than in PMNs despite identical receptor sequences. In addition, distinct signaling in monocyte and PMNs was further supported by different responses to LXA_4 in these cell types. LXA_4 modulates mitogen-activated protein kinase activities in mesangial cells in a PTX-insensitive manner (McMahon et al., 2000), suggesting the presence of an additional ALX receptor subtype and/or signaling pathway for ALX. Since the ALX receptor has been shown to switch recognition and function with certain chemotactic peptides, the G-proteins and intracellular pathways involved may prove to be a difficult but fascinating area to explore. One of the problems presently confronting investigators in this area of research is the availability of the ligands. Studies on G-protein and intracellular messengers are presently limited (Kang et al., 2000), since stable analogs for LXA_4 and LXB_4 have only recently become available.

E. Summary

Within the last few years, a considerable effort at the molecular level has been undertaken to identify the leukotriene receptors. However, data involving chimeric constructs of the leukotriene receptors have only recently been reported (Gaudreau et al., 2002). In contrast,

there are several observations that warrant further investigation. For example, the mouse CysLT₁ cloned receptor is activated by all three native ligands and antagonized by MK-571. However, the ligand profile for the mCysLT₁ is quite different from that observed in the human CysLT₁ receptor, since the mouse CysLT₁ receptor exhibited little response to LTC₄. An explanation for this difference is not readily apparent. In addition, MK-571 potentiated Ca²⁺ mobilization in CHO cells transfected with mCysLT₁ long isoform cDNA (Maekawa et al., 2001). The exact reason for this specific effect has not been explored. Recently, Ogasawara et al. (2002) reported different pharmacological properties of the CysLT₂ receptor between human and mouse, and they also showed that mCysLT₁ and mCysLT₂ tissue expression was different in some strains of mice. These data suggest that receptor distributions may be different within and between species.

IV. Properties and Significance of Leukotriene Receptors

Well before the cloning of the leukotriene receptors, a considerable amount of indirect evidence had already suggested their existence. To date, the molecular database has confirmed the pharmacological database. However, a more thorough appreciation of ligand-receptor interactions under both physiological and pathophysiological conditions can only be achieved by an assessment of both these databases. Therefore, an effort has been made in this section to provide the evidence that reported the properties and significance of leukotriene receptors and to highlight those observations that suggested other receptor subtypes may exist.

A. BLT Functional and Radioligand Binding Studies

As early as 1974, certain monohydroxy eicosanoids, including the platelet arachidonate lipoxygenase product (12-HETE), were shown to be neutrophil chemoattractants (Turner et al., 1975) as well as other monohydroxy products (5-HETE, 12-HETE) (Goetzl and Pickett, 1980; Powell et al., 1996). In addition, certain derivatives of LTB₄, namely, 12-oxo-LTB₄ and 20-hydroxy-LTB₄ were reported to induce chemotaxis (Yokomizo et al., 1993, 1996). However, each required higher concentrations than LTB₄ to induce chemotaxis in experiments when cells were placed in isolated Boyden-type chambers (Ford-Hutchinson et al., 1980). Furthermore, radioligand binding studies (Goldman and Goetzl, 1982; Snyder et al., 1984; Goldman et al., 1985; Gorman et al., 1985; Falcone and Aharony, 1990; Maghni et al., 1991) provided more support for the existence and distribution of specific receptors for LTB₄. The differences in relative potencies between LTB₄ and the mono-HETEs (LTB₄ ≫ HETE) also provided evidence and facilitated the identification and classification of the cognate receptor. However, the leukocyte surface receptors on neutrophils involved in chemo-

taxis by which other mono-HETEs activate these cells (Powell et al., 1996; Falgoutret and Riendeau, 2000) have not been identified.

B. Cysteinyl-Leukotriene Functional Studies

1. Airway Smooth Muscle Contraction. Drazen et al. (1980) reported that LTC₄ and LTD₄ were equipotent in guinea pig tracheal smooth muscle preparations whereas LTD₄ was a more selective peripheral airway agonist, since LTD₄ exhibited a 100-fold greater potency than LTC₄ in the lung parenchymal strip suggesting the presence of two CysLT receptors. The heterogeneity of leukotriene receptors received further support from Krell et al. (1983) who showed that the potency of LTD₄ in the guinea pig lung parenchymal tissues (pEC₅₀, 9.2) was significantly different from that observed in the tracheal preparations (pEC₅₀, 7.8). In addition, Fleisch et al. (1982) demonstrated that the potency of LTD₄ was different in tissue preparations derived from various organs. These investigators reported that in the guinea pig ileum LTD₄ had a pEC₅₀ of 9.2, whereas in the trachea from this species the pEC₅₀ was 8.5. However, when contractile studies were performed on human airways, results showed that LTC₄, LTD₄, and LTE₄ were equipotent as contractile agonists (Buckner et al., 1990; Labat et al., 1992) with pEC₅₀ values of approximately 10 nM, that is, about one-thousandth of the pEC₅₀ values for histamine in the same tissues (Dahlén et al., 1980). However, LTE₄ was markedly less active than LTC₄ and LTD₄ in these tissues (Buckner et al., 1986). A comparison of the cys-LT agonist potencies between different tissues illustrates that considerable variation exists in functional assays (pEC₅₀ values 11 to 7) (Jones et al., 1984; Hand and Schwalm, 1987; Gardiner et al., 1990; Gieske et al., 1990; Norman et al., 1990; DeLegge et al., 1993). Based solely upon agonist potencies, one is unable to surmise whether either a single or several receptors are present in these preparations. This requires additional information from structural, operational, and molecular biological studies. However, the data suggest a marked difference between LTE₄ and the other cys-LTs. Interestingly, human vascular preparations exhibited exquisite sensitivity to these mediators (Allen et al., 1994).

Results using the SRS-A receptor antagonist, FPL 55712, further supported the hypothesis for the existence of distinct receptors for the cys-LTs. Drazen et al. (1980) reported a pK_B value for FPL 55712 of 6.9 in the lung parenchymal tissues when LTD₄ was used as the agonist with a Schild plot slope of 0.72; similar results were demonstrated by Krell et al. (1981). Furthermore, these latter investigators showed that the potency of FPL 55712 (pK_B) for antagonism of LTD₄-induced contraction in the parenchyma decreased with increasing concentrations of this antagonist, suggesting that in this tissue either factors may be released which were not blocked by FPL 55712 or the profile of antagonism of this compound was not competitive. In the guinea pig tra-

chea, Krell et al. (1981) calculated a pK_B (6.4) for FPL 55712 for inhibition of LTD_4 contraction. When these preparations were contracted with LTC_4 , the pK_B for FPL 55712 was similar to that obtained against LTD_4 . In contrast, when tracheal tissues were treated with indomethacin the pK_B obtained against LTC_4 was 5.7. Thus, in the trachea, while FPL 55712 antagonized both LTD_4 -induced and LTC_4 -induced contractions, the pK_B values differed (6.4 and 5.7, respectively). In parenchymal tissues, contractions to LTD_4 , but not those to LTC_4 , were inhibited. Fleisch et al. (1982) reported their results with FPL 55712 against LTD_4 contractions in different tissues and demonstrated pK_B values of 6.1 and 6.3 in parenchyma and trachea whereas in the ileum the value was 7.1; the pA_2 values were calculated to be 7.3 for the ileum, 6.5 for the trachea, and 6.0 for the parenchyma. These data also indicated that the dissociation constants for FPL 55712 derived from the trachea and parenchyma were markedly different from that obtained in the ileum. Additional experiments with FPL 55712 supported the notion that different leukotriene receptors existed, since FPL 55712 failed to antagonize the LTC_4 contractions in the lung parenchymal strips and exhibited a pK_B of 9.2 against LTD_4 -induced contractions in the guinea pig trachea. Since FPL 55712 was reported to have effects other than leukotriene antagonism, including PDE inhibition (Welton et al., 1981), the development of other specific compounds was required to establish more definitive evidence for the presence of at least two receptors. Many compounds with much greater potency (K_i values of about 0.1–10 nM; pA_2 values or pK_B values of 9.5–7.5) and markedly improved selectivity compared with FPL 55712 have been pharmacologically identified and comprehensively characterized (Jones et al., 1983; Buckner et al., 1986; Tucker and Weichman, 1986; Hay et al., 1987; Krell et al., 1987, 1990; Muccitelli et al., 1987; Jones et al., 1989, 1995; Torphy et al., 1989; Labat et al., 1992; Gardiner et al., 1994). Collectively, the results with these chemically distinct antagonists (Table 5) provided pertinent pharmacological support for the presence of two receptors in the various tissue preparations.

2. Vascular Smooth Muscle Contraction. Hanna et al. (1981) reported that cys-LTs contracted not only isolated human airways but also human pulmonary veins and noted that the maximum responses on human pulmonary arteries were small (Schellenberg and Foster, 1984; Bourdillat et al., 1987). Berkowitz et al. (1984) studied vascular preparations from several species (rat, rabbit, and guinea pig) and observed small contractions in guinea pig pulmonary veins, the inferior vena cava and jugular vein; the effects of LTC_4 were not examined. In the rabbit renal vein, LTC_4 - and LTD_4 -induced contractions (Kito et al., 1981), although only small or no contractile responses, were observed in pulmonary and portal veins. Gleason et al. (1983) also reported that LTD_4 -induced contractions in the guinea pig-isolated

pulmonary artery were small. Together these observations suggested that a number of vascular preparations exhibited little or no contractile response to the cys-LTs. Whether this lack of contraction was due to the absence of receptors or to factors that are released and mask the response has not been systematically studied (Bäck et al., 2002). However, in human vascular preparations, Allen et al. (1992) reported a pronounced constriction by LTC_4 and LTD_4 in human-isolated saphenous veins and the agonists were equipotent.

Nishiye et al. (1988) showed that FPL 55712 and ONO-RS-411 blocked the LTD_4 contractions in the guinea pig basilar artery demonstrating a single specific receptor. However, in human pulmonary veins, unlike those in human airways, the contractions induced by cys-LTs were not affected by the CysLT₁ receptor-selective antagonists MK-571 and ICI 198615 (Labat et al., 1992; Gorenne et al., 1996). These results suggested the presence of distinct CysLT receptors on the human pulmonary veins compared with either human bronchus or trachea (Jones et al., 1982; Buckner et al., 1986). In contrast, Rinkema et al. (1993) showed that the LTD_4 contractions in the guinea pig inferior vena cava were blocked by tomelukast and WY 48252. However, the contractions induced by LTC_4 were blocked in a biphasic fashion by these two antagonists, that is, the low concentrations of LTC_4 were not affected by the antagonists suggesting two CysLT receptors. Therefore, in some species, such as the guinea pig, vascular smooth muscle may contain either one or two CysLT receptors associated with the contractions. Whether the CysLT receptors in the human pulmonary veins, which are resistant to these CysLT₁ receptor antagonists, are the same as the receptors in the guinea pig vena cava, which are activated by LTC_4 , remains to be established. Recently, Bäck et al. (2000b) have reported that contractions induced by LTC_4 in porcine pulmonary arteries were resistant to MK-571 (CysLT₁ antagonist) as well as BAY u9773 (dual antagonist, CysLT₁/CysLT₂), suggesting that another receptor may be associated with this tissue response.

3. Vascular Smooth Muscle Relaxation. Secrest et al. (1985) reported that, in canine renal arteries where tone had been induced by a contractile agonist, LTD_4 provoked relaxations. Similar results were obtained with LTC_4 and LTD_4 in guinea pig pulmonary artery (Sakuma et al., 1987; Sakuma and Levi, 1988) and isolated human saphenous veins (Allen et al., 1992), and LTD_4 in isolated human pulmonary arteries and veins (Ortiz et al., 1995). LTD_4 relaxed canine renal blood vessels and was approximately 100-fold more potent on veins than arteries. Whether or not this latter difference is due to receptor density or receptor-effector coupling is presently unknown. LTC_4 was not examined in these tissues. In canine splanchnic venous capacitance vessels similar relaxations for LTC_4 and LTD_4 have been observed. Furthermore, in canine coronary arteries both LTC_4 and LTD_4 relaxed the tissues with the latter agonist being 10

times more potent. The data reported indicate that the agonist potencies for relaxation of human vascular preparations (pEC_{50} values, approximately, 11–10) (Allen et al., 1992; Ortiz et al., 1995) are markedly different from the potencies required to produce contractions in the same tissue (pEC_{50} values, approximately, 9–7) (Schellenberg and Foster, 1984; Bourdillat et al., 1987; Allen et al., 1992; Labat et al., 1992; Ortiz et al., 1995; Stanke-Labesque et al., 2000). Such data suggest that either the receptors on the endothelium associated with the relaxation are different from the receptors responsible for the contraction or the G-protein second messengers may be coupled more efficiently. In addition, CysLT₁ receptor antagonism of contractions in preparations from the human lung (Table 7) suggest that the receptor present in vascular smooth muscle may be different from that in the human airways. Interestingly, the LTD₄-induced relaxation of isolated human pulmonary arteries and veins has also been reported to be resistant to CysLT₁ antagonists, MK-571 and ICI 198,615 (Ortiz et al., 1995). The question remains as to whether or not the receptor present on the endothelium and associated with the relaxation is the same as that present on the vascular smooth muscle and responsible for the contraction.

Secrest et al. (Secrest et al., 1985; Secrest and Chapnick, 1988) reported that the endothelium-dependent relaxations were produced by activation of a specific receptor since the response was attenuated by FPL 55712. Sakuma and Levi (1988) observed that the relaxations induced by LTC₄ and LTD₄ in the isolated guinea pig thoracic aorta were antagonized with similar potency by the CysLT₁ receptor antagonist, ICI 198,615, suggesting that both agonists activate a single receptor (CysLT₁). This antagonism was also observed in the guinea pig pulmonary artery. Therefore, on the endothelium of guinea pig arterial preparations, a single receptor was present (Sakuma and Levi, 1988), and activation was associated with relaxation. In contrast, this does not appear to be the case in the canine renal arteries and

veins (Pawloski and Chapwick, 1993a; Pawloski and Chapwick, 1993b) or in the human pulmonary arteries and veins (Ortiz et al., 1995). In the latter study, the endothelium present on the human pulmonary veins contains two CysLT receptors: a CysLT₂-type responsible for relaxation and a CysLT₁ receptor associated with the release of a contractile factor (Ortiz et al., 1995).

4. Cardiovascular Effects. Subsequent to the early investigations (Levi and Burke, 1980; Levi et al., 1980), which reported that partially purified SRS markedly reduced coronary blood flow, a number of other investigators have explored the cardiovascular effects of the cys-LTs in a variety of different animal models. The early studies have shown that the cys-LTs are potent vasoconstrictors of the coronary vasculature in both isolated perfused hearts (Woodman and Dusting, 1982; Letts and Piper, 1983; Letts et al., 1983) and in a number of species in vivo (Michelassi et al., 1982; Panzenbeck and Kaley, 1983; Fiedler et al., 1984, 1987; Leffler et al., 1984; Fiedler and Abram, 1987; Kopia et al., 1987; Michelassi et al., 1987) including human (Marone et al., 1988). In sheep and pigs, cys-LTs induced not only coronary vasoconstriction but also ischemia and impaired left ventricular function (Michelassi et al., 1982; Ezra et al., 1983; Fiedler et al., 1985). In isolated perfused guinea pig heart preparations (Terashita et al., 1982; Letts and Piper, 1983; Roth et al., 1985), LTC₄ and LTD₄ caused a reduction in myocardial contractility concomitant with the vasoconstriction. In perfused rat hearts (Bittl et al., 1985), LTD₄ caused not only a reduction in flow but also reduced the spontaneous heart rate to a greater extent than contractility, suggesting an action on conductivity in this species (Feuerstein et al., 1981; Zukowska-Grojec et al., 1982; Zukowska-Grojec et al., 1984; Tomoike et al., 1987).

Burke et al. (1982) also showed that FPL 55712 antagonized the cardiodepressant effects of the cys-LTs suggesting that these effects were via activation of a specific receptor and noted that the potency of FPL 55712 was greater against ventricular contraction induced by LTD₄ compared with those of LTC₄, suggesting that there may be two receptors present in the guinea pig heart. These investigators also reported that in the human heart, the negative inotropic effect of cys-LTs was similar to that of the guinea pig and the rank order potency (LTD₄ > LTC₄ > LTE₄) was the same for both species. In other studies using isolated guinea pig cardiac muscle preparations, the data have been contradictory. Letts and Piper (1983) reported no myocardial effects whereas other investigators (Falcone et al., 1991a,b) have demonstrated positive inotropic affects.

The use of potent and selective CysLT₁ receptor antagonists provided an alternate approach for establishing the effects of cys-LTs in the perfused heart preparations. McLeod and Piper (1991) reported a differential antagonistic effect against LTC₄ and LTD₄. ICI 198,615 selectively antagonized LTD₄-induced increases in coro-

TABLE 7
CysLT₁ antagonists: effects in human lung preparations

Tissue	Agonists	MK 571	ICI 198,615	BAYu9773
Contraction				
Bronchus	LTC ₄	8.6–8.3	9.8–8.5	5.4
	LTD ₄	8.8–8.5	9.2–8.2	6.8–6.2
Pulmonary artery	LTC ₄	NS (7.20)	NS (7.02)	NS (6.26)
	LTD ₄	NS (NS)	NS (NS)	NS (NS)
Pulmonary vein	LTC ₄	NS	NS	6.7–5.8
	LTD ₄	NS	NS	6.8–6.5
Relaxation				
Pulmonary artery	LTD ₄	NS	NS	ND
Pulmonary vein	LTD ₄	NS	NS	ND

The range of pK_B values are shown. NS indicates no significant displacement of the concentration effect curves. ND indicates no data available. Numbers in parentheses are the pK_B values derived from tissues (Walch et al., 2002) where the endothelium had been removed and the preparations treated with indomethacin. The other values are from references: Hay et al., 1987; Jones et al., 1989; Buckner et al., 1990; Labat et al., 1992; Ortiz et al., 1995; Gorenne et al., 1996; Bäck et al., 2000b.

nary vascular resistance with no significant effect against LTC₄. Pobilukast antagonized both LTC₄ and LTD₄ responses whereas MK-571 was without effect on cardiac vascular resistance. However, ICI 198,615 antagonized both LTC₄- and LTD₄-induced ventricular contractility. These data suggested that the reduction in contractility by LTC₄ may be more sensitive to the actions of the antagonists when compared with their action against the vasoconstrictory effect of LTC₄. The failure of MK-571 to antagonize the coronary vasoconstriction was also observed against ventricular contractility induced by LTC₄ and LTD₄. In contrast, Falcone et al. (1990) showed specific binding of [³H]ICI 198,615 to guinea pig cardiac ventricular membranes, suggesting the existence of CysLT₁ receptors in this tissue, whereas Egan et al. (1989) demonstrated that selective antagonism of the leukotriene responses did not reduce myocardial effects. Although the data from animal studies remain controversial, the results derived from the guinea pig indicate that the antagonist profile in the guinea pig heart and lung preparations were not similar and suggested that the receptors in cardiac vessels may be different from those in the airways of this species.

Marone et al. (1988) reported the effects of cys-LTs following intracoronary injection in man. These investigators showed that low doses of exogenous LTD₄ and LTC₄ induced immediate changes of cardiovascular function consisting of a fall in blood pressure, a rise in heart rate, and sympathoadrenergic activation. These initial alterations were followed by a marked vasoconstriction of small resistance coronary vessels. In another study, Albazzaz et al. (1989) assessed the cardiopulmonary effects of leukotriene C₄ inhalation in nonasthmatic and asthmatic patients. Although the mean cardiac output decreased without changes in blood pressure and heart rate in both subjects, there was difficulty in separating the cardiac output modifications from the relative contribution of the pulmonary vasoconstriction, bronchoconstriction, and reduced cardiac output, which followed the challenge. The use of the potent and selective CysLT₁ receptor antagonists for such in vivo studies in man may provide an alternative for understanding the role of these mediators in cardiovascular disease.

