Paracrine signals

- Proteins, lipids that diffuse short distances
- Include growth factors and cytokines
- Secreted or synthesized as membrane-bound precursor (pro-peptide) that is proteolytically processed to produce soluble signal molecule
- Interact with cell surface receptors on target cell to induce a reponse
- Among the hundreds of known paracrine signals, several major families include:
  - oFibroblast growth factor (FGF)
  - oEpidermal growth factor (EGF)
  - oHedgehog
  - oWnt
  - oTGF-β (Transforming Growth factor)
- The names usually do not reflect the wide array of functions each growth factor performs
- Many growth factors can induce different responses in different cells. The response is controlled by the responding cell.

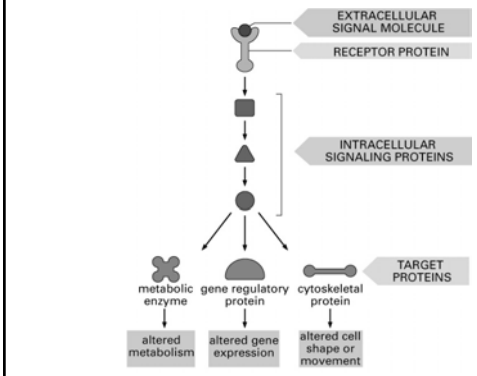


Figure 15–1. Molecular Biology of the Cell, 4th Edition.

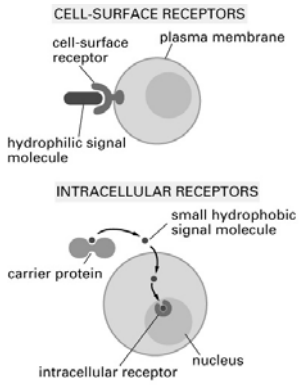


Figure 15–3. Molecular Biology of the Cell, 4th Edition

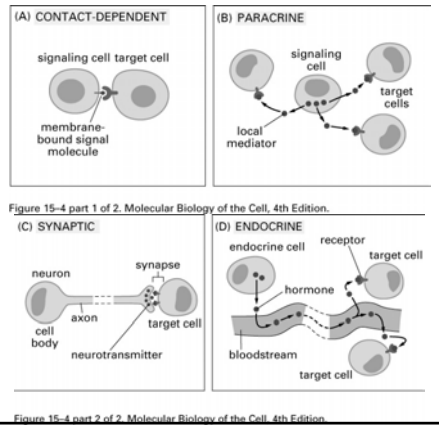


Figure 15–4 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Figure 15–4 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

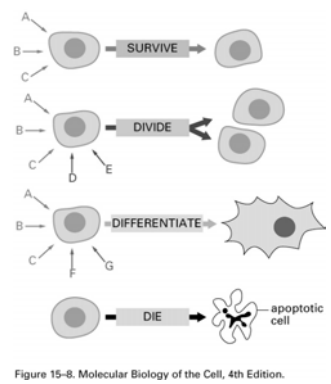


Figure 15–8. Molecular Biology of the Cell, 4th Edition.

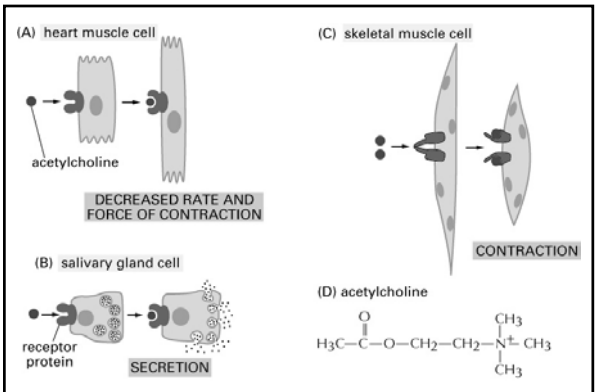


Figure 15–9. Molecular Biology of the Cell, 4th Edition.

Autocrine signalling