5. Diverse Effects of Cysteinyl-Leukotrienes. Human umbilical vein endothelial cells (HUVECs) have been reported to produce (Sjostrom et al., 2001) as well as respond to all cys-LTs (Datta et al., 1995) by releasing von Willebrand Factor as well as an increased expression of P-selectin. The effects were blocked by the CysLT₁ receptor antagonist, pobilukast. These investigators demonstrated a concomitant surface expression of P-selectin and release of high-molecular weight von Willebrand Factor by the cys-LTs due to stimulation of exocytosis of the Weibel-Palade body pools rather than promoting the constitutive release from endothelial cells. In contrast, Pedersen et al. (1997) showed that the P-selectin secretion induced by LTD₄ and LTC₄ was not

inhibited by three different CysLT₁ receptor antagonists (zafirlukast, SB 205,312 and pobilukast). However, in this latter study the pool of P-selectin monitored by their assay was not described. The difference between the effects of the CysLT₁ antagonists may depend on which pool of P-selectin was assayed rather than a subset of receptors.

There are several reports that the cys-LTs increase microvascular permeability in guinea pig airways (Woodward et al., 1983a,b; Evans et al., 1989; Obata et al., 1992; Bochnowicz and Underwood, 1995); the mechanism may involve contraction of endothelial cells resulting in gaps in the endothelium of venules (Joris et al., 1987). This influence of the cys-LTs, which appeared to involve both direct and indirect pathways, was inhibited by either FPL 55712 (Woodward et al., 1983a) or pranlukast (Nakagawa et al., 1992; Bochnowicz and Underwood, 1995) indicating that a CysLT₁ receptor was involved.

There is increasing evidence that the cys-LTs may also influence inflammatory cell function since they enhance the recruitment of eosinophils. For example, inhaled LTE₄ (Laitinen et al., 1993) or LTD₄ selectively increased airway eosinophil numbers when assessed in bronchoalveolar lavage from humans (Smith et al., 1993). Similar results were obtained with LTC₄ or LTD₄ in guinea pigs (Foster and Chan, 1991; Underwood et al., 1996), with single administration of inhaled LTD₄ elicited a marked increase in eosinophils, which was maintained for at least 4 weeks (Underwood et al., 1996). The receptor responsible for this phenomenon of the cys-LTs appears to be CysLT₁ receptor (Foster and Chan, 1991; Underwood et al., 1996) and, at least in guinea pigs, appeared to involve IL-5 (Underwood et al., 1996), a key cytokine in eosinophil proliferation, activation, and survival. Pobilukast inhibited LTD₄-induced chemotaxis of peripheral blood eosinophils from nonasthmatic volunteers (Spada et al., 1994). Furthermore, the CysLT₁ receptor antagonists pranlukast, MK-571, ICI 198,615 inhibited antigen-induced eosinophil influx in rats (Salmon et al., 1999), guinea pigs (Foster and Chan, 1991), and cynomolgus monkeys (Turner et al., 1994).

The effects of the cys-LTs on mucus secretion have been explored only to a limited degree. Initial studies involved the incorporation of radiolabels such as [³H]glucosamine into secretory cells (Shelhamer et al., 1980; Coles et al., 1983; Kim et al., 1989, 1993). Although the cys-LTs were shown to increase release of radiolabeled material from the secretory elements of the human bronchus, there is little information on the effects of selective antagonists (Lundgren et al., 1990). LTC₄ or LTD₄ induced an increase in mucus secretion from cultured human airway mucosal explants, an effect which was antagonized by FPL 55712 (Marom et al., 1982; Coles et al., 1983). Pobilukast inhibited the aerosolized LTD₄-induced increase in epithelial mucus secretion in guinea pig airways in vitro (Hoffstein et al.,

1990), suggesting that the effects of the cys-LTs on mucus secretion are CysLT₁ receptor-mediated. Labat et al. (1999) have reported that challenge of human bronchial preparations with LTD₄ failed to elicit increased amounts of the MUC5AC gene product, suggesting that there may or may not be alterations in the secretory activity of human airways depending upon which secretory product is monitored.

Panettieri et al. (1998) reported that although LTD₄ alone had no significant effect on DNA synthesis in human cultured airway smooth muscle cells, there was a marked potentiation in the airway smooth muscle proliferation induced by the classical mitogens, epidermal growth factor, or thrombin. This effect of LTD₄ was inhibited by either pranlukast or pobilukast, but not zafirlukast, suggesting activation of a CysLT receptor other than CysLT₁ (Panettieri et al., 1998). Evidence for an influence of the cys-LTs on airway smooth muscle proliferation is derived from the results of two in vivo studies in rats following chronic antigen challenge where airway smooth muscle proliferation and hyperreactivity (Wang et al., 1993) or eosinophil infiltration (Salmon et al., 1999) were attenuated by the CysLT₁ receptor antagonists, MK-571 (Wang et al., 1993) or pranlukast (Salmon et al., 1999).

There is also evidence in guinea pigs that the cys-LTs may influence lung function by modulating the afferent nervous system, specifically enhancing activity of capsaicin-sensitive sensory C fibers (Stewart et al., 1984; Udem and Weinreich, 1993; Udem et al., 1993). For example, LTD₄ enhanced the release of substance P (Bloomquist and Kream, 1990) and potentiated tachykinin-mediated, nerve-induced responses (Ellis and Udem, 1991) in guinea pig trachea. Vagal nerve-induced contraction and microvascular permeability in guinea pig airways are inhibited by CysLT₁ receptor antagonists (Ellis and Udem, 1991), indicating that the effects of the cys-LTs on tachykinin-containing sensory C fibers are mediated via the CysLT₁ receptor in guinea pig airways.

There are a number of other observations concerning the cys-LTs that require further investigation. Few studies have been performed to examine these mediators in angiogenesis (Tsopanoglou et al., 1994) where pobilukast has been reported to block responses to LTD₄, albeit with less potency than those to LTC₄. The effects of these mediators on liver metabolism has been overlooked but an investigation has demonstrated that both LTD₄ and LTC₄ are equipotent in altering glucose and lactate output, and this modification is blocked by CGP 35949, a CysLT₁ antagonist (Iwai and Jungermann, 1988, 1989). In addition, investigations on the actions of the cys-LTs on renal function (Chapnick, 1984; Filep et al., 1985; Piper et al., 1985; Petric and Ford-Hutchinson, 1995), the pulmonary circulation (Smedegard et al., 1982; Kadowitz and Hyman, 1984; Jones and Masson, 1985; Malik et al., 1985; Garcia et al., 1987;

Ohtaka et al., 1987; Schreiber et al., 1987), systemic circulation (Pfeffer et al., 1983), and on the microcirculation (Dahlén et al., 1981; Bisgaard et al., 1982, 1985, 1986; Soter et al., 1983; Chan and Ford-Hutchinson, 1985; Hua et al., 1985) to established specific CysLT receptors have not been pursued.

C. CysLT Radioligand Binding Studies

The presence of heterogeneous CysLT receptors was also supported by data from experiments using classical radioligand binding techniques. Cheng et al. (1985) provided evidence that the CysLT receptors were widely distributed in different organs, tissues, and species. Furthermore, autoradiographic studies with the labeled agonists have also demonstrated the presence of CysLT receptors in lung (Carstairs et al., 1988), endothelial cells (Chau et al., 1986), uterus (Chegini and Rao, 1988b), corpus lutea (Chegini and Rao, 1988a), as well as brain (Goffinet, 1986). In addition, a number of smooth muscle or transformed cells in culture were also shown to possess these receptors (Krilis et al., 1983; Mong et al., 1988; Frey et al., 1993). The radioligand binding studies have not only demonstrated the presence of specific sites for [³H]LTC₄, [³H]LTD₄, and [³H]LTE₄ binding but also have shown that there is often a preferential binding for one ligand and not the other (Mong et al., 1984a, 1985; Cheng et al., 1985). These latter observations suggested that certain tissues may contain a single class of receptor responsible for the physiological actions of cys-LTs. Although a considerable amount of evidence demonstrated that membrane preparations from a number of tissues bind all the radiolabeled agonists, there are data suggesting the existence of at least two sites: one associated with [³H]LTC₄ and one that binds [³H]LTD₄ and [³H]LTE₄ (Cheng and Townley, 1984b).

However, three major points became apparent from the radioligand binding studies that need to be highlighted. First, interpretation of the results of [³H]LTC₄ binding assays were complicated by the rapid metabolic transformation of LTC₄ to LTD₄ if this was not controlled by enzymatic inhibition. Inhibitors of this metabolism must frequently be incorporated in [³H]LTC₄ binding studies. In addition, recent binding studies (Ravasi et al., 2000) have also suggested the necessity to include S-decyl-glutathione (a high-affinity ligand for nonreceptor LTC₄ binding sites) in the membrane preparations. Second, there was often no correlation between the [³H]LTC₄ binding studies and the functional studies (contraction) as in the case of either the guinea pig ileum (Norman et al., 1990), guinea pig lung strip (Mong et al., 1985; Norman et al., 1987), or guinea pig uterus (Weichman and Tucker, 1982; Levinson, 1984). In contrast, a correlation was observed between the antagonist effects in the [³H]LTD₄ binding studies and the ability of the antagonists to block LTD₄-induced contractions (Fig. 6). Furthermore, in the rat lung the radioligand binding data provided evidence for the presence of two sites

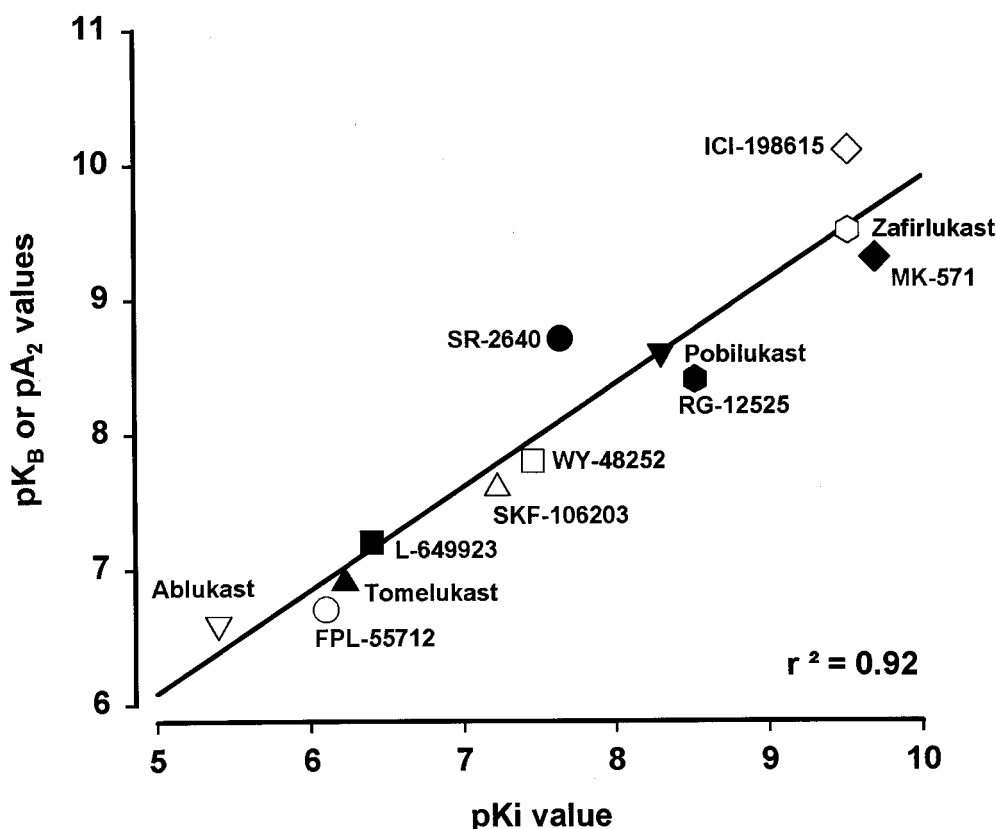


FIG. 6. Cysteinyl-leukotriene antagonists in binding versus functional assays. Data (pK_B/pA_2 values) are derived from guinea pig tissues contracted with LTD_4 and compared with results (pK_i values) obtained in [3H]LTD $_4$ radioligand binding assays performed on membrane fractions from the same species. The different CysLT $_1$ receptor antagonists are presented. There was a significant correlation ($r^2 = 0.92$). The values are derived and modified from Shaw and Krell (1991).

(Pong et al., 1983; Metters et al., 1991) whereas the functional data (contraction) suggested a single receptor was associated with the contractions with LTC $_4$ as the full agonist (Norman et al., 1994; Gardiner et al., 1994). Third, the number of [3H]LTC $_4$ binding sites in most tissues was in excess of 10 pmol/mg of protein whereas the number of binding sites for [3H]LTD $_4$ was markedly lower. These observations suggested that [3H]LTC $_4$ may bind to other cellular entities, such as glutathione *S*-transferase (GST) as has been reported by several investigators (Sun et al., 1987; Metters et al., 1994; Bannenberg et al., 1999). In addition, LTC $_4$ may also bind to specific exporters in human eosinophils (Lam et al., 1992), to ATP-dependent carriers in rat liver (Ishikawa et al., 1990; Keppler et al., 1992), and to multidrug-resistant protein 1 (Qian et al., 2001). The GST data (Sun et al., 1987) supported the original observations of Mong et al. (1985) who demonstrated that the glutathione conjugates (SK&F 103,050 and SK&F 103,024), which were devoid of contractile activity in the guinea pig lung and trachea, exhibited a high affinity to the specific site labeled by [3H]LTC $_4$. Furthermore, these glutathione conjugates did not antagonize the LTC $_4$ -induced contractions in the guinea pig trachea. These observations suggest [3H]LTC $_4$ binding to GST may mask the LTC $_4$ receptors present in the preparations

and thereby explain the reason for the lower affinity for LTC $_4$ binding sites when compared with the functional data. However, the evidence reported by several groups in functional studies have shown that in guinea pig tracheal preparations the LTC $_4$ -induced contractions, in the presence of inhibitors of γ -glutamyl transpeptidase (Snyder and Krell, 1984; Charette and Jones, 1987), are resistant to the classical CysLT $_1$ antagonists suggesting a distinct receptor for LTC $_4$.

1. [3H]LTC $_4$ Binding Sites. Despite the [3H]LTC $_4$ binding to nonreceptor proteins, which is now well established, [3H]LTC $_4$ binding has been demonstrated in the central nervous system (Schalling et al., 1986), brain capillaries (Mayhan et al., 1986; Black et al., 1987), endothelial cell membranes (Muller et al., 1987), as well as human erythrocytes (Ghiglieri-Bertez et al., 1986) and leukocytes (Baud et al., 1987b). Unfortunately, the significance of this [3H]LTC $_4$ binding is presently unknown. Cheng et al. (1985) showed that membrane preparations from the guinea pig uterus did not bind [3H]LTD $_4$, data that were also confirmed in kinetic binding experiments (Mong et al., 1985). The [3H]LTC $_4$ binding K_d value (10 nM) in the membrane fractions in the uterus (Levinson, 1984) was similar to that reported for binding to intact cultured smooth muscle cells from the hamster vas deferens (Krilis et al., 1983) but slightly

lower than that reported for the guinea pig and rat lung membranes (Hogaboom et al., 1983; Pong et al., 1983). Similar concentrations of LTC₄ competed for [³H]LTC₄ binding in the guinea pig ileal muscle (Nicosia et al., 1984). Together these data are consistent with the notion that a specific high-affinity receptor was present in the uterus. Further studies have been performed using brain homogenates, which contain the highest activity of [³H]LTC₄ binding sites, and the potency order of the cys-LTs for inhibition of the brain [³H]LTC₄ binding is identical to their inhibitory order in the uterine homogenate. In addition, these studies have demonstrated that the relative ability of LTC₄, LTD₄, and LTE₄ to inhibit [³H]LTC₄ binding in the brain and uterine membrane fraction was the same as their ability to reduce binding in the smooth muscle cell and lung homogenates (Krilis et al., 1983, 1984; Pong et al., 1983; Cheng et al., 1985). In contrast, the IC₅₀ of LTC₄ to inhibit brain [³H]LTC₄ binding differs from that value obtained in the uterine homogenate. This suggests a difference between the dissociation constant (K_d) of brain and uterine [³H]LTC₄ binding sites. Data from initial studies are comparable with these results and have shown that the K_d of lung [³H]LTC₄ binding apparently differed from that demonstrated in the smooth muscle cell (Krilis et al., 1983; Pong et al., 1983). Whether this difference is due to the existence of high- and low-affinity LTC₄ receptor sites (Krilis et al., 1983) awaits further studies. Interestingly, Cheng and Townley (1984a) also demonstrated that LTC₄ was more potent than LTD₄ in the ability to produce uterine contractions. These results would suggest that in the guinea pig uterine preparations [³H]LTC₄ binds to a receptor that may be responsible for cys-LT-induced uterine contraction in this species.

2. [³H]LTD₄ Binding Sites. The initial data from radioligand binding in a variety of membrane preparations demonstrated the presence of [³H]LTD₄ binding sites. There was also a correlation between the tissue distribution and the binding sites for [³H]LTD₄ and [³H]LTE₄ (Cheng et al., 1985). In addition, the apparent affinity constants for agonists and antagonists (Mong et al., 1985) suggested that [³H]LTD₄ and [³H]LTE₄ bound to the same receptor in guinea pig lung preparations. Aharony et al. (1989) provided the pertinent evidence that this was indeed the case in the guinea pig lung. However, these investigators demonstrated that not only was the rank order inhibition potency similar when obtained against either ligand but also the absolute potency for LTC₄, LTD₄, and LTE₄ was higher against [³H]LTE₄ than that observed with [³H]LTD₄. These results suggested a discriminative binding of [³H]LTE₄ toward a subset of high-affinity LTD₄ receptors. Although ICI 198,615 exhibited equal potency for inhibiting both [³H]LTE₄ and [³H]LTD₄, there were subtle differences; namely, LTD₄ was significantly less potent than ICI 198,615 for inhibiting [³H]LTD₄ binding whereas no such difference was observed against

[³H]LTE₄. Furthermore, the rank order antagonist potency against [³H]LTD₄ was ICI 198,615 analog > ICI 198,615 > zafirlukast whereas against [³H]LTE₄ the antagonists were equipotent. These investigators also reported that there was a higher sensitivity to inhibition by sodium ions and GTP analogs for [³H]LTE₄ binding compared with [³H]LTD₄. These data provided evidence for the existence of heterogeneous LTD₄ receptors. This suggestion has received some support from Shirley and Cheng (1991) who demonstrated that either LTD₄ (K_i = 0.49 nM) or ICI 198,615 (K_i = 6.89 nM) interacted with a single homogeneous [³H]LTD₄ binding site, whereas the competitive binding results of either LTC₄ (in the presence of AT-125) or LTE₄ exhibited high- and low-affinity [³H]LTD₄ binding sites.

While ligand binding studies have indicated that tissues may contain different receptors (Rovati et al., 1985, 1992), the binding sites for the same radioligand in one tissue may also be different from that observed in another preparation. In guinea pig lung membranes, [³H]LTD₄ exhibits a K_d of ~0.43 nM whereas in the membranes obtained from the guinea pig myocardium the K_d is 3.4 nM. In the latter study, Hogaboom et al. (1985a,b) reported that the myocardial [³H]LTD₄ binding sites were neither modulated by guanine nucleotides nor divalent cations and the sulfhydryl-reducing reagents altered the maximum number of sites without any effect on binding affinity. Unfortunately the significance of these myocardium sites has not been explored.

In a study involving [³H]ICI 198,615, Aharony et al. (1988) reported that a CysLT receptor (which corresponds to the current CysLT₁ receptor) was coupled to a G-protein and exhibited high- and low-affinity states. In addition, Metters and Zamboni (1993) identified the CysLT₁ receptor as a single polypeptide with a molecular mass of 45 kDa using direct photoaffinity labeling. These investigators demonstrated that this protein was inhibited by both agonist and antagonists with potencies (IC₅₀) similar to those reported in radioligand binding studies. Furthermore, both cations and nucleotide analogs modulated the photolabeled protein. This latter characteristic was analogous to [³H]LTD₄ specific binding in the guinea pig lung membranes.

D. Evidence for Additional CysLT Receptor Subtypes

The reported functional data suggest that other CysLT receptors may exist. Initial reports have shown that the LTC₄ contractions of the guinea pig trachea are resistant to the classical CysLT₁ antagonists (Snyder and Krell, 1984). Furthermore, in the guinea pig lung preparation, MK-571 exhibited a low affinity for the cys-LTs (pK_B 5.8) and BAY u9773 did not block the cys-LT contractions (Gardiner et al., 1993; Sakata and Bäck, 2002), data which do not fit the classical CysLT₁ profile. One interpretation is that there may be a CysLT₁ receptor subtype. In addition, Tudhope et al. (1994) observed a residual contraction in the guinea pig

lung strip subsequent to CysLT₁/CysLT₂ antagonism by BAY u9773. Recently, Bäck et al. (2000a,b) have reported that the contractions to LTC₄ in porcine and human pulmonary arteries were not antagonized by either MK-571 or BAY u9773, data which further suggest the presence of another CysLT receptor responsible for the LTC₄-induced contractions. These latter observations have received support from the work of Northacker et al. (2000) who demonstrated that BAY u9773 was a selective CysLT₂ agonist. Although BAY u9773 contracted human pulmonary veins (Labat et al., 1992) in porcine and human pulmonary arterial preparations, this compound did not modify basal tone. These data suggest that the CysLT receptor associated with the contractions of human pulmonary arteries may be different from those receptors characterized as CysLT₁ and CysLT₂. These observations have been extended by Walch et al. (2002) who suggested a "novel CysLT receptor" based on the data in the human pulmonary artery (Table 7).

Panettieri et al. (1998) demonstrated an enhanced LTD₄ proliferation of human smooth muscle cells in the presence of epidermal growth factor. These investigators suggested that the CysLT₁ receptor associated with proliferation may be different from the CysLT₁ receptor activated in human airways responsible for contraction. This interpretation was based on the low affinity of the well known CysLT₁ antagonists, pranlukast and poblukast, which blocked the effect of LTD₄ in this assay. In addition, the authors also reported that zafirlukast did not work at a high concentration. Together these data do not suggest that based only on zafirlukast the receptor is different but that all of the antagonists were less active at concentrations that caused a significant shift of LTD₄ contractions in the human airways. However, the contractile response at CysLT₁ receptors in human airways was also induced by LTC₄ and LTE₄, the proliferative response should therefore also be induced by these ligands as well. Until such data are presented, the results are preliminary and not conclusive for the existence of another receptor subtype.

Using molecular techniques, Ogasawara et al. (2002) demonstrated different pharmacological characteristics of mCysLT₂ and hCysLT₂. These investigators reported that pranlukast, which inhibited hCysLT₁, antagonized mCysLT₂ responses as determined by Ca²⁺ mobilization and receptor-induced promoter activation. Furthermore, the distribution of mRNA expression of mCysLT₁ and mCysLT₂ in tissues between different strains of mice was also different. These results provide further evidence that other CysLT receptor subtypes may exist as has previously been suggested (Mellor et al., 2001). Indeed the up-regulation of the CysLT₁ receptor by different cytokines (Mellor et al., 2001; Thivierge et al., 2000, 2001) raises the further question as to whether or not the "induced receptor" is similar to the "constitutive receptor".

Recently, results generated from radioligand binding studies in human lung membrane preparations suggested that [³H]LTC₄ binding (Capra et al., 1998) may be associated with a specific LTC₄ receptor different from the classical CysLT₁ and CysLT₂ receptors (Ravasi et al., 2000, 2002). These latter investigators demonstrated that the LTC₄ contractions of human lung parenchyma were via a classical CysLT₁ receptor activation whereas the [³H]LTC₄ binding (high-affinity site) was insensitive to GTP. Furthermore, the CysLT₁ receptor antagonists, zafirlukast and iralukast, did not interact with this site.

Although, the above results suggest that other receptor subtypes may be present in different preparations, this hypothesis is based solely on indirect pharmacological assessment and should be interpreted cautiously. Extension of the current CysLT receptor classification requires additional investigation, namely, structural, operational, and molecular biological information.

E. Clinical Studies Involving CysLT Receptors

Most molecules that have been identified as antagonists of the CysLT₁ receptor in isolated guinea pig tissue antagonize the effects of LTC₄ and LTD₄ to an equal extent (Buckner et al., 1986, 1990) in human isolated bronchial tissues. These data suggest that in healthy human medium-sized airways CysLT₁ is the only leukotriene receptor responsible for the contractile response. These data have also received some support from Civelli et al. (1987) who demonstrated that the number of [³H]LTC₄ binding sites in membrane preparations derived from human segmental bronchi at different levels of the respiratory tract were similar even though there was large interindividual variation. Furthermore, these investigators reported that [³H]LTC₄ may actually label LTD₄ sites as well, since LTD₄ bound with higher affinity to the lower capacity class site. Although certainly not conclusive, these latter observations suggest that this uniform distribution of sites may reflect the functional receptors reported by other investigators (Buckner et al., 1986; Labat et al., 1992).

Inhalation of aerosols generated from solutions of cysteinyl leukotrienes results in airway obstruction manifested as decreased specific airway conductance (S_{Gaw}) or as decreased flow rates (measured from partial or full expiratory flow-volume curves) (Drazen, 1986); LTC₄ and LTD₄ are nearly equipotent contractile agonists in intact humans. Nebulizer concentrations on the order of 10 μ M are required to reduce the maximal expiratory flow rate (measured from a partial flow-volume curve, V_{30-P}) by 30% in nonasthmatic subjects. Approximately 30-fold greater concentrations of LTE₄ are required for an equivalent physiological effect in nonasthmatic subjects. Comparable nebulizer concentrations are required for reduction of a similar magnitude in the S_{Gaw} . Compared with a reference agonist in nonasthmatic subjects (with V_{30-P} or S_{Gaw} as the index of airway obstruction),

LTC₄ and LTD₄ are approximately 2000 times more potent than histamine, whereas LTE₄ is 30 to 100 times more potent than histamine as a bronchoconstrictor agonist (Weiss et al., 1982; Griffin et al., 1983; Barnes et al., 1984; Smith et al., 1985; Adelroth et al., 1986; Greenberger et al., 1986; Kern et al., 1986; Davidson et al., 1987). Complete dose-response curves show that the plateau response to LTD₄ is greater than that to methacholine (Bel et al., 1989). Since no such difference is evident with dose-response curves for 2- to 4-mm isolated bronchi, these findings are consistent with the hypothesis that airways from physically distinct loci (i.e., small versus large airways) respond to both stimuli (LTD₄ and methacholine) in normal human subjects.

Patients with asthma also exhibit bronchoconstrictor responses when they inhale aerosols generated from solutions of cys-LTs (Griffin et al., 1983; Adelroth et al., 1986; Davidson et al., 1987; Pichurko et al., 1989; Smith et al., 1993); the aerosol generator concentrations of LTC₄, LTD₄, and LTE₄ required to decrease the V_{30-P} by approximately 30% are about 30- to 100-fold less than is required in normal human subjects. The bronchoconstrictor responses of asthmatic subjects to these leukotrienes are all manifest within 3 to 5 min after aerosol inhalation; the duration of the effect is related to the severity of the bronchospasm but is on the order of 20 to 30 min when the decrement in the V_{30-P} is 30%.

Thus the predominant biological effect of administration of leukotrienes by inhalation is bronchoconstriction mediated by the CysLT₁ receptor. There may be other effects when leukotrienes are administered systemically but at present there are inadequate data to make a statement.

F. Summary

The development of selective antagonist for specific CysLT cell surface receptors was undertaken to harness the detrimental aspects of the inflammatory process specifically in asthma. This research has led to compounds with clinical benefits in asthmatic patients and the milestones in the sequence of events which have led to this therapy are presented in Table 8.

Therefore, based on the CysLT₁ antagonists, certain therapeutic goals have been achieved. However, the evidence to suggest that the metabolites of the 5-lipoxygenase enzymatic pathway activate other CysLT receptors, which may have further implications in pathophysiological conditions, needs to be addressed. Unfortunately, there are no selective antagonists for the CysLT₂ receptor. Recent molecular studies have shown that this receptor is expressed in several human tissues (heart, brain, peripheral blood leukocytes) but an exploration of their role in these tissues remains markedly compromised until selective pharmacological compounds become available.

Interestingly, there is some evidence from vascular studies that a CysLT receptor may be induced during

TABLE 8
Milestones in the development of CysLT₁ therapeutic antagonists

- 1938, identification of SRS (Feldberg and Kellaway, 1938)
- 1951, SRS-A generated by the human lung (Schild et al., 1951)
- 1973, SRS-A antagonist (Augstein et al., 1973)
- 1976–1980, structures and synthetic pathways for the leukotrienes (Corey et al., 1980; Samuelsson, 1983)
- 1980–1990, CysLT selective antagonists (Laboratories: Merck, Smith Kline & French, Abbott, Eli Lilly)
- 1985–1995, existence of two receptors in the human lung: a single CysLT₁ receptor in airways responsible for smooth muscle contraction (Buckner et al., 1986) and a CysLT₂ receptor in the human pulmonary vasculature associated with vasoconstriction (Labat et al., 1992)
- 1995, first CysLT₁ antagonist marketed (Pranlukast)
- 1999, hCysLT₁ receptor cloned expressed and characterized (Lynch et al., 1999; Sarau et al., 1999)
- 2000, hCysLT₂ receptor cloned expressed and characterized (Heise et al., 2000; Nothacker et al., 2000; Takasaki et al., 2000).

pathological conditions, such as atherosclerosis (Allen et al., 1993, 1998) since cys-LT-induced contractions were only observed in tissues derived from patients with this disease. In addition, the potency of the cys-LTs are markedly altered during aging (Duncan and Douglas, 1985). Whether these data reflect modification in receptor number, affinity, or second messenger pathways has never been pursued. Recently, Mellor et al. (2001) have reported that the CysLT₁ receptor may function as a UDP pyrimidinergic receptor. These latter results indicate that the eicosanoid GPCRs may exhibit a dual receptor role. UDP acting with the CysLT₁ receptor is analogous in some respects to the peptides that can act at the ALX. These observations provide future perspectives for studies in receptor classification.

G. Lipoxins Receptors

1. Functional and Radioligand Binding Studies. Although LXs are ancient molecular structures conserved and produced by fish as well as frog blood cells (Rowley et al., 1994), the potential physiologic roles of LX and related compounds have not been widely tested outside of the context of inflammation and the immune system. The principal actions reported for LX focus on leukocytes, in particular neutrophils, monocytes, and eosinophils in vitro and in vivo, suggesting that these cells are the major targets. The potency of action of LX on human leukocytes is in the nanomolar to subnanomolar range. Experiments with LXA₄ were initially performed on guinea pig lung strips to establish structure-activity relationships. These early reports indicated stereospecificity, that is, the 5*S*,6*R*-orientation of the two hydroxyl groups positioned immediately adjacent to the carboxylic end of the conjugated tetraene was essential for the contractile activity in the submicromolar range. These stereospecific requirements suggested the presence of specific LXA₄ recognition sites. Although this evidence was essential in establishing the biological activities evoked by LXA₄, the use of isolated lung strip

as a target tissue model for endogenous LX actions in view of recent findings may be misleading. These compounds, in particular LXA₄, act in the subnanomolar ranges to initiate protective actions. In this regard, LXA₄ possesses human leukocyte-selective actions that implicate lipoxins as endogenous stop signals or chalone (Serhan, 1994, 1997). In addition, LXA₄ stimulates rapid lipid remodeling and release of arachidonic acid in human PMN, which is sensitive to PTX treatment (Grandordy et al., 1990; Nigam et al., 1990). These results point to the involvement of a GPCR and indicate that LXA₄ stimulates selective functions of human leukocytes (PMN and monocytes) that are markedly different from the leukotrienes.

LXA₄, in addition to activating ALX to regulate leukocyte motility, may also interact at CysLT receptors (Badr et al., 1989; Fiore et al., 1992; McMahon et al., 2000; Gronert et al., 2001) and block LTD₄ binding to mesangial cells (Badr et al., 1989) and isolated HUVECs (Takano et al., 1997). HUVECs specifically bind [³H]LXA₄ with a *K_d* of 11 nM, which can be inhibited by LTD₄ and the CysLT₁ receptor antagonist, pobilukast (Fiore et al., 1993). This receptor is inducible in HUVECs and identical to the CysLT₁ (Gronert et al., 2001). Therefore, LXA₄ interacts with at least two classes of cell surface receptors: one specific for LXA₄ on leukocytes, the other shared by LTD₄ that is present on HUVEC and mesangial cells, namely, CysLT₁.

In human PMNs, subcellular fractionation showed that [³H]LXA₄ binding sites are associated with plasma membrane and endoplasmic reticulum (42.1%) and granule (34.5%) as well as nuclear-enriched fractions (23.3%), a distribution distinct from [³H]LTB₄ binding (Fiore et al., 1992). LXA₄ was also reported recently to bind to the Ah receptor, which is a ligand-activated transcription factor (Schaldach et al., 1999). The finding that LXA₄ blocks either PAF- or fMLP-stimulated eosinophil chemotaxis (Soyombo et al., 1994) suggests that functional ALX is also present on eosinophils.

ALX activation on human PMNs evokes inhibition of LTB₄- and fMLP-stimulated PMN adhesion (by down-regulating CD11/CD18), chemotaxis and transmigration as well as degranulation (Serhan, 1997). In human enterocytes (T84), LX analogs inhibit TNF α -induced IL-8 release (Gronert et al., 1998) as well as pathogen-induced IL-8 secretion at the mRNA level (Gewirtz et al., 1998). In addition, LXA₄ analogs inhibit rat leukocyte rolling and adherence by attenuating P-selectin expression in mesenteric microvasculature (Scalia et al., 1997). When applied to mouse ears, LX analogs markedly reduced LTB₄-induced PMN infiltration and vascular permeability (Takano et al., 1998). Moreover, LXA₄ inhibits PMN recruitment to inflamed glomeruli in vivo (Papayanni et al., 1995), further supporting the anti-inflammatory actions of LX to activate "stop signaling" via the ALX receptor.

To further investigate ALX receptors and their function in inflammation, analogs based on the native structures of LXA₄ and LXB₄ were designed to resist rapid inactivation (Serhan et al., 1995; Takano et al., 1998). Methyl groups were placed on carbon-15 and carbon-5 of LXA₄ and LXB₄ structures, respectively, to block dehydrogenation (Serhan et al., 1995; Clish et al., 2000). 15(*R,S*)-methyl-LXA₄ is a racemic stable analog of both LXA₄ and the aspirin-triggered 15-epi-LXA₄. Additional analogs of LXA₄ were synthesized with a phenoxy group bonded to carbon-16 and replacing the ω -end of the molecule. This design permits 16-phenoxy-LXA₄ to resist potential ω -oxidation and to be protected from dehydrogenation and potential ω -oxidation in vivo by the stearic hindrance of the bulky aromatic ring. The actions of LX analogs were tested in vitro and in vivo and proved to be potent LX mimetics (Clish et al., 1999, 2000). Furthermore, the topical application of LX and 15-epi-LX analogs in a mouse ear model of acute inflammation demonstrated that these analogs are potent inhibitors of LTB₄- and phorbol 12-myristate 13-acetate-initiated neutrophil-recruitment as well as PMN-mediated vascular injury (Takano et al., 1997, 1998). These LX mimetics proved to be more potent as topical agents than LTB₄ receptor antagonists within the range of the clinically used anti-inflammatory steroid dexamethasone (Takano et al., 1998). In addition, in a rat model of endothelial dysfunction characterized by reduced nitric oxide release and up-regulation of adhesion molecules such as P-selectin, superfusion with LX analogs dramatically inhibited leukocyte rolling (Scalia et al., 1997). Thus, these analogs of LX and 15-epi-LX serve as potent topically active agents that inhibit PMN recruitment and PMN-mediated changes in vascular permeability.

In addition to LXA₄ and aspirin-triggered LXA₄ analogs, ALX is activated by and competed with a series of rogue synthetic peptides that are also chemotactic (Chiang et al., 2000). CHO cells with G_{q/o} expressing ALX can chemotax in response to either the synthetic peptide MMK-1 or 15-epi-LXA₄ analog. CHO cell chemotaxis to 15-epi-LXA₄ was not only ligand-dependent but also G_{q/o}-dependent in that without G_{q/o} neither LXA₄ nor 15-epi-LXA₄ or their analogs could stimulate chemotaxis. Several synthetic nonphysiological peptides interact with ALX-related receptors such as FPRL2 (Christophe et al., 2001). The bioactive LX and 15-epi-LX analogs, as well as select small peptides, each competed for specific [³H]LXA₄ binding with recombinant human ALX, and the *N*-glycosylation of the receptor proved essential for peptide but not LXA₄ recognition. Several chimeric receptors (Chiang et al., 2000) were constructed from receptors with opposing functions, namely ALX and BLT₁. Results with these chimeric receptors also showed that the seven-transmembrane segment and adjacent regions of ALX are essential for LXA₄ recognition, and additional regions of ALX are required for high-affinity binding of the peptide ligands. These

initial findings indicated that a single seven-transmembrane receptor can switch recognition as well as function with certain chemotactic peptides from stimulatory to inhibitory with aspirin-triggered lipoxins (ATL) and LX (lipid ligands). This evidence also raises the possibility that ALX activation by LX or ATL can protect the host from potentially deleterious PMN-induced responses associated with innate immunity and direct effector responses to sites of tissue injury by recognition of mitochondrial-derived peptide protein fragments. Hence, activation of ALX by LXA₄, aspirin-triggered 15-epi-LXA₄ or their analogs can dampen inflammation by evoking a stop signal as well as blocking either LTC₄ or LTD₄ responses at CysLT₁ receptors (Gronert et al., 2001). The *in vivo* combination of these sites of LXA₄ action (ALX and CysLT₁) favor resolution of inflammation and protection from acute PMN-mediated tissue injury.

2. Summary. The ability to inhibit the expression of pro-inflammatory messengers, regulate trafficking, and sequestration of leukocytes as well as antagonize the CysLT₁ receptor, illustrate that LX and ATL by activating specific receptors have clearly established roles in a variety of vascular-related inflammatory phenomenon. Of considerable interest is the ability of the stable aspirin-triggered lipoxin analogs (ATLa) to antagonize the LTD₄ activation of a CysLT₁ receptor in the mouse ear model (Gronert et al., 2001). Previous investigators (Ortiz et al., 1995) have reported that LTD₄, via CysLT₁ receptor activation, released a contractile factor from endothelial cells in human pulmonary vessels. This factor masked the functional relaxations associated with nitric oxide. Whether or not the CysLT₁ receptor antagonized by ATLa in the murine vascular model is identical to that present on the endothelium of the human pulmonary vessels remains to be determined. However, lipoxin activation of an ALX receptor also inhibits LTD₄-induced mesangial cell proliferation (McMahon et al., 2000). These investigators demonstrated that two receptor subtypes were present in these cells, namely, an ALX receptor and an ALX/CysLT shared receptor. Recently, McMahon et al. (2002) have extended these observations to include further cross-talk between receptors, since leukotrienes and lipoxins also interact with PDGF receptor β in mesangial cell proliferation. These data provide evidence of the complex mechanisms involved in mediator-receptor activation associated with cellular proliferation.

V. General Conclusions

The classification of the receptors that are activated by leukotrienes and lipoxins was initially derived from data obtained in functional investigations. The pharmacological studies provided substantial evidence for the existence of specific and distinct receptors that were activated by these lipid mediators and are now well

supported by the recombinant systems. However, the molecular cellular mechanisms for the contractions of vascular smooth muscle by LTC₄ and LTD₄, which are resistant to the classical CysLT₁ antagonists has yet to be elucidated. Since the observations of Labat et al. (1992), no known selective antagonists has been reported. Without such compounds the identification of CysLT receptors is severely compromised and our nomenclature for the receptors associated with vascular inflammation will be difficult to postulate. In fact, the CysLT receptor on the human pulmonary endothelium is also resistant to the classical CysLT antagonists (Ortiz et al., 1995). Presently, there is no way of identifying the receptor associated with the relaxations induced by LTD₄ in human pulmonary vessels from the receptor responsible for the contractions induced by this same ligand.

In addition to these areas of research, future work in splice variants of the different receptors may lead to a better understanding of the cellular signal transduction pathways subsequent to activation by the ligand. Presently, little information is available based on this approach.

The use of transgenic animals have clearly demonstrated that BLT₁ plays a role in the recruitment of eosinophils to the inflamed site as well as a gender-associated effect related to mouse survival in an anaphylactic model. These results strongly suggest that BLT₁ receptors are intimately linked with acute inflammation *in vivo*. One of the most promising aspects of knockout mice is the uncovering of new receptors or the possible roles for receptor subtypes. The role of CysLT receptors in these knockout animals remains to be elucidated.

The recent evidence provided by Ogasawara et al. (2002) concerning the pharmacological differences between hCysLT₂ and mCysLT₂ together with CysLT receptor distribution in different mouse strains suggests that the choice of the animal model and the extrapolation to human inflammation warrants considerable reflection. Furthermore, in human airways activation of the CysLT₁ receptor is responsible for the bronchoconstriction both in normal and asthmatic subjects. However, there is no evidence for airway constriction in mice (Martin et al., 1988; Richter and Sirois, 2000), and the mCysLT₁ receptor has only been implicated in constriction of the microvascular smooth muscles leading to increased permeability and extravasation. Although the genomic organization of the mCysLT₁ has been reported by Maekawa et al. (2001), there is a need for a similar exploration of the human CysLT₁ receptor.

The relative potencies of LTC₄, LTD₄, and LTE₄ in a number of functional studies demonstrated that LTE₄ is generally less potent and markedly less efficient in activating the CysLT receptors. The receptor studies involving cells transfected with specific CysLT receptors have confirmed this observation. However, there are now very few investigations that are based on the use of

LTE₄. This is unfortunate, since there are several reports suggesting that this ligand may activate another receptor (Snyder and Krell, 1984; Mong et al., 1985; Sjolander et al., 1990; Sakata and Bäck, 2002) or may not activate the known classical CysLT receptors (Walch et al., 2002). Although these observations are only suggestive, care should be taken so as not to overlook these pertinent published data, which may offer interesting leads to our understanding the leukotriene receptors.

Young (1989) postulated that a hydrophobic pocket existed at the receptor for the tetraene chain (the unstable triene moiety found in LTC₄ or LTD₄). An earlier report (Snyder and Bernstein, 1987) had demonstrated that several leukotriene analogs, where the heptyl (C₇H₁₅) was positioned either para, meta, or ortho on the phenyl ring altered the pharmacological potency profile of the analog. Whether or not the use of stable but pharmacologically active analogs of the cys-LTs would provide clearer results for the presence of two separate CysLT receptors in functional studies based on agonist potency has received little attention. Such tools may be of some benefit in stereochemical investigations for the identification of receptorial proteins. Interestingly, the functional identification and classification of the ALX receptor has been systematically evaluated using the LX stable analogs.

The identification and classification of the CysLT receptors has been based essentially on the ability of antagonists to block or not to block a physiological response. Although this classification has proven to be reliable at the functional and more recently at the molecular levels, future studies need to evaluate not only several antagonists but also the different ligands, especially when the functional profiles of the antagonists do not appear to match what has previously been reported.

Based on investigations involving ALX and BLT receptors, there are several phenomenon that need to be highlighted. "Receptor induction" as seen in animal models of vascular inflammation (mouse ear model) (Gronert et al., 2001) has now been widely accepted. These data demonstrated that receptor message is markedly increased in tissues or cells where a pro-inflammatory agent has been used as a primer. In addition, other receptor subtypes may be induced, as in the case of the human mast cell, where IL-4 induced a receptor with a pharmacological profile different from the classical CysLT receptors (Mellor et al., 2001). These data warrant further attention since inducible receptors may not be identical to constitutive receptors (receptors that are present under normal conditions). In addition, the G-protein second messenger systems must equally be explored. Along similar lines, there is an intriguing observation that at least one receptor, namely, CysLT₁ may act as a pyrimidinergic (UDP) receptor (Mellor et al., 2001) suggesting that GPCRs may be quite versatile at the ligand-G-protein interface. Although the significance of these latter findings remains to be established,

both observations provide fruitful areas for future work. Furthermore, the observations that LTB₄ is associated with an excessive activation of leukocytes as well as the 5-LO pathway suggests a role for BLT receptor "tone" and indicates that receptor expression amplifies pro-inflammatory circuits in vivo.

In the last few years there has been some controversy as to whether or not the BLT receptor may act as a coreceptor for macrophage-trophic human immunodeficiency virus (HIV) of the type 1 strains. Owman et al. (1998) reported that this receptor mediated entry of primary HIV isolates into CD14 cells. However, results from transfected cells apparently raise some question as to such a role for the BLT₁ receptor (Martin et al., 1999). Whether or not BLT₁ antagonists can be used to prevent HIV infection warrants perhaps further pursuit.

Although there is considerable optimism about these recent developments in lipid mediator receptor classification, only by further work in this area, as well as in vascular inflammation models, will a potential for therapeutic exploitation be realized.

References

- Accomazzo MR, Rovati GE, Vigano T, Hernandez A, Bonazzi A, Bolla M, Fumagalli F, Viappiani S, Galbiati E, Ravasi S, et al. (2001) Leukotriene D₄-induced activation of smooth-muscle cells from human bronchi is partly Ca²⁺-independent. *Am J Respir Crit Care Med* **163**:266–272.
- Adelroth E, Morris MM, Hargreave FE, and O'Byrne PM (1986) Airway responsiveness to leukotrienes C₄ and D₄ and to methacholine in patients with asthma and normal controls. *N Engl J Med* **315**:480–484.
- Aharony D, Catanese CA, and Falcone RC (1989) Kinetic and pharmacologic analysis of [³H]leukotriene E₄ binding to receptors on guinea pig lung membranes: evidence for selective binding to a subset of leukotriene D₄ receptors. *J Pharmacol Exp Ther* **248**:581–588.
- Aharony D, Falcone RC, Yee YK, Hesp B, Giles RE, and Krell RD (1988) Biochemical and pharmacological characterization of the binding of the selective peptide-leukotriene antagonist, 3H-ICI 198, 615, to leukotriene D₄ receptors in the guinea-pig lung membranes. *Ann NY Acad Sci* **524**:162–180.
- Akbar GK, Dasari VR, Webb TE, Ayyanathan K, Pillarisetti K, Sandhu AK, Athwal RS, Daniel JL, Ashby B, Barnard EA, et al. (1996) Molecular cloning of a novel P₂ purinoceptor from human erythroleukemia cells. *J Biol Chem* **271**:18363–18367.
- Albazzaz MK, Patel KR, Shakir S, Dargie HJ, and Reid JM (1989) Effect of inhaled leukotriene C₄ on cardiopulmonary function. *Am Rev Respir Dis* **139**:188–193.
- Allen SP, Chester AH, Dashwood MR, Tadjkarimi S, Piper PJ, and Yacoub MH (1994) Preferential vasoconstriction to cysteinyl leukotrienes in the human saphenous vein compared with the internal mammary artery. Implications for graft performance. *Circulation* **90**:515–524.
- Allen SP, Chester AH, Piper PJ, Sampson AP, Akl ES, and Yacoub MH (1992) Effects of leukotrienes C₄ and D₄ on human isolated saphenous veins. *Br J Clin Pharmacol* **34**:409–414.
- Allen SP, Dashwood MR, Chester AH, Tadjkarimi S, Collins M, Piper PJ, and Yacoub MH (1993) Influence of atherosclerosis on the vascular reactivity of isolated human epicardial coronary arteries to leukotriene C₄. *Cardioscience* **4**:47–54.
- Allen SP, Dashwood MR, Morrison K, and Yacoub MH (1998) Differential leukotriene constrictor responses in human atherosclerotic coronary arteries. *Circulation* **97**:2406–2413.
- Augstein J, Farmer JB, Lee TB, Sheard P, and Tattersall ML (1973) Selective inhibitor of slow reacting substance of anaphylaxis. *Nat New Biol* **245**:215–217.
- Bach MK, Brashler JR, and Gorman RR (1977) On the structure of slow reacting substance of anaphylaxis: evidence of biosynthesis from arachidonic acid. *Prostaglandins* **14**:21–38.
- Bäck M, Norel X, Walch L, Gascard J, de Montpreville V, Dahlen S, and Brink C (2000b) Prostacyclin modulation of contractions of the human pulmonary artery by cysteinyl-leukotrienes. *Eur J Pharmacol* **401**:389–395.
- Bäck M, Norel X, Walch L, Gascard J, Mazmanian G, Dahlen S, and Brink C (2000a) Antagonist resistant contractions of the porcine pulmonary artery by cysteinyl-leukotrienes. *Eur J Pharmacol* **401**:381–388.
- Bäck M, Walch L, Norel X, Gascard JP, Mazmanian G, and Brink C (2002) Modulation of vascular tone and reactivity by nitric oxide in porcine pulmonary arteries and veins. *Acta Physiol Scand* **174**:9–15.
- Badr KF, DeBoer DK, Schwartzberg M, and Serhan CN (1989) Lipoxin A₄ antagonizes cellular and in vivo actions of leukotriene D₄ in rat glomerular mesangial cells: evidence for competition at a common receptor. *Proc Natl Acad Sci USA* **86**:3438–3442.
- Bandeira-Melo C, Serra MF, Diaz BL, Cordeiro RSB, Silva PMR, Lenzi HL, Bakhle YS, Serhan CN, and Martins MA (2000) Cyclooxygenase-2-derived prostaglandin E₂ and lipoxin A₄ accelerate resolution of allergic edema in *Angiostrongylus*

- costaricensis-infected rats: relationship with concurrent eosinophilia. *J Immunol* **164**:1029–1036.
- Bannenberg G, Dahlen SE, Luijckink M, Lundqvist G, and Morgenstern R (1999) Leukotriene C4 is a tight-binding inhibitor of microsomal glutathione transferase-1. Effects of leukotriene pathway modifiers. *J Biol Chem* **274**:1994–1999.
- Bao L, Gerard NP, Eddy RL Jr, Shows TB, and Gerard C (1992) Mapping of genes for the human C5a receptor (C5AR), human FMLP receptor (FPR) and two FMLP receptor homologue orphan receptors (FPRH1, FPRH2) to chromosome 19. *Genomics* **13**:437–440.
- Barnes NC, Piper PJ, and Costello JF (1984) Comparative effects of inhaled leukotriene C4, leukotriene D4 and histamine in normal human subjects. *Thorax* **39**:500–504.
- Baud L, Goetzel EJ, and Koo CH (1987a) Stimulation by leukotriene D4 of increases in the cytosolic concentration of calcium in dimethylsulfoxide-differentiated HL-60 cells. *J Clin Invest* **80**:983–991.
- Baud L, Koo CH, and Goetzel EJ (1987b) Specificity and cellular distribution of human polymorphonuclear leucocyte receptors for leukotriene C4. *Immunology* **62**:53–59.
- Beckman BS, Despinasse BP, and Spriggs L (1992) Actions of lipoxins A4 and B4 on signal transduction events in Friend erythroleukemia cells. *Proc Soc Exp Biol Med* **201**:169–173.
- Bel EH, van der Veen H, Dijkman JH, and Sterk PJ (1989) The effect of inhaled budesonide on the maximal degree of airway narrowing to leukotriene D4 and methacholine in normal subjects in vivo. *Am Rev Respir Dis* **139**:427–431.
- Berkowitz BA, Zabko-Potapovich B, Valocik R, and Gleason JG (1984) Effects of the leukotrienes on the vasculature and blood pressure of different species. *J Pharmacol Exp Ther* **229**:105–112.
- Bisgaard H, Kristensen J, and Sondergaard J (1982) The effect of leukotriene C4 and D4 on cutaneous blood flow in humans. *Prostaglandins* **23**:797–801.
- Bisgaard H, Lerche A, and Kristensen JK (1985) Leukotriene- and histamine-induced increases in vascular permeability and interstitial transport in the skin. *J Invest Dermatol* **84**:427–429.
- Bisgaard H, Olsson P, and Bende M (1986) Effect of leukotriene D4 on nasal mucosal blood flow, nasal airway resistance and nasal secretion in humans. *Clin Allergy* **16**:289–297.
- Bittl JA, Pfeffer MA, Lewis RA, Mehrotra MM, Corey EJ, and Austen KF (1985) Mechanism of the negative inotropic action of leukotrienes C4 and D4 on isolated rat heart. *Cardiovasc Res* **19**:426–432.
- Black KL, Betz AL, and Ar DB (1987) Leukotriene C4 receptors in isolated brain capillaries. *Adv Prostaglandin Thromboxane Leukotriene Res* **17A**:508–511.
- Bloomquist EI and Kream RM (1990) Release of substance P from guinea pig trachea leukotriene D4. *Exp Lung Res* **16**:645–659.
- Bochnowicz S and Underwood DC (1995) Dose-dependent mediation of leukotriene D4-induced airway microvascular leakage and bronchoconstriction in the guinea pig. *Prostaglandins Leukotrienes Essent Fatty Acids* **52**:403–411.
- Borgeat P, Hamberg M, and Samuelsson B (1976) Transformation of arachidonic acid and homo-gamma-linolenic acid by rabbit polymorphonuclear leukocytes. Monohydroxy acids from novel lipoxygenases. *J Biol Chem* **251**:7816–7820.
- Borgeat P and Samuelsson B (1979a) Arachidonic acid metabolism in polymorphonuclear leukocytes: unstable intermediate in formation of dihydroxy acids. *Proc Natl Acad Sci USA* **76**:3213–3217.
- Borgeat P and Samuelsson B (1979b) Metabolism of arachidonic acid in polymorphonuclear leukocytes. Structural analysis of novel hydroxylated compounds. *J Biol Chem* **254**:7865–7869.
- Borgeat P and Samuelsson B (1979c) Transformation of arachidonic acid by rabbit polymorphonuclear leukocytes. Formation of a novel dihydroxyicosatetraenoic acid. *J Biol Chem* **254**:2643–2646.
- Bouchelouche PN, Ahnfelt-Ronne I, and Thomsen MK (1990) LTD4 increases cytosolic free calcium and inositol phosphates in human neutrophils: inhibition by the novel LTD4 receptor antagonist, SR2640 and possible relation to modulation of chemotaxis. *Agents Actions* **29**:299–307.
- Bourdillat B, Hays-Legrand I, Labat C, Raffestin B, Norel X, Benveniste J, and Brink C (1987) Effects of various pharmacological agents on isolated human bronchial and pulmonary arterial and venous muscle preparations contracted by leukotriene D4. *Fundam Clin Pharmacol* **1**:433–444.
- Bray MA, Ford-Hutchinson AW, Shipley ME, and Smith MJ (1980) Calcium ionophore A23187 induces release of chemokinetic and aggregating factors from polymorphonuclear leucocytes. *Br J Pharmacol* **71**:507–512.
- Brezinski DA and Serhan CN (1991) Characterization of lipoxins by combined gas chromatography and electron-capture negative ion chemical ionization mass spectrometry: formation of lipoxin A4 by stimulated human whole blood. *Biol Mass Spectrom* **20**:45–52.
- Brocklehurst WE (1960) The release of histamine and formation of a slow reacting substance (SRS-A) during anaphylactic shock. *J Physiol (Lond)* **151**:416–435.
- Buckner CK, Fedyna JS, Robertson JL, Will JA, England DM, Krell RD, and Saban R (1990) An examination of the influence of the epithelium on contractile responses to peptidoleukotrienes and blockade by ICI 204, 219 in isolated guinea pig trachea and human intralobar airways. *J Pharmacol Exp Ther* **252**:77–85.
- Buckner CK, Krell RD, Laravuso RB, Coursin DB, Bernstein PR, and Will JA (1986) Pharmacological evidence that human intralobar airways do not contain different receptors that mediate contractions to leukotriene C4 and leukotriene D4. *J Pharmacol Exp Ther* **237**:558–562.
- Burke JA, Levi R, Guo ZG, and Corey EJ (1982) Leukotrienes C4, D4 and E4: effects on human and guinea-pig cardiac preparations in vitro. *J Pharmacol Exp Ther* **221**:235–241.
- Capra V, Bolla M, Belloni PA, Mezzetti M, Folco GC, Nicosia S, and Rovati GE (1998) Pharmacological characterization of the cysteinyl-leukotriene antagonists CGP 45715A (iralukast) and CGP 57698 in human airways in vitro. *Br J Pharmacol* **123**:590–598.
- Carstairs JR, Norman P, Abram TS, and Barnes PJ (1988) Autoradiographic localization of leukotriene C4 and D4 binding sites in guinea-pig lung. *Prostaglandins* **35**:503–513.
- Chan CC and Ford-Hutchinson A (1985) Effects of synthetic leukotrienes on local blood flow and vascular permeability in porcine skin. *J Invest Dermatol* **84**:154–157.
- Chapnick BM (1984) Divergent influences of leukotrienes C4, D4 and E4 on mesenteric and renal blood flow. *Am J Physiol* **246**:H518–H524.
- Charette L and Jones TR (1987) Effects of L-serine borate on antagonism of leukotriene C4-induced contractions of guinea-pig trachea. *Br J Pharmacol* **91**:179–188.
- Chau LY, Hoover RL, Austen KF, and Lewis RA (1986) Subcellular distribution of leukotriene C4 binding units in cultured bovine aortic endothelial cells. *J Immunol* **137**:1985–1992.
- Chegini N and Rao CV (1988a) The presence of leukotriene C4-binding sites in bovine corpora lutea of pregnancy. *Biol Reprod* **39**:929–935.
- Chegini N and Rao CV (1988b) Quantitative light microscopic autoradiographic study on [3H]leukotriene C4 binding to nonpregnant bovine uterine tissue. *Endocrinology* **122**:1732–1736.
- Cheng JB, Lang D, Bewtra A, and Townley RG (1985) Tissue distribution and functional correlation of [3H]leukotriene C4 and [3H]leukotriene D4 binding sites in guinea-pig uterus and lung preparations. *J Pharmacol Exp Ther* **232**:80–87.
- Cheng JB and Townley RG (1984a) Effect of the serine-borate complex on the relative ability of leukotriene C4, D4 and E4 to inhibit lung and brain [3H] leukotriene D4 and [3H] leukotriene C4 binding: demonstration of the agonists' potency order for leukotriene D4 and leukotriene C4 receptors. *Biochem Biophys Res Commun* **119**:612–617.
- Cheng JB and Townley RG (1984b) Evidence for a similar receptor site for binding of [3H] leukotriene E4 and [3H] leukotriene D4 to the guinea-pig crude lung membrane. *Biochem Biophys Res Commun* **122**:949–954.
- Chiang N, Fierro IM, Gronert K, and Serhan CN (2000) Activation of lipoxin A(4) receptors by aspirin-triggered lipoxins and select peptides evokes ligand-specific responses in inflammation. *J Exp Med* **191**:1197–1208.
- Chiang N, Gronert K, Clish CB, O'Brien JA, Freeman MW, and Serhan CN (1999) Leukotriene B4 receptor transgenic mice reveal novel protective roles for lipoxins and aspirin-triggered lipoxins in reperfusion. *J Clin Invest* **104**:309–316.
- Christie PE, Spur BW, and Lee TH (1992) The effects of lipoxin A4 on airway responses in asthmatic subjects. *Am Rev Respir Dis* **145**:1281–1284.
- Christophe T, Karlsson A, Dugave C, Rabiet MJ, Boulay F, and Dahlgren C (2001) The synthetic peptide Trp-Lys-Tyr-Met-Val-Met-NH2 specifically activates neutrophils through FPRL1/lipoxin A4 receptors and is an agonist for the orphan monocyte-expressed chemoattractant receptor FPRL2. *J Biol Chem* **276**:21585–21593.
- Civelli M, Oliva D, Mezzetti M, and Nicosia S (1987) Characteristics and distribution of specific binding sites for leukotriene C4 in human bronchi. *J Pharmacol Exp Ther* **242**:1019–1024.
- Clària J, Lee MH, and Serhan CN (1996) Aspirin-triggered lipoxins (15-epi-LX) are generated by the human lung adenocarcinoma cell line (A549)-neutrophil interactions and are potent inhibitors of cell proliferation. *Mol Med* **2**:583–596.
- Clark MA, Cook M, Mong S, and Crooke ST (1985) The binding of leukotriene C4 and leukotriene D4 to membranes of a smooth muscle cell line (BC3H1) and evidence that leukotriene induced contraction in these cells is mediated by thromboxane, protein and RNA syntheses. *Eur J Pharmacol* **116**:207–220.
- Clish CB, Levy BD, Chiang N, Tai HH, and Serhan CN (2000) Oxidoreductases in lipoxin A4 metabolic inactivation: a novel role for 15-onoprostaglandin 13-reductase/leukotriene B4 12-hydroxydehydrogenase in inflammation. *J Biol Chem* **275**:25372–25380.
- Clish CB, O'Brien JA, Gronert K, Stahl GL, Petasis NA, and Serhan CN (1999) Local and systemic delivery of a stable aspirin-triggered lipoxin prevents neutrophil recruitment in vivo. *Proc Natl Acad Sci USA* **96**:8247–8252.
- Coleman RA, Eglen RM, Jones RL, Narumiyi S, Shimizu T, Smith WL, Dahlen SE, Drazen JM, Gardiner PJ, Jackson WT, et al. (1995) Prostanoid and leukotriene receptors: a progress report from the IUPHAR working parties on classification and nomenclature. *Adv Prostaglandin Thromboxane Leukotriene Res* **23**:283–285.
- Coles SJ, Neill KH, Reid LM, Austen KF, Nii Y, Corey EJ, and Lewis RA (1983) Effects of leukotrienes C4 and D4 on glycoprotein and lysozyme secretion by human bronchial mucosa. *Prostaglandins* **25**:155–170.
- Corey EJ, Clark DA, Goto G, Marfat A, Mioskowski C, Samuelsson B, and Hammarström S (1980) Stereospecific total synthesis of a "slow reacting substance" of anaphylaxis, leukotriene C-1. *J Am Chem Soc* **102**:1436–1439.
- Crooke ST, Mattern M, Sarau HM, Winkler JD, Balcarek J, Wong A, and Bennett CF (1989) The signal transduction system of the leukotriene D4 receptor. *Trends Pharmacol Sci* **10**:103–107.
- Crooke ST, Sarau H, Saussy D, Winkler J, and Foley J (1990) Signal transduction processes for the LTD4 receptor. *Adv Prostaglandin Thromboxane Leukotriene Res* **20**:127–137.
- Dahlén SE, Björk J, Hedqvist P, Arfors KE, Hammarström S, Lindgren JA, and Samuelsson B (1981) Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: in vivo effects with relevance to the acute inflammatory response. *Proc Natl Acad Sci USA* **78**:3887–3891.
- Dahlén SE, Hedqvist P, Hammarström S, and Samuelsson B (1980) Leukotrienes are potent constrictors of human bronchi. *Nature (Lond)* **288**:484–486.
- Datta YH, Romano M, Jacobson BC, Golan DE, Serhan CN, and Ewenstein BM (1995) Peptido-leukotrienes are potent agonists of von Willebrand factor secretion and P-selectin surface expression in human umbilical vein endothelial cells. *Circulation* **92**:3304–3311.
- Davidson AB, Lee TH, Scanlon PD, Solway J, McFadden ER Jr, Ingram RH Jr, Corey EJ, Austen KF, and Drazen JM (1987) Bronchoconstrictor effects of leukotriene E4 in normal and asthmatic subjects. *Am Rev Respir Dis* **135**:333–337.
- DeLegge M, Murthy KS, Grider JR, and Makhlouf GM (1993) Characterization of distinct receptors for the peptidyl leukotrienes LTC4 and LTD4/LTE4 coupled to the same signaling pathway in isolated gastric muscle cells. *J Pharmacol Exp Ther* **266**:857–863.

- Devchand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ, and Wahli W (1996) The PPAR α -leukotriene B $_4$ pathway to inflammation control. *Nature (Lond)* **384**:39–43.
- Didsbury JR, UHING RJ, Tomhave E, Gerard C, Gerard N, and Snyderman R (1991) Receptor class desensitization of leukocyte chemoattractant receptors. *Proc Natl Acad Sci USA* **88**:11564–11568.
- Drazen JM (1986) Inhalation challenge with sulfidopeptide leukotrienes in human subjects. *Chest* **89**:414–419.
- Drazen JM, Austen KF, Lewis RA, Clark DA, Goto G, Marfat A, and Corey EJ (1980) Comparative airway and vascular activities of leukotrienes C-1 and D in vivo and in vitro. *Proc Natl Acad Sci USA* **77**:4354–4358.
- Duncan PG and Douglas JS (1985) Influences of gender and maturation on responses of guinea-pig airway tissues to LTD $_4$. *Eur J Pharmacol* **112**:423–427.
- Egan JW, Griswold DE, Hillegass LM, Newton JF, Eckardt RD, Slivjak MJ, and Smith EF 3rd (1989) Selective antagonism of peptidoleukotriene responses does not reduce myocardial damage or neutrophil accumulation following coronary artery occlusion with reperfusion. *Prostaglandins* **37**:597–613.
- Ellis JL and Udem BJ (1991) Role of peptidoleukotrienes in capsaicin-sensitive sensory fibre-mediated responses in guinea-pig airways. *J Physiol (Lond)* **436**:469–484.
- Evans TW, Rogers DF, Aursudkij B, Chung KF, and Barnes PJ (1989) Regional and time-dependent effects of inflammatory mediators on airway microvascular permeability in the guinea pig. *Clin Sci (Lond)* **76**:479–485.
- Ezra D, Boyd LM, Feuerstein G, and Goldstein RE (1983) Coronary constriction by leukotriene C $_4$, D $_4$ and E $_4$ in the intact pig heart. *Am J Cardiol* **51**:1451–1454.
- Falcone RC and Aharony D (1990) Modulation of ligand binding to leukotriene B $_4$ receptors on guinea pig lung membranes by sulfhydryl modifying reagents. *J Pharmacol Exp Ther* **255**:565–571.
- Falcone RC, Aharony D, and Orzechowski RF (1991a) Evidence for leukotriene D $_4$ receptors in guinea pig left atria. *J Pharmacol Exp Ther* **258**:199–206.
- Falcone RC, Moore WC, Aharony D, Scott AL, Siegl PK, and Orzechowski RF (1991b) Leukotriene D $_4$ increases both the force of contraction and polyphosphoinositide formation in guinea-pig papillary muscle. *Eur J Pharmacol* **204**:223–226.
- Falcone RC, Orzechowski RF, and Aharony D (1990) Specific binding of 3H-ICI 198, 615, a potent LTD $_4$ antagonist, to guinea pig cardiac ventricular membranes. *Prostaglandins* **39**:241–258.
- Falgueyret J and Riendeau D (2000) LTA(4)-derived 5-oxo-eicosatetraenoic acid: pH-dependent formation and interaction with the LTB $_4$ receptor of human polymorphonuclear leukocytes. *Biochim Biophys Acta* **1484**:51–58.
- Feinmark SJ and Cannon PJ (1986) Endothelial cell leukotriene C $_4$ synthesis results from intercellular transfer of leukotriene A $_4$ synthesized by polymorphonuclear leukocytes. *J Biol Chem* **261**:16466–16472.
- Feldberg W, Holden HF, and Kellaway CH (1938) The formation of lysicithin and a muscle-stimulating substance by snake venoms. *J Physiol (Lond)* **94**:232–248.
- Feldberg W and Kellaway CH (1938) Liberation of histamine and formation of lysicithin-like substances by cobra venom. *J Physiol (Lond)* **94**:187–226.
- Feuerstein G, Zukowska-Grojec Z, and Kopin IJ (1981) Cardiovascular effects of leukotriene D $_4$ in SHR and WKY rats. *Eur J Pharmacol* **76**:107–110.
- Fiedler VB and Abram TS (1987) Effects of intracoronary leukotriene D $_4$ infusion on the coronary circulation, hemodynamics and prostanoid release in porcine experiments. *Cardiology* **74**:89–99.
- Fiedler VB, Mardin M, and Abram TS (1984) Leukotriene D $_4$ -induced vasoconstriction of coronary arteries in anaesthetized dogs. *Eur Heart J* **5**:253–260.
- Fiedler VB, Mardin M, and Abram TS (1987) Comparison of cardiac and hemodynamic effects of platelet-activating factor-acether and leukotriene D $_4$ in anesthetized dogs. *Basic Res Cardiol* **82**:197–208.
- Fiedler VB, Mardin M, Gardiner PJ, and Abram TS (1985) Leukotrienes on porcine hemodynamics and prostanoid release. *Int J Cardiol* **8**:451–463.
- Fierro IM and Serhan CN (2001) A novel inhibitor of angiogenesis: aspirin-triggered 15R lipoxin A $_4$. *FASEB J* **15**:A949.
- Figuerola DJ, Breyer RM, Defoe SK, Kargman S, Daugherty BL, Waldburger K, Liu Q, Clements M, Zeng Z, O'Neill GP, et al. (2001) Expression of the cysteinyl leukotriene 1 receptor in normal human lung and peripheral blood leukocytes. *Am J Respir Crit Care Med* **163**:226–233.
- Filep J, Rigger B, and Frolich JC (1985) Vascular and renal effects of leukotriene C $_4$ in conscious rats. *Am J Physiol* **249**:F739–F744.
- Fiore S, Maddox JF, Perez HD, and Serhan CN (1994) Identification of a human cDNA encoding a functional high affinity lipoxin A $_4$ receptor. *J Exp Med* **180**:253–260.
- Fiore S, Romano M, Reardon EM, and Serhan CN (1993) Induction of functional lipoxin A $_4$ receptors in HL-60 cells. *Blood* **81**:3395–3403.
- Fiore S, Ryeom SW, Weller PF, and Serhan CN (1992) Lipoxin recognition sites. Specific binding of labeled lipoxin A $_4$ with human neutrophils. *J Biol Chem* **267**:16168–16176.
- Fiore S and Serhan CN (1995) Lipoxin A $_4$ receptor activation is distinct from that of the formyl peptide receptor in myeloid cells: inhibition of CD11/18 expression by lipoxin A $_4$ -lipoxin A $_4$ receptor interaction. *Biochemistry* **34**:16678–16686.
- Fleisch JH, Rinkema LE, and Baker SR (1982) Evidence for multiple leukotriene D $_4$ receptors in smooth muscle. *Life Sci* **31**:577–581.
- Ford-Hutchinson AW, Bray MA, Doig MV, Shipley ME, and Smith MJ (1980) Leukotriene B $_4$, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature (Lond)* **286**:264–265.
- Foster A and Chan CC (1991) Peptide leukotriene involvement in pulmonary eosinophil migration upon antigen challenge in the actively sensitized guinea pig. *Int Arch Allergy Appl Immunol* **96**:279–284.
- Frey EA, Nicholson DW, and Metters KM (1993) Characterization of the leukotriene D $_4$ receptor in dimethylsulphoxide-differentiated U937 cells: comparison with the leukotriene D $_4$ receptor in human lung and guinea-pig lung. *Eur J Pharmacol* **244**:239–250.
- Garcia JG, Noonan TC, Jubiz W, and Malik AB (1987) Leukotrienes and the pulmonary microcirculation. *Am Rev Respir Dis* **136**:161–169.
- Gardiner PJ, Abram TS, and Cuthbert NJ (1990) Evidence for two leukotriene receptor types in the guinea-pig isolated ileum. *Eur J Pharmacol* **182**:291–299.
- Gardiner PJ, Abram TS, Tudhope SR, Cuthbert NJ, Norman P, and Brink C (1994) Leukotriene receptors and their selective antagonists. *Adv Prostaglandin Thromboxane Leukotriene Res* **22**:49–61.
- Gardiner PJ, Norman P, Cuthbert NJ, Tudhope SR, and Abram TS (1993) Characterisation of the peptido-leukotriene receptor PL2 on the ferret spleen strip. *Eur J Pharmacol* **238**:19–26.
- Gaudreau R, Le Guill C, Metaoui S, Lemire S, Stankova J, and Rola-Pleszczynski M (1998) Signalling through the leukotriene B $_4$ receptor involves both α 1 and α 16, but not α 14 or α 11 G-protein subunits. *Biochem J* **335**:15–18.
- Gaudreau R, Le Guill C, Venne MH, Stankova J, and Rola-Pleszczynski M (2002) Threonine 308 within a putative casein kinase 2 site of the cytoplasmic tail of leukotriene B(4) receptor (BLT1) is crucial for ligand-induced, G-protein-coupled receptor-specific kinase 6-mediated desensitization. *J Biol Chem* **277**:31567–31576.
- Gewirtz AT, McCormick B, Neish AS, Petasis NA, Gronert K, Serhan CN, and Madara JL (1998) Pathogen-induced chemokine secretion from model intestinal epithelium is inhibited by lipoxin A $_4$ analogs. *J Clin Invest* **101**:1860–1869.
- Ghiglieri-Bertez C, Cristol JP, and Bonne C (1986) High-affinity binding site for leukotriene C $_4$ in human erythrocytes. *Biochim Biophys Acta* **879**:97–102.
- Gieske TH, Sabol JS, and Raddatz R (1990) MDL 28, 753, an agonist of LTD $_4$ but an antagonist of LTC $_4$ in longitudinal muscle of guinea pig ileum. *J Pharmacol Exp Ther* **254**:192–197.
- Gleason JG, Ku TW, McCarthy ME, Weichman BM, Holden D, Osborn RR, Zabko-Potapovich B, Berkowitz B, and Wasserman MA (1983) 2-nor-leukotriene analogs: antagonists of the airway and vascular smooth muscle effects of leukotriene C $_4$, D $_4$ and E $_4$. *Biochem Biophys Res Commun* **117**:732–739.
- Godson C and Brady HR (2000) Lipoxins: novel anti-inflammatory therapeutics? *Curr Opin Investig Drugs* **1**:380–385.
- Goetzl EJ and Pickett WC (1980) The human PMN leukocyte chemotactic activity of complex hydroxy-eicosatetraenoic acids (HETEs). *J Immunol* **125**:1789–1791.
- Goffinet AM (1986) Leukotriene C $_4$ binding sites in the mouse forebrain: autoradiographic demonstration. *C R Acad Sci III* **302**:633–636.
- Goldman DW, Gifford LA, Olson DM, and Goetzl EJ (1985) Transduction by leukotriene B $_4$ receptors of increases in cytosolic calcium in human polymorphonuclear leukocytes. *J Immunol* **135**:525–530.
- Goldman DW and Goetzl EJ (1982) Specific binding of leukotriene B $_4$ to receptors on human polymorphonuclear leukocytes. *J Immunol* **129**:1600–1604.
- Gorenne I, Labat C, Gascard JP, Norel X, Nashashibi N, and Brink C (1998) Leukotriene D $_4$ contractions in human airways are blocked by SK&F 96365, an inhibitor of receptor-mediated calcium entry. *J Pharmacol Exp Ther* **284**:549–552.
- Gorenne I, Norel X, and Brink C (1996) Cysteinyl leukotriene receptors in the human lung: what's new? *Trends Pharmacol Sci* **17**:342–345.
- Gorman RR, Ruppel PL, and Lin AH (1985) Evidence for leukotriene B $_4$ receptors in human neutrophils. *Adv Prostaglandin Thromboxane Leukotriene Res* **15**:661–665.
- Grandordy BM, Lacroix H, Mavoungou E, Krilis S, Crea AE, Spur BW, and Lee TH (1990) Lipoxin A $_4$ inhibits phosphoinositide hydrolysis in human neutrophils. *Biochem Biophys Res Commun* **167**:1022–1029.
- Greenberger PA, Smith LJ, Patterson R, Krell RD, Roberts M, Harris KE, and Shaughnessy MA (1986) Comparison of cutaneous and bronchial reactivity to leukotriene D $_4$ in humans. *J Lab Clin Med* **108**:70–75.
- Griffin M, Weiss JW, Leitch AG, McFadden ER Jr, Corey EJ, Austen KF, and Drazen JM (1983) Effects of leukotriene D on the airways in asthma. *N Engl J Med* **308**:436–439.
- Gronert K, Gewirtz A, Madara JL, and Serhan CN (1998) Identification of a human erythrocyte lipoxin A $_4$ receptor that is regulated by interleukin (IL)-13 and interferon gamma and inhibits tumor necrosis factor alpha-induced IL-8 release. *J Exp Med* **187**:1285–1294.
- Gronert K, Martinsson-Niskanen T, Ravasi S, Chiang N, and Serhan CN (2001) Selectivity of recombinant human leukotriene D(4), leukotriene B(4) and lipoxin A(4) receptors with aspirin-triggered 15-epi-LXA(4) and regulation of vascular and inflammatory responses. *Am J Pathol* **158**:3–9.
- Gronroos E, Andersson T, Schippert A, Zheng L, and Sjolander A (1996) Leukotriene D $_4$ -induced mobilization of intracellular Ca $^{2+}$ in epithelial cells is critically dependent on activation of the small GTP-binding protein Rho. *Biochem J* **316**:239–245.
- Gronroos E, Schippert A, Engstrom M, and Sjolander A (1995) The regulation of leukotriene D $_4$ -induced calcium influx in human epithelial cells involves protein tyrosine phosphorylation. *Cell Calcium* **17**:177–186.
- Gronroos E, Thodeti CK, and Sjolander A (1998) Leukotriene D $_4$ induces a rapid increase in cAMP in the human epithelial cell line, Int 407: a potential role for this signal in the regulation of calcium influx through the plasma membrane. *Cell Calcium* **24**:9–16.
- Hachicha M, Pouliot M, Petasis NA, and Serhan CN (1999) Lipoxin (LX) $_4$ and aspirin-triggered 15-epi-LXA $_4$ inhibit tumor necrosis factor 1 α -initiated neutrophil responses and trafficking: regulators of a cytokine-chemokine axis. *J Exp Med* **189**:1923–1929.
- Hall RA, Premont RT, and Lefkowitz RJ (1999) Heptahelical receptor signaling: beyond the G protein paradigm. *J Cell Biol* **145**:927–932.
- Hand JM and Schwalm SF (1987) Pharmacological comparison of L-serine borate and glutathione as inhibitors of metabolism of LTC $_4$ to LTD $_4$ by the isolated guinea pig trachea. *Prostaglandins* **33**:709–716.
- Hanna CJ, Bach MK, Pare PD, and Schellenberg RR (1981) Slow-reacting substances (leukotrienes) contract human airway and pulmonary vascular smooth muscle in vitro. *Nature (Lond)* **290**:343–344.
- Hansson G, Lindgren JA, Dahlen SE, Hedqvist P, and Samuelsson B (1981) Identification and biological activity of novel omega-oxidized metabolites of leukotriene B $_4$ from human leukocytes. *FEBS Lett* **130**:107–112.
- Haribabu B, Verghese MW, Steeber DA, Sellers DD, Bock CB, and Snyderman R

- (2000) Targeted disruption of the leukotriene B₄ receptor in mice reveals its role in inflammation and platelet-activating factor-induced anaphylaxis. *J Exp Med* **192**:433–438.
- Hay DW, Muccitelli RM, Tucker SS, Vickery-Clark LM, Wilson KA, Gleason JG, Hall RF, Wasserman MA, and Torphy TJ (1987) Pharmacologic profile of SK&F 104353: a novel, potent and selective peptidoleukotriene receptor antagonist in guinea pig and human airways. *J Pharmacol Exp Ther* **243**:474–481.
- Heise CE, O'Dowd BF, Figueroa DJ, Sawyer N, Nguyen T, Im DS, Stocco R, Bellefeuille JN, Abramovitz M, Cheng R, et al. (2000) Characterization of the human cysteinyl leukotriene 2 receptor. *J Biol Chem* **275**:30531–30536.
- Henderson WR, Jorg A, and Klebanoff SJ (1982) Eosinophil peroxidase-mediated inactivation of leukotrienes B₄, C₄ and D₄. *J Immunol* **128**:2609–2613.
- Hirai H, Tanaka K, Yoshie O, Ogawa K, Kenmotsu K, Takamori Y, Ichimasa M, Sugamura K, Nakamura M, Takano S, et al. (2001) Prostaglandin D₂ selectively induces chemotaxis in T helper type 2 cells, eosinophils and basophils via seven-transmembrane receptor CRTH2. *J Exp Med* **193**:255–261.
- Hoffstein ST, Malo PE, Bugelski P, and Wheelodon EB (1990) Leukotriene D₄ (LTD₄) induces mucus secretion from goblet cells in the guinea pig respiratory epithelium. *Exp Lung Res* **16**:711–725.
- Hogaboom GK, Mong S, Stadel JM, and Crooke ST (1985a) Characterization of [3H]leukotriene D₄ binding sites in guinea-pig ventricular myocardium. *J Pharmacol Exp Ther* **233**:686–693.
- Hogaboom GK, Mong S, Stadel JM, and Crooke ST (1985b) Characterization of guinea pig myocardial leukotriene C₄ binding sites. Regulation by cations and sulphydryl-directed reagents. *Mol Pharmacol* **27**:236–245.
- Hogaboom GK, Mong S, Wu HL, and Crooke ST (1983) Peptidoleukotrienes: distinct receptors for leukotriene C₄ and D₄ in the guinea-pig lung. *Biochem Biophys Res Commun* **116**:1136–1143.
- Hoshino M, Izumi T, and Shimizu T (1998) Leukotriene D₄ activates mitogen-activated protein kinase through a protein kinase Cα-Raf-1-dependent pathway in human monocytic leukemia THP-1 cells. *J Biol Chem* **273**:4878–4882.
- Hua XY, Dahlen SE, Lundberg JM, Hammarstrom S, and Hedqvist P (1985) Leukotrienes C₄, D₄ and E₄ cause widespread and extensive plasma extravasation in the guinea pig. *Naunyn-Schmiedeberg's Arch Pharmacol* **330**:136–141.
- Huang WW, Garcia-Zepeda EA, Sauty A, Oettgen HC, Rothenberg ME, and Luster AD (1998) Molecular and biological characterization of the murine leukotriene B₄ receptor expressed on eosinophils. *J Exp Med* **188**:1063–1074.
- Hui Y, Yang G, Galczinski H, Figueroa DJ, Austin CP, Copeland NG, Gilbert DJ, Jenkins NA, and Funk CD (2001) The murine cysteinyl leukotriene 2 (CysLT₂) receptor. cDNA and genomic cloning, alternative splicing and in vitro characterization. *J Biol Chem* **276**:47489–47495.
- Igarashi T, Yokomizo T, Tsutsumi O, Taketani Y, Shimizu T, and Izumi T (1999) Characterization of the leukotriene B₄ receptor in porcine leukocytes. Separation and reconstitution with heterotrimeric GTP-binding proteins. *Eur J Biochem* **259**:419–425.
- Ishikawa T, Muller M, Klunemann C, Schaub T, and Keppler D (1990) ATP-dependent primary active transport of cysteinyl leukotrienes across liver canalicular membrane. Role of the ATP-dependent transport system for glutathione S-conjugates. *J Biol Chem* **265**:19279–19286.
- Iwai M and Jungermann K (1988) Leukotrienes increase glucose and lactate output and decrease flow in perfused rat liver. *Biochem Biophys Res Commun* **151**:283–290.
- Iwai M and Jungermann K (1989) Mechanism of action of cysteinyl leukotrienes on glucose and lactate balance and on flow in perfused rat liver. Comparison with the effects of sympathetic nerve stimulation and noradrenaline. *Eur J Biochem* **180**:273–281.
- Jakschik BA, Falkenhein S, and Parker CW (1977) Precursor role of arachidonic acid in release of slow reacting substance from rat basophilic leukemia cells. *Proc Natl Acad Sci USA* **74**:4577–4581.
- Jones T, Denis D, Hall R, and Ethier D (1983) Pharmacological study of the effects of leukotrienes C₄, D₄, E₄ and F₄ on guinea pig trachealis: interaction with FPL-55712. *Prostaglandins* **26**:833–843.
- Jones TR, Davis C, and Daniel EE (1982) Pharmacological study of the contractile activity of leukotriene C₄ and D₄ on isolated human airway smooth muscle. *Can J Physiol Pharmacol* **60**:638–643.
- Jones TR, Denis D, and Comptois P (1984) Study of mechanisms mediating contraction to leukotriene C₄, D₄ and other bronchoconstrictors on guinea pig trachea. *Prostaglandins* **27**:939–959.
- Jones TR, Labelle M, Belley M, Champion E, Charette L, Evans J, Ford-Hutchinson AW, Gauthier JY, Lord A, Masson P, et al. (1995) Pharmacology of montelukast sodium (Singulair), a potent and selective leukotriene D₄ receptor antagonist. *Can J Physiol Pharmacol* **73**:191–201.
- Jones TR and Masson P (1985) Comparative study of the pulmonary effects of intravenous leukotrienes and other bronchoconstrictors in anaesthetized guinea pigs. *Prostaglandins* **29**:799–817.
- Jones TR, Zamboni R, Belley M, Champion E, Charette L, Ford-Hutchinson AW, Frenette R, Gauthier JY, Leger S, Masson P, et al. (1989) Pharmacology of L-660, 711 (MK-571): a novel potent and selective leukotriene D₄ receptor antagonist. *Can J Physiol Pharmacol* **67**:17–28.
- Joris I, Majno G, Corey EJ, and Lewis RA (1987) The mechanism of vascular leakage induced by leukotriene E₄. Endothelial contraction. *Am J Pathol* **126**:19–24.
- Kadowitz PJ and Hyman AL (1984) Analysis of responses to leukotriene D₄ in the pulmonary vascular bed. *Circ Res* **55**:707–717.
- Kamohara M, Takasaki J, Matsumoto M, Matsumoto S, Saito T, Soga T, Matsushime H, and Furuichi K (2001) Functional characterization of cysteinyl leukotriene CysLT₂ receptor on human coronary artery smooth muscle cells. *Biochem Biophys Res Commun* **287**:1088–1092.
- Kamohara M, Takasaki J, Matsumoto M, Saito T, Ohishi T, Ishii H, and Furuichi K (2000) Molecular cloning and characterization of another leukotriene B₄ receptor. *J Biol Chem* **275**:27000–27004.
- Kang Y, Taddeo B, Varai G, Varga J, and Fiore S (2000) Mutations of serine 235–237 and tyrosine 302 residues in the human lipoxin A₄ receptor intracellular domains result in sustained signaling. *Biochemistry* **39**:13551–13557.
- Kato K, Yokomizo T, Izumi T, and Shimizu T (2000) Cell-specific transcriptional regulation of human leukotriene B₄ receptor gene. *J Exp Med* **192**:413–420.
- Kellaway CH and Trethewie CH (1940) The liberation of a slow reacting smooth-muscle stimulating substance in anaphylaxis. *Q J Exp Physiol* **30**:121–145.
- Keppler D, Muller M, Klunemann C, Guhlmann A, Krauss K, Muller J, Berger U, Leier I, and Mayatepek E (1992) Transport and in vivo elimination of cysteinyl leukotrienes. *Adv Enzyme Regul* **32**:107–116.
- Kern R, Smith LJ, Patterson R, Krell RD, and Bernstein PR (1986) Characterization of the airway response to inhaled leukotriene D₄ in normal subjects. *Am Rev Respir Dis* **133**:1127–1132.
- Kim KC, Nassiri J, and Brody JS (1989) Mechanisms of airway goblet cell mucin release: studies with cultured tracheal surface epithelial cells. *Am J Respir Cell Mol Biol* **1**:137–143.
- Kim KC, Zheng QX, and Van-Seuning I (1993) Involvement of a signal transduction mechanism in ATP-induced mucin release from cultured airway goblet cells. *Am J Respir Cell Mol Biol* **8**:121–125.
- Kimura K, Noguchi E, Shibasaki M, Arinami T, Yokouchi Y, Takeda K, Yamakawa-Kobayashi K, Matsui A, and Hamaguchi H (1999) Linkage and association of atopic asthma to markers on chromosome 13 in the Japanese population. *Hum Mol Genet* **8**:1487–1490.
- Kito G, Okuda H, Ohkawa S, Terao S, and Kikuchi K (1981) Contractile activities of leukotrienes C₄ and D₄ on vascular strips from rabbits. *Life Sci* **29**:1325–1332.
- Kopia GA, Valocik RE, Torphy TJ, Cieslinski LB, Sarau HM, Foley JJ, and Wasserman MA (1987) Inhibition of leukotriene D₄-induced coronary vasoconstriction by leukotriene antagonists in the anesthetized dog. *J Pharmacol Exp Ther* **241**:174–180.
- Krell RD, Aharony D, Buckner CK, Keith RA, Kusner EJ, Snyder DW, Bernstein PR, Matassa VG, Yee YK, Brown FJ, et al. (1990) The preclinical pharmacology of ICI 204, 219. A peptide leukotriene antagonist. *Am Rev Respir Dis* **141**:978–987.
- Krell RD, Giles RE, Yee YK, and Snyder DW (1987) In vivo pharmacology of ICI 198, 615: a novel, potent and selective peptide leukotriene antagonist. *J Pharmacol Exp Ther* **243**:557–564.
- Krell RD, Osborn R, Vickery L, Falcone K, O'Donnell M, Gleason J, Kinzig C, and Bryan D (1981) Contraction of isolated airway smooth muscle by synthetic leukotrienes C₄ and D₄. *Prostaglandins* **22**:387–409.
- Krell RD, Tsai BS, Berdoulay A, Barone M, and Giles RE (1983) Heterogeneity of leukotriene receptors in guinea-pig trachea. *Prostaglandins* **25**:171–178.
- Kriliis S, Lewis RA, Corey EJ, and Austen KF (1983) Specific receptors for leukotriene C₄ on a smooth muscle cell line. *J Clin Invest* **72**:1516–1519.
- Kriliis S, Lewis RA, Corey EJ, and Austen KF (1984) Specific binding of leukotriene C₄ to ileal segments and subcellular fractions of ileal smooth muscle cells. *Proc Natl Acad Sci USA* **81**:4529–4533.
- Kuehl FA Jr, DeHaven RN, and Pong SS (1984) Lung tissue receptors for sulfidopeptide leukotrienes. *J Allergy Clin Immunol* **74**:378–381.
- Kuhns DB, Young HA, Gallin EK, and Gallin JI (1998) Ca²⁺-dependent production and release of IL-8 in human neutrophils. *J Immunol* **161**:4332–4339.
- Labat C, Bara J, Gascard JP, Sosse-Alaoui H, Thomas de Montpreville V, Yeadon M, and Brink C (1999) M1/MUC5AC mucin released by human airways in vitro. *Eur Respir J* **14**:390–395.
- Labat C, Ortiz JL, Norel X, Gorenne I, Verley J, Abram TS, Cuthbert NJ, Tudhope SR, Norman P, Gardiner P, et al. (1992) A second cysteinyl leukotriene receptor in human lung. *J Pharmacol Exp Ther* **263**:800–805.
- Laitinen LA, Laitinen A, Haahela T, Vilka V, Spur BW, and Lee TH (1993) Leukotriene E₄ and granulocytic infiltration into asthmatic airways. *Lancet* **341**:989–990.
- Lam BK, Xu K, Atkins MB, and Austen KF (1992) Leukotriene C₄ uses a probenecid-sensitive export carrier that does not recognize leukotriene B₄. *Proc Natl Acad Sci USA* **89**:11598–11602.
- Lee CW, Lewis RA, Tauber AI, Mehrotra M, Corey EJ, and Austen KF (1983) The myeloperoxidase-dependent metabolism of leukotrienes C₄, D₄ and E₄ to 6-trans-leukotriene B₄ diastereoisomers and the subclass-specific S-diastereoisomeric sulfoxides. *J Biol Chem* **258**:15004–15010.
- Lee TH, Horton CE, Kyan-Aung U, Haskard D, Crea AE, and Spur BW (1989) Lipoxin A₄ and lipoxin B₄ inhibit chemotactic responses of human neutrophils stimulated by leukotriene B₄ and N-formyl-L-methionyl-L-leucyl-L-phenylalanine. *Clin Sci (Lond)* **77**:195–203.
- Leffler CW, Mitchell JA, and Green RS (1984) Cardiovascular effects of leukotrienes in neonatal piglets. Role in hypoxic pulmonary vasoconstriction? *Circ Res* **55**:780–787.
- Letts LG, Newman DL, Greenwald SE, and Piper PJ (1983) Effects of intra-coronary administration of leukotriene D₄ in the anesthetized dog. *Prostaglandins* **26**:563–572.
- Letts LG and Piper PJ (1983) Cardiac actions of leukotrienes B₄, C₄, D₄ and E₄ in guinea pig and rat in vitro. *Adv Prostaglandin Thromboxane Leukotriene Res* **11**:391–395.
- Levi R and Burke JA (1980) Cardiac anaphylaxis: SRS-A potentiates and extends the effects of released histamine. *Eur J Pharmacol* **62**:41–49.
- Levi R, Burke JA, Holland BA, and Green PJ (1980) Immediate hypersensitivity reactions of the heart: reduction of tachycardia and arrhythmias by the SRS-A antagonist FPL 55712. *Immunopharmacology* **2**:173–176.
- Levinson SL (1984) Peptidoleukotriene binding in guinea pig uterine membrane preparations. *Prostaglandins* **28**:229–240.
- Levy BD, Fokin VV, Clark JM, Wakelam MJ, Petasis NA, and Serhan CN (1999) Polyisoprenyl phosphate (PIPP) signaling regulates phospholipase D activity: a 'stop' signaling switch for aspirin-triggered lipoxin A₄. *FASEB J* **13**:903–911.
- Levy BD, Petasis NA, and Serhan CN (1997) Polyisoprenyl phosphates in intracellular signalling. *Nature (Lond)* **389**:985–990.
- Lewis RA, Austen KF, Drazen JM, Clark DA, Marfat A, and Corey EJ (1980) Slow

- reacting substances of anaphylaxis: identification of leukotrienes C-1 and D from human and rat sources. *Proc Natl Acad Sci USA* **77**:3710–3714.
- Lundgren JD, Kaliner M, Logun C, and Shelhamer JH (1990) Platelet activating factor and tracheobronchial respiratory glycoconjugate release in feline and human explants: involvement of the lipoxygenase pathway. *Agents Actions* **30**:329–337.
- Luscinskas FW, Nicolaou KC, Webber SE, Veale CA, Gimbrone MA Jr, and Serhan CN (1990) Ca²⁺ mobilization with leukotriene A₄ and epoxytetraenes in human neutrophils. *Biochem Pharmacol* **39**:355–365.
- Lynch KR, O'Neill GP, Liu Q, Im DS, Sawyer N, Metters KM, Coulombe N, Abramovitz M, Figueroa DJ, Zeng Z, et al. (1999) Characterization of the human cysteinyl leukotriene CysLT₁ receptor. *Nature (Lond)* **399**:789–793.
- MacIouf JA and Murphy RC (1988) Transcellular metabolism of neutrophil-derived leukotriene A₄ by human platelets. A potential cellular source of leukotriene C₄. *J Biol Chem* **263**:174–181.
- Maddox JF, Colgan SP, Clish CB, Petasis NA, Fokin VV, and Serhan CN (1998) Lipoxin B₄ regulates human monocyte/neutrophil adherence and motility: design of stable lipoxin B₄ analogs with increased biologic activity. *FASEB J* **12**:487–494.
- Maddox JF, Hachicha M, Takano T, Petasis NA, Fokin VV, and Serhan CN (1997) Lipoxin A₄ stable analogs are potent mimetics that stimulate human monocytes and THP-1 cells via a G-protein-linked lipoxin A₄ receptor. *J Biol Chem* **272**:6972–6978.
- Maddox JF and Serhan CN (1996) Lipoxin A₄ and B₄ are potent stimuli for human monocyte migration and adhesion: selective inactivation by dehydrogenation and reduction. *J Exp Med* **183**:137–146.
- Maekawa A, Kanaoka Y, Lam BK, and Austen KF (2001) Identification in mice of two isoforms of the cysteinyl leukotriene 1 receptor that result from alternative splicing. *Proc Natl Acad Sci USA* **98**:2256–2261.
- Maghni K, de Brum-Fernandes AJ, Földes-Filep E, Gaudry M, Borgeat P, and Sirois P (1991) Leukotriene B₄ receptors on guinea pig alveolar eosinophils. *J Pharmacol Exp Ther* **258**:784–789.
- Malik AB, Noonan TC, Selig WM, and Garcia JG (1985) Effects of exogenous leukotrienes on the pulmonary circulation. *Prog Clin Biol Res* **199**:221–235.
- Marom Z, Shelhamer JH, Bach MK, Morton DR, and Kaliner M (1982) Slow-reacting substances, leukotrienes C₄ and D₄, increase the release of mucus from human airways in vitro. *Am Rev Respir Dis* **126**:449–451.
- Marone G, Giordano A, Cirillo R, Triggiani M, and Vigorito C (1988) Cardiovascular and metabolic effects of peptide leukotrienes in man. *Ann NY Acad Sci* **524**:321–333.
- Martin TR, Gerard NP, Galli SJ, and Drazen JM (1988) Pulmonary responses to bronchoconstrictor agonists in the mouse. *J Appl Physiol* **64**:2318–2323.
- Martin V, Ronde P, Unett D, Wong A, Hoffman TL, Edinger AL, Doms RW, and Funk CD (1999) Leukotriene binding, signaling and analysis of HIV coreceptor function in mouse and human leukotriene B₄ receptor-transfected cells. *J Biol Chem* **274**:8597–8603.
- Martin V, Sawyer N, Stocco R, Unett D, Lerner MR, Abramovitz M, and Funk CD (2001) Molecular cloning and functional characterization of murine cysteinyl-leukotriene 1 (CysLT₁) receptors. *Biochem Pharmacol* **62**:1193–1200.
- Massoumi R and Sjölander A (2001) Leukotriene D₄ affects localisation of vinculin in intestinal epithelial cells via distinct tyrosine kinase and protein kinase C controlled events. *J Cell Sci* **114**:1925–1934.
- Masuo M, Reardon S, Ikebe M, and Kitazawa T (1994) A novel mechanism for the Ca²⁺-sensitizing effect of protein kinase C on vascular smooth muscle: inhibition of myosin light chain phosphatase. *J Gen Physiol* **104**:265–286.
- Mattern MR, Nambi P, Bartus JO, Mirabelli CK, Crooke ST, and Johnson RK (1991) Regulation of topoisomerase I and II activities by cyclic nucleotide- and phospholipid-dependent protein kinases. Effects of interactions between the two transduction pathways. *Receptor* **1**:181–190.
- Mayhan WG, Sahagun G, Spector R, and Heistad DD (1986) Effects of leukotriene C₄ on the cerebral microvasculature. *Am J Physiol* **251**:H471–H474.
- McLeod JD and Piper PJ (1991) Effect of ICI 198, 615, SK-F 104, 353, MK-571 and CGP45715A on cysteinyl leukotriene-induced responses in guinea-pig heart. *Prostaglandins* **41**:395–406.
- McMahon B, Mitchell D, Shattock R, Martin F, Brady HR, and Godson C (2002) Lipoxin, leukotriene and PDGF receptors cross-talk to regulate mesangial cell proliferation. *FASEB J* **16**:1817–1819.
- McMahon B, Stenson C, McPhillips F, Fanning A, Brady HR, and Godson C (2000) Lipoxin A₄ antagonizes the mitogenic effects of leukotriene D₄ in human renal mesangial cells. Differential activation of MAP kinases through distinct receptors. *J Biol Chem* **275**:27566–27575.
- Mellor EA, Maekawa A, Austen KF, and Boyce JA (2001) Cysteinyl leukotriene receptor 1 is also a pyrimidinergic receptor and is expressed by human mast cells. *Proc Natl Acad Sci USA* **98**:7964–7969.
- Metters KM, Frey EA, and Ford-Hutchinson AW (1991) Characterization of the leukotriene D₄ receptor in hyperreactive rat lung. *Eur J Pharmacol* **194**:51–61.
- Metters KM, Gareau Y, Lord A, Rochette C, and Sawyer N (1994) Characterization of specific binding sites for cysteinyl leukotrienes in sheep lung. *J Pharmacol Exp Ther* **270**:399–406.
- Metters KM and Zamboni RJ (1993) Photoaffinity labeling of the leukotriene D₄ receptor in guinea pig lung. *J Biol Chem* **268**:6487–6495.
- Michelassi F, Landa L, Hill RD, Lowenstein E, Watkins WD, Petka AJ, and Zapol WM (1982) Leukotrien D₄: a potent coronary vasoconstrictor associated with impaired ventricular contraction. *Science (Wash DC)* **217**:841–843.
- Michelassi F, Shahinian HK, and Ferguson MK (1987) Effects of leukotrienes B₄, C₄ and D₄ on rat mesenteric microcirculation. *J Surg Res* **42**:475–482.
- Miki I, Watanabe T, Nakamura M, Seyama Y, Ui M, Sato F, and Shimizu T (1990) Solubilization and characterization of leukotriene B₄ receptor-GTP binding protein complex from porcine spleen. *Biochem Biophys Res Commun* **166**:342–348.
- Mita H, Hasegawa M, Saito H, and Akiyama K (2001) Levels of cysteinyl leukotriene receptor mRNA in human peripheral leukocytes: significantly higher expression of cysteinyl leukotriene receptor 2 mRNA in eosinophils. *Clin Exp Allergy* **31**:1660–1664.
- Møllerup J, Jørgensen ST, Hougaard C, and Hoffmann EK (2001) Identification of a murine cysteinyl leukotriene receptor by expression in *Xenopus laevis* oocytes. *Biochim Biophys Acta* **1517**:455–459.
- Mong S, Hoffman K, Wu HL, and Crooke ST (1987) Leukotriene-induced hydrolysis of inositol lipids in guinea pig lung: mechanism of signal transduction for leukotriene-D₄ receptors. *Mol Pharmacol* **31**:35–41.
- Mong S, Wu HL, Clark MA, Stadel JM, Gleason JG, and Crooke ST (1984a) Identification of leukotriene D₄ specific binding sites in the membrane preparation isolated from guinea pig lung. *Prostaglandins* **28**:805–822.
- Mong S, Wu HL, Hogaboom GK, Clark MA, Stadel JM, and Crooke ST (1984b) Regulation of ligand binding to leukotriene D₄ receptors: effects of cations and guanine nucleotides. *Eur J Pharmacol* **106**:241–253.
- Mong S, Wu HL, Scott MO, Lewis MA, Clark MA, Weichman BM, Kinzig CM, Gleason JG, and Crooke ST (1985) Molecular heterogeneity of leukotriene receptors: correlation of smooth muscle contraction and radioligand binding in guinea-pig lung. *J Pharmacol Exp Ther* **234**:316–325.
- Mong S, Wu HL, Wong A, Sarau HM, and Crooke ST (1988) Leukotriene D₄ receptor-mediated phosphoinositol hydrolysis and calcium mobilization in rat basophilic leukemic cells. *J Pharmacol Exp Ther* **247**:803–813.
- Morris HR, Taylor GW, Piper PJ, and Toppins JR (1980) Structure of slow-reacting substance of anaphylaxis from guinea-pig lung. *Nature (Lond)* **285**:104–106.
- Muccitelli RM, Tucker SS, Hay DW, Torphy TJ, and Wasserman MA (1987) Is the guinea pig trachea a good in vitro model of human large and central airways? Comparison on leukotriene-, methacholine-, histamine- and antigen-induced contractions. *J Pharmacol Exp Ther* **243**:467–473.
- Muller A, Ghiglieri-Bertez C, Modat G, and Bonne C (1987) Specific binding of leukotriene C₄ to endothelial cell membranes. *Prostaglandins Leukotrienes Med* **26**:233–240.
- Murphy PM, Özbek T, Kenney RT, Tiffany HL, McDermott D, and Francke U (1992) A structural homologue of the N-formyl peptide receptor. Characterization and chromosome mapping of a peptide chemoattractant receptor family. *J Biol Chem* **267**:7637–7643.
- Murphy RC, Hammarstrom S, and Samuelsson B (1979) Leukotriene C: a slow-reacting substance from murine mastocytoma cells. *Proc Natl Acad Sci USA* **76**:4275–4279.
- Nakagawa N, Obata T, Kobayashi T, Okada Y, Nambu F, Terawaki T, and Aishita H (1992) In vivo pharmacologic profile of ONO-1078: a potent, selective and orally active peptide leukotriene (LT) antagonist. *Jpn J Pharmacol* **60**:217–225.
- Nicosia S, Crowley HJ, Oliva D, and Welton AF (1984) Binding sites for 3H-LTC₄ in membranes from guinea pig ileal longitudinal muscle. *Prostaglandins* **27**:483–494.
- Nigam S, Fiore S, Luscinskas FW, and Serhan CN (1990) Lipoxin A₄ and lipoxin B₄ stimulate the release but not the oxygenation of arachidonic acid in human neutrophils: dissociation between lipid remodeling and adhesion. *J Cell Physiol* **143**:512–523.
- Nishiye E, Itoh T, and Kuriyama H (1988) Some effects of leukotriene D₄ on the mechanical properties of the guinea-pig basilar artery. *Br J Pharmacol* **93**:591–600.
- Nomura H, Nielsen BW, and Matsushima K (1993) Molecular cloning of cDNAs encoding a LD78 receptor and putative leukocyte chemotactic peptide receptors. *Int Immunol* **5**:1239–1249.
- Norman P, Abram TS, Cuthbert NJ, and Gardiner PJ (1990) The inhibition of [3H]leukotriene D₄ binding to guinea-pig lung membranes. The correlation of binding affinity with activity on the guinea-pig ileum. *Eur J Pharmacol* **182**:301–312.
- Norman P, Abram TS, Cuthbert NJ, Tudhope SR, and Gardiner PJ (1994) Characterisation of leukotriene receptors on rat lung strip. *Eur J Pharmacol* **271**:73–78.
- Norman P, Abram TS, Kluender HC, Gardiner PJ, and Cuthbert NJ (1987) The binding of [3H]leukotriene C₄ to guinea-pig lung membranes. The lack of correlation of LTC₄ functional activity with binding affinity. *Eur J Pharmacol* **143**:323–334.
- Nothacker HP, Wang Z, Zhu Y, Reinscheid RK, Lin SH, and Civelli O (2000) Molecular cloning and characterization of a second human cysteinyl leukotriene receptor: discovery of a subtype selective agonist. *Mol Pharmacol* **58**:1601–1608.
- Obata T, Kobayashi T, Okada Y, Nakagawa N, Terawaki T, and Aishita H (1992) Effect of a peptide leukotriene antagonist, ONO-1078 on antigen-induced airway microvascular leakage in actively sensitized guinea pigs. *Life Sci* **51**:1577–1583.
- Ogasawara H, Ishii S, Yokomizo T, Kakinuma T, Komine M, Tamaki K, Shimizu T, and Izumi T (2002) Characterization of mouse cysteinyl leukotriene receptors mCysLT₁ and mCysLT₂: differential pharmacological properties and tissue distribution. *J Biol Chem* **277**:18763–18768.
- Ohtaka H, Tsang JY, Foster A, Hogg JC, and Schellenberg RR (1987) Comparative effects of leukotrienes on porcine pulmonary circulation in vitro and in vivo. *J Appl Physiol* **63**:582–588.
- Oliva D, Accomazzo MR, Giovanazzi S, and Nicosia S (1994) Correlation between leukotriene D₄-induced contraction and cytosolic calcium elevation: a quantitative and simultaneous evaluation in smooth muscle. *J Pharmacol Exp Ther* **268**:159–166.
- Orange RP, Murphy RC, Karnovsky ML, and Austen KF (1973) The physicochemical characteristics and purification of slow-reacting substance of anaphylaxis. *J Immunol* **110**:760–770.
- Ortiz JL, Gorenne I, Cortijo J, Seller A, Labat C, Sarria B, Abram TS, Gardiner PJ, Morcillo E, and Brink C (1995) Leukotriene receptors on human pulmonary vascular endothelium. *Br J Pharmacol* **115**:1382–1386.
- Owman C, Garzino-Demo A, Cocchi F, Popovic M, Sabirsh A, and Gallo RC (1998) The leukotriene B₄ receptor functions as a novel type of coreceptor mediating entry of primary HIV-1 isolates into CD4-positive cells. *Proc Natl Acad Sci USA* **95**:9530–9534.
- Panettieri RA, Tan EM, Ciocca V, Luttmann MA, Leonard TB, and Hay DW (1998) Effects of LTD₄ on human airway smooth muscle cell proliferation, matrix expression and contraction in vitro: differential sensitivity to cysteinyl leukotriene receptor antagonists. *Am J Respir Cell Mol Biol* **19**:453–461.

- Panzenbeck MJ and Kaley G (1983) Leukotriene D4 reduces coronary blood flow in the anesthetized dog. *Prostaglandins* **25**:661–670.
- Papayianni A, Serhan CN, Phillips ML, Rennke HG, and Brady HR (1995) Transcellular biosynthesis of lipoxin A4 during adhesion of platelets and neutrophils in experimental immune complex glomerulonephritis. *Kidney Int* **47**:1295–1302.
- Parker CW, Huber MM, Hoffman MK, and Falkenhein SF (1979) Characterization of the two major species of slow reacting substance from rat basophilic leukemia cells as glutathionyl thioethers of eicosatetraenoic acids oxygenated at the 5 position. Evidence that peroxy groups are present and important for spasmogenic activity. *Prostaglandins* **18**:673–686.
- Paruchuri S, Hallberg B, Juhas M, Larsson C, and Sjolander A (2002) Leukotriene D(4) activates MAPK through a Ras-independent but PKCepsilon-dependent pathway in intestinal epithelial cells. *J Cell Sci* **115**:1883–1893.
- Pawloski JR and Chapnick BM (1993a) Antagonism of LTD4-evoked relaxation in canine renal artery and vein. *Am J Physiol* **265**:H980–H985.
- Pawloski JR and Chapnick BM (1993b) Leukotrienes C4 and D4 are potent endothelium-dependent relaxing agents in canine splanchnic venous capacitance vessels. *Circ Res* **73**:395–404.
- Pedersen KE, Bochner BS, and Undem BJ (1997) Cysteinyl leukotrienes induce P-selectin expression in human endothelial cells via a non-CysLT1 receptor-mediated mechanism. *J Pharmacol Exp Ther* **281**:655–662.
- Perez HD, Holmes R, Kelly E, McClary J, and Andrews WH (1992) Cloning of a cDNA encoding a receptor related to the formyl peptide receptor of human neutrophils. *Gene* **118**:303–304.
- Perretti M, Chiang N, La M, Fierro IM, Marullo S, Getting SJ, Solito E, and Serhan CN (2002) Endogenous lipid- and peptide-derived anti-inflammatory pathways generated with glucocorticoid and aspirin treatment activate the lipoxin A(4) receptor. *Nat Med* **8**:1296–1302.
- Petric R and Ford-Hutchinson A (1995) Inhibition of leukotriene biosynthesis improves renal function in experimental glomerulonephritis. *J Lipid Mediat Cell Signal* **11**:231–240.
- Pfeffer MA, Pfeffer JM, Lewis RA, Braunwald E, Corey EJ, and Austen KF (1983) Systemic hemodynamic effects of leukotrienes C4 and D4 in the rat. *Am J Physiol* **244**:H628–H633.
- Pichurko BM, Ingram RH Jr, Sperling RI, Lafleur JE, Corey EJ, Austen KF, and Drazen JM (1989) Localization of the site of the bronchoconstrictor effects of leukotriene C4 compared with that of histamine in asthmatic subjects. *Am Rev Respir Dis* **140**:334–339.
- Piper PJ, Stanton AW, and McLeod LJ (1985) The actions of leukotrienes C4 and D4 in the porcine renal vascular bed. *Prostaglandins* **29**:61–73.
- Pong SS, DeHaven RN, Kuehl FA Jr, and Egan RW (1983) Leukotriene C4 binding to rat lung membranes. *J Biol Chem* **258**:9616–9619.
- Popov GK, Nekrasov AS, Khshivo AL, Pochinskii AG, and Lankin VZ (1989) [Effects of lipoxin B on colony-forming capacity of human peripheral blood mononuclear cells in diffusion chambers]. *Biull Eksp Biol Med* **107**:80–83.
- Powell WS, Rokach J, Khanapure SP, Manna S, Hashefi M, Gravel S, Macleod RJ, Falck JR, and Bhatt RK (1996) Effects of metabolites of leukotriene B4 on human neutrophil migration and cytosolic calcium levels. *J Pharmacol Exp Ther* **276**:728–736.
- Powell WS, Wainwright S, and Gravelle F (1989) Metabolism of leukotriene B4 and related substances by polymorphonuclear leukocytes. *Adv Prostaglandin Thromboxane Leukotriene Res* **19**:112–115.
- Qian YM, Qiu W, Gao M, Westlake CJ, Cole SP, and Deeley RG (2001) Characterization of binding of leukotriene C4 by human multidrug resistance protein 1: evidence of differential interactions with NH2- and COOH-proximal halves of the protein. *J Biol Chem* **276**:38636–38644.
- Qiu F-H, Devchand PR, Wada K, and Serhan CN (2001) Aspirin-triggered lipoxin A₄ and lipoxin A₄ up-regulate transcriptional corepressor NAB1 in human neutrophils. *FASEB J* **15**:2736–2738.
- Raud J, Palmertz U, Dahlen SE, and Hedqvist P (1991) Lipoxins inhibit microvascular inflammatory actions of leukotriene B4. *Adv Exp Med Biol* **314**:185–192.
- Ravasi S, Capra V, Mezzetti M, Nicosia S, and Rovati GE (2000) A kinetic binding study to evaluate the pharmacological profile of a specific leukotriene C(4) binding site not coupled to contraction in human lung parenchyma. *Mol Pharmacol* **57**:1182–1189.
- Ravasi S, Capra V, Panigalli T, Rovati GE, and Nicosia S (2002) Pharmacological differences among CysLT(1) receptor antagonists with respect to LTC(4) and LTD(4) in human lung parenchyma. *Biochem Pharmacol* **63**:1537–1546.
- Resnati M, Pallavicini I, Wang JM, Oppenheim J, Serhan CN, Romano M, and Blasi F (2002) The fibrinolytic receptor for urokinase activates the G protein-coupled chemotactic receptor FPRL1/LXA4R. *Proc Natl Acad Sci USA* **99**:1359–1364.
- Richter M and Sirois P (2000) Effects of eicosanoids, neuromediators and bioactive peptides on murine airways. *Eur J Pharmacol* **389**:225–234.
- Rinkema LE, Roman CR, VanAlstyne EL, Spaethe SM, and Fleisch JH (1993) Contraction of guinea pig inferior vena cava by eicosanoids. *Naunyn-Schmiedeberg's Arch Pharmacol* **348**:520–525.
- Rokach J, Girard Y, Guindon Y, Atkinson JG, Larue M, Young RN, Masson P, and Holme G (1980) The synthesis of a leukotriene with SRS-like activity. *Tetrahedron Lett* **21**:1485–1488.
- Rola-Pleszczynski M, Thivierge M, Gagnon N, Lacasse C, and Stankova J (1993) Differential regulation of cytokine and cytokine receptor genes by PAF, LTB4 and PGE2. *J Lipid Mediators* **6**:175–181.
- Romano M, Maddox JF, and Serhan CN (1996) Activation of human monocytes and the acute monocytic leukemia cell line (THP-1) by lipoxins involves unique signaling pathways for lipoxin A4 versus lipoxin B4: evidence for differential Ca2+ mobilization. *J Immunol* **157**:2149–2154.
- Roth DM, Lefer DJ, Hock CE, and Lefer AM (1985) Effects of peptide leukotrienes on cardiac dynamics in rat, cat and guinea pig hearts. *Am J Physiol* **249**:H477–H484.
- Rovati GE, Giovanazzi S, Mezzetti M, and Nicosia S (1992) Heterogeneity of binding sites for ICI 198, 615 in human lung parenchyma. *Biochem Pharmacol* **44**:1411–1415.
- Rovati GE, Oliva D, Sautebin L, Folco GC, Welton AF, and Nicosia S (1985) Identification of specific binding sites for leukotriene C4 in membranes from human lung. *Biochem Pharmacol* **34**:2831–2837.
- Rowley AF, Lloyd-Evans P, Barrow SE, and Serhan CN (1994) Lipoxin biosynthesis by trout macrophages involves the formation of epoxide intermediates. *Biochemistry* **33**:856–863.
- Sakata K and Bäck M (2002) Receptor preferences of cysteinyl-leukotrienes in the guinea pig lung parenchyma. *Eur J Pharmacol* **436**:119–126.
- Sakuma I, Gross SS, and Levi R (1987) Peptidoleukotrienes induce an endothelium-dependent relaxation of guinea pig main pulmonary artery and thoracic aorta. *Prostaglandins* **34**:685–696.
- Sakuma I and Levi R (1988) Vasomotor effects of leukotrienes C4 and D4 on cavian pulmonary artery and aorta. Characterization and mechanisms. *Ann NY Acad Sci* **524**:91–102.
- Sala A, Rossini G, Buccellati C, Berti F, Folco G, and Maclof J (1993) Formation of sulphidepeptide-leukotrienes by cell-cell interaction causes coronary vasoconstriction in isolated, cell-perfused heart of rabbit. *Br J Pharmacol* **110**:1206–1212.
- Salmon M, Walsh DA, Huang TJ, Barnes PJ, Leonard TB, Hay DW, and Chung KF (1999) Involvement of cysteinyl leukotrienes in airway smooth muscle cell DNA synthesis after repeated allergen exposure in sensitized Brown Norway rats. *Br J Pharmacol* **127**:1151–1158.
- Samuelsson B (1983) Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science (Wash DC)* **220**:568–575.
- Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, and Serhan CN (1987) Leukotrienes and lipoxins: structures, biosynthesis and biological effects. *Science (Wash DC)* **237**:1171–1176.
- Sarau HM, Ames RS, Chambers J, Ellis C, Elshourbagy N, Foley JJ, Schmidt DB, Muccitelli RM, Jenkins O, Murdock PR, et al. (1999) Identification, molecular cloning, expression and characterization of a cysteinyl leukotriene receptor. *Mol Pharmacol* **56**:657–663.
- Saussy DL Jr, Sarau HM, Foley JJ, Mong S, and Crooke ST (1989) Mechanisms of leukotriene E4 partial agonist activity at leukotriene D4 receptors in differentiated U-937 cells. *J Biol Chem* **264**:19845–19855.
- Scalia R, Gefen J, Petasis NA, Serhan CN, and Lefer AM (1997) Lipoxin A4 stable analogs inhibit leukocyte rolling and adherence in the rat mesenteric microvasculature: role of P-selectin. *Proc Natl Acad Sci USA* **94**:9967–9972.
- Schaldach CM, Riby J, and Bjeldanes LF (1999) Lipoxin A4: a new class of ligand for the Ah receptor. *Biochemistry* **38**:7594–7600.
- Schalling M, Neil A, Terenius L, Lindgren JA, Miamoto T, Hokfelt T, and Samuelsson B (1986) Leukotriene C4 binding sites in the rat central nervous system. *Eur J Pharmacol* **122**:251–257.
- Schellenberg RR and Foster A (1984) Differential activity of leukotrienes upon human pulmonary vein and artery. *Prostaglandins* **27**:475–482.
- Schild HO, Hawkins DF, Mongar JL, and Herxheimer H (1951) Reactions of isolated human asthmatic lung and bronchial tissue, to a specific antigen. *Lancet* **261**:376–382.
- Schreiber MD, Heymann MA, and Soifer SJ (1987) The differential effects of leukotriene C4 and D4 on the pulmonary and systemic circulations in newborn lambs. *Pediatr Res* **21**:176–182.
- Secrest RJ and Chapnick BM (1988) Endothelial-dependent relaxation induced by leukotrienes C4, D4 and E4 in isolated canine arteries. *Circ Res* **62**:983–991.
- Secrest RJ, Olsen EJ, and Chapnick BM (1985) Leukotriene D4 relaxes canine renal and superior mesenteric arteries. *Circ Res* **57**:323–329.
- Serhan CN (1994) Lipoxin biosynthesis and its impact in inflammatory and vascular events. *Biochim Biophys Acta* **1212**:1–25.
- Serhan CN (1997) Lipoxins and novel aspirin-triggered 15-epi-lipoxins (ATL): a jungle of cell-cell interactions or a therapeutic opportunity? *Prostaglandins* **53**:107–137.
- Serhan CN, Fierro IM, Chiang N, and Pouliot M (2001) Cutting edge: nociceptin stimulates neutrophil chemotaxis and recruitment: inhibition by aspirin-triggered-15-epi-lipoxin A4. *J Immunol* **166**:3650–3654.
- Serhan CN, Fiore S, and Levy BD (1994) Cell-cell interactions in lipoxin generation and characterization of lipoxin A4 receptors. *Ann N Y Acad Sci* **744**:166–180.
- Serhan CN, Hamberg M, and Samuelsson B (1984) Lipoxins: novel series of biologically active compounds formed from arachidonic acid in human leukocytes. *Proc Natl Acad Sci USA* **81**:5335–5339.
- Serhan CN, Maddox JF, Petasis NA, Akritopoulou-Zanze I, Papayianni A, Brady HR, Colgan SP, and Madara JL (1995) Design of lipoxin A4 stable analogs that block transmigration and adhesion of human neutrophils. *Biochemistry* **34**:14609–14615.
- Serhan CN, Radin A, Smolen JE, Korchak H, Samuelsson B, and Weissmann G (1982) Leukotriene B4 is a complete secretagogue in human neutrophils: a kinetic analysis. *Biochem Biophys Res Commun* **107**:1006–1012.
- Shak S and Goldstein IM (1985) Leukotriene B4 omega-hydroxylase in human polymorphonuclear leukocytes. Partial purification and identification as a cytochrome P-450. *J Clin Invest* **76**:1218–1228.
- Shaw A and Krell RD (1991) Peptide leukotrienes: current status of research. *J Med Chem* **34**:1235–1242.
- Shelhamer JH, Marom Z, and Kaliner M (1980) Immunologic and neuropharmacologic stimulation of mucous glycoprotein release from human airways in vitro. *J Clin Invest* **66**:1400–1408.
- Shirley JT and Cheng JB (1991) Competition of leukotrienes and ICI-198, 615 for [3H]LTD4 binding sites in guinea pig lung membranes suggests the involvement of two LTD4 receptor subtypes. *J Pharmacol Exp Ther* **258**:531–536.
- Showell HJ, Conklyn MJ, Alpert R, Hingorani GP, Wright KF, Smith MA, Stam E, Salter ED, Scampori DN, Meltzer S, et al. (1998) The preclinical pharmacological profile of the potent and selective leukotriene B4 antagonist CP-195543. *J Pharmacol Exp Ther* **285**:946–954.
- Simon MI, Strathmann MP, and Gautam N (1991) Diversity of G proteins in signal transduction. *Science (Wash DC)* **252**:802–808.
- Sjolander A, Gronroos E, Hammarstrom S, and Andersson T (1990) Leukotriene D4 and E4 induce transmembrane signaling in human epithelial cells. Single cell

- analysis reveals diverse pathways at the G-protein level for the influx and the intracellular mobilization of Ca^{2+} . *J Biol Chem* **265**:20976–20981.
- Sjostrom M, Jakobsson PJ, Heimburger M, Palmblad J, and Haeggstrom JZ (2001) Human umbilical vein endothelial cells generate leukotriene C₄ via microsomal glutathione S-transferase type 2 and express the CysLT(1) receptor. *Eur J Biochem* **268**:2578–2586.
- Smedegard G, Hedqvist P, Dahlen SE, Revenas B, Hammarstrom S, and Samuelsson B (1982) Leukotriene C₄ affects pulmonary and cardiovascular dynamics in monkey. *Nature (Lond)* **295**:327–329.
- Smith LJ, Glass M, and Minkwitz MC (1993) Inhibition of leukotriene D₄-induced bronchoconstriction in subjects with asthma: a concentration-effect study of ICI 204, 219. *Clin Pharmacol Ther* **54**:430–436.
- Smith LJ, Greenberger PA, Patterson R, Krell RD, and Bernstein PR (1985) The effect of inhaled leukotriene D₄ in humans. *Am Rev Respir Dis* **131**:368–372.
- Snyder DW, Aharony D, Dobson P, Tsai BS, and Krell RD (1984) Pharmacological and biochemical evidence for metabolism of peptide leukotrienes by guinea-pig airway smooth muscle in vitro. *J Pharmacol Exp Ther* **231**:224–229.
- Snyder DW and Bernstein PR (1987) Pharmacologic profile of chemically stable analogs of peptide leukotrienes. *Eur J Pharmacol* **138**:397–405.
- Snyder DW and Krell RD (1984) Pharmacological evidence for a distinct leukotriene C₄ receptor in guinea-pig trachea. *J Pharmacol Exp Ther* **231**:616–622.
- Soberman RJ, Harper TW, Murphy RC, and Austen KF (1985) Identification and functional characterization of leukotriene B₄ 20-hydroxylase of human polymorphonuclear leukocytes. *Proc Natl Acad Sci USA* **82**:2292–2295.
- Soter NA, Lewis RA, Corey EJ, and Austen KF (1983) Local effects of synthetic leukotrienes (LTC₄, LTD₄, LTE₄ and LTB₄) in human skin. *J Invest Dermatol* **80**:115–119.
- Soyombo O, Spur BW, and Lee TH (1994) Effects of lipoxin A₄ on chemotaxis and degranulation of human eosinophils stimulated by platelet-activating factor and N-formyl-L-methionyl-L-leucyl-L-phenylalanine. *Allergy* **49**:230–234.
- Spada CS, Nieves AL, Krauss AH, and Woodward DF (1994) Comparison of leukotriene B₄ and D₄ effects on human eosinophil and neutrophil motility in vitro. *J Leukoc Biol* **55**:183–191.
- Stanke-Labesque F, Devillier P, Bedouch P, Cracowski JL, Chavanon O, and Bessard G (2000) Angiotensin II-induced contractions in human internal mammary artery: effects of cyclooxygenase and lipoxygenase inhibition. *Cardiovasc Res* **47**:376–383.
- Stenke L, Mansour M, Edenius C, Reizenstein P, and Lindgren JA (1991) Formation and proliferative effects of lipoxins in human bone marrow. *Biochem Biophys Res Commun* **180**:255–261.
- Stewart AG, Thompson DC, and Fennessy MR (1984) Involvement of capsaicin-sensitive afferent neurones in a vagal-dependent interaction between leukotriene D₄ and histamine on bronchomotor tone. *Agents Actions* **15**:500–508.
- Sun FF, Chau LY, and Austen KF (1987) Binding of leukotriene C₄ by glutathione transferase: a reassessment of biochemical and functional criteria for leukotriene receptors. *Fed Proc* **46**:204–207.
- Sweatman WJF and Collier HOJ (1968) Effects of prostaglandins on human bronchial muscle. *Nature (Lond)* **217**:69.
- Tager AM, Dufour JH, Goodarzi K, Bercury SD, von Andrian UH, and Luster AD (2000) BLTR mediates leukotriene B₄-induced chemotaxis and adhesion and plays a dominant role in eosinophil accumulation in a murine model of peritonitis. *J Exp Med* **192**:439–446.
- Takano T, Clish CB, Gronert K, Petasis N, and Serhan CN (1998) Neutrophil-mediated changes in vascular permeability are inhibited by topical application of aspirin-triggered 15-epi-lipoxin A₄ and novel lipoxin B₄ stable analogues. *J Clin Invest* **101**:819–826.
- Takano T, Fiore S, Maddox JF, Brady HR, Petasis NA, and Serhan CN (1997) Aspirin-triggered 15-epi-lipoxin A₄ (LXA₄) and LXA₄ stable analogues are potent inhibitors of acute inflammation: evidence for anti-inflammatory receptors. *J Exp Med* **185**:1693–1704.
- Takasaki J, Kamohara M, Matsumoto M, Saito T, Sugimoto T, Ohishi T, Ishii H, Ota T, Nishikawa T, Kawai Y, et al. (2000) The molecular characterization and tissue distribution of the human cysteinyl leukotriene CysLT(2) receptor. *Biochem Biophys Res Commun* **274**:316–322.
- Terashita Z, Fukui H, Nishikawa K, Hirata M, and Kikuchi S (1982) Effects of arachidonic acid and bradykinin on the coronary flow, release of PGI₂ and cardiac functions in the perfused guinea-pig heart. *Jpn J Pharmacol* **32**:351–358.
- Thivierge M, Doty M, Johnson J, Stankova J, and Rola-Pleszczynski M (2000) IL-5 up-regulates cysteinyl leukotriene 1 receptor expression in HL-60 cells differentiated into eosinophils. *J Immunol* **165**:5221–5226.
- Thivierge M, Stankova J, and Rola-Pleszczynski M (2001) IL-13 and IL-4 up-regulate cysteinyl leukotriene 1 receptor expression in human monocytes and macrophages. *J Immunol* **167**:2855–2860.
- Thodeti CK, Adolfsson J, Juhas M, and Sjolander A (2000) Leukotriene D₄ triggers an association between gbetagamma subunits and phospholipase C-gamma1 in intestinal epithelial cells. *J Biol Chem* **275**:9849–9853.
- Toda A, Yokomizo T, Masuda K, Nakao A, Izumi T, and Shimizu T (1999) Cloning and characterization of rat leukotriene B₄ receptor. *Biochem Biophys Res Commun* **262**:806–812.
- Toh H, Ichikawa A, and Narumiya S (1995) Molecular evolution of receptors for eicosanoids. *FEBS Lett* **361**:17–21.
- Tomoike H, Egashira K, Yamada A, Hayashi Y, and Nakamura M (1987) Leukotriene C₄- and D₄-induced diffuse peripheral constriction of swine coronary artery accompanied by ST elevation on the electrocardiogram: angiographic analysis. *Circulation* **76**:480–487.
- Torphy TJ, Newton JF, Wasserman MA, Vickery-Clark L, Osborn RR, Bailey LS, Yodis LA, Underwood DC, and Hay DW (1989) The bronchopulmonary pharmacology of SK&F 104353 in anesthetized guinea pigs: demonstration of potent and selective antagonism of responses to peptidoleukotrienes. *J Pharmacol Exp Ther* **249**:430–437.
- Tryselius Y, Nilsson NE, Kotarsky K, Olde B, and Owman C (2000) Cloning and characterization of cDNA encoding a novel human leukotriene B₄ receptor. *Biochem Biophys Res Commun* **274**:377–382.
- Tsopanglou NE, Pipili-Synetos E, and Maragoudakis ME (1994) Leukotrienes C₄ and D₄ promote angiogenesis via a receptor-mediated interaction. *Eur J Pharmacol* **258**:151–154.
- Tucker SS and Weichman BM (1986) Differential rank order of potency for antagonism of LTC₄- and LTD₄-induced contractions of guinea pig trachea. *Prostaglandins Leukotrienes Med* **23**:37–44.
- Tudhope SR, Cuthbert NJ, Abram TS, Jennings MA, Maxey RJ, Thompson AM, Norman P, and Gardiner PJ (1994) BAY u9773, a novel antagonist of cysteinyl-leukotrienes with activity against two receptor subtypes. *Eur J Pharmacol* **264**:317–323.
- Turner CR, Smith WB, Andresen CJ, Swindell AC, and Watson JW (1994) Leukotriene D₄ receptor antagonism reduces airway hyperresponsiveness in monkeys. *Pulm Pharmacol* **7**:49–58.
- Turner SR, Tainer JA, and Lynn WS (1975) Biogenesis of chemotactic molecules by the arachidonate lipoxygenase system of platelets. *Nature (Lond)* **257**:680–681.
- Undem BJ, Hubbard W, and Weinreich D (1993) Immunologically induced neuro-modulation of guinea pig nodose ganglion neurons. *J Auton Nerv Syst* **44**:35–44.
- Undem BJ and Weinreich D (1993) Electrophysiological properties and chemosensitivity of guinea pig nodose ganglion neurons in vitro. *J Auton Nerv Syst* **44**:17–33.
- Underwood DC, Osborn RR, Newsholme SJ, Torphy TJ, and Hay DW (1996) Persistent airway eosinophilia after leukotriene (LT) D₄ administration in the guinea pig: modulation by the LTD₄ receptor antagonist, pranlukast, or an interleukin-5 monoclonal antibody. *Am J Respir Crit Care Med* **154**:850–857.
- Walch L, Norel X, Bäck M, Gascard J-P, Dahlen S-E, and Brink C (2002) Pharmacological evidence for a novel cysteinyl-leukotriene receptor subtype in human pulmonary artery smooth muscle. *Br J Pharmacol* **137**:1339–1345.
- Wang CG, Du T, Xu LJ, and Martin JG (1993) Role of leukotriene D₄ in allergen-induced increases in airway smooth muscle in the rat. *Am Rev Respir Dis* **148**:413–417.
- Wang S, Gustafson E, Pang L, Qiao X, Behan J, Maguire M, Bayne M, and Laz T (2000) A novel hepatointestinal leukotriene B₄ receptor. Cloning and functional characterization. *J Biol Chem* **275**:40686–40694.
- Watanabe T, Shimizu T, Miki I, Sakanaka C, Honda Z, Seyama Y, Teramoto T, Matsushima T, Ui M, and Kurokawa K (1990) Characterization of the guinea pig lung membrane leukotriene D₄ receptor solubilized in an active form. Association and dissociation with an islet-activating protein-sensitive guanine nucleotide-binding protein. *J Biol Chem* **265**:21237–21241.
- Weichman BM and Tucker SS (1982) Contraction of guinea pig uterus by synthetic leukotrienes. *Prostaglandins* **24**:245–253.
- Weiss JW, Drazen JM, Coles N, McFadden ER Jr, Weller PF, Corey EJ, Lewis RA, and Austen KF (1982) Bronchoconstrictor effects of leukotriene C in humans. *Science (Wash DC)* **216**:196–198.
- Welton AF, Hope WC, Tobias LD, and Hamilton JG (1981) Inhibition of antigen-induced histamine release and thromboxane synthase by FPL 55712, a specific SRA-A antagonist? *Biochem Pharmacol* **30**:1378–1382.
- Woo CH, You HJ, Cho SH, Eom YW, Chun JS, Yoo YJ, and Kim JH (2002) Leukotriene B₄ stimulates Rac-ERK cascade to generate reactive oxygen species that mediates chemotaxis. *J Biol Chem* **277**:8572–8578.
- Woodman OL and Dusting GJ (1982) Coronary vasoconstriction induced by leukotrienes in the anesthetized dog. *Eur J Pharmacol* **86**:125–128.
- Woodward DF, Wasserman MA, and Weichman BM (1983a) Investigation of leukotriene involvement in the vasopermeability response associated with guinea pig tracheal anaphylaxis: comparison with cutaneous anaphylaxis. *Eur J Pharmacol* **93**:9–19.
- Woodward DF, Weichman BM, Gill CA, and Wasserman MA (1983b) The effect of synthetic leukotrienes on tracheal microvascular permeability. *Prostaglandins* **25**:131–142.
- Ye RD, Cavanagh SL, Quehenberger O, Prossnitz ER, and Cochrane CG (1992) Isolation of a cDNA that encodes a novel granulocyte N-formyl peptide receptor. *Biochem Biophys Res Commun* **184**:582–589.
- Yokomizo T, Izumi T, Chang K, Takawa Y, and Shimizu T (1997) A G-protein-coupled receptor for leukotriene B₄ that mediates chemotaxis. *Nature (Lond)* **387**:620–624.
- Yokomizo T, Izumi T, and Shimizu T (2001a) Co-expression of two LTB₄ receptors in human mononuclear cells. *Life Sci* **68**:2207–2212.
- Yokomizo T, Izumi T, and Shimizu T (2001b) Leukotriene B₄: metabolism and signal transduction. *Arch Biochem Biophys* **385**:231–241.
- Yokomizo T, Izumi T, Takahashi T, Kasama T, Kobayashi Y, Sato F, Taketani Y, and Shimizu T (1993) Enzymatic inactivation of leukotriene B₄ by a novel enzyme found in the porcine kidney. Purification and properties of leukotriene B₄ 12-hydroxydehydrogenase. *J Biol Chem* **268**:18128–18135.
- Yokomizo T, Kato K, Hagiya H, Izumi T, and Shimizu T (2001c) Hydroxyeicosanoids bind to and activate the low affinity leukotriene B₄ receptor, BLT2. *J Biol Chem* **276**:12454–12459.
- Yokomizo T, Kato K, Terawaki K, Izumi T, and Shimizu T (2000) A second leukotriene B₄ receptor, BLT2. A new therapeutic target in inflammation and immunological disorders. *J Exp Med* **192**:421–432.
- Yokomizo T, Ogawa Y, Uozumi N, Kume K, Izumi T, and Shimizu T (1996) cDNA cloning, expression and mutagenesis study of leukotriene B₄ 12-hydroxydehydrogenase. *J Biol Chem* **271**:2844–2850.
- Young RN (1989) Structural analysis of sulfido-peptide leukotrienes: application to the design of potent and specific antagonists of leukotriene D₄. *Adv Prostaglandin Thromboxane Leukotriene Res* **19**:643–646.
- Zukowska-Grojec Z, Bayorh MA, Kopin IJ, and Feuerstein G (1982) Leukotriene D₄: cardiovascular and sympathetic effects in spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. *J Pharmacol Exp Ther* **223**:183–189.
- Zukowska-Grojec Z, Pamnani M, and Feuerstein G (1984) Cardiovascular and sympathetic effects of leukotriene D₄ in renovascular and spontaneously hypertensive rats. *Clin Exp Hypertens [A]* **6**:1575–1590.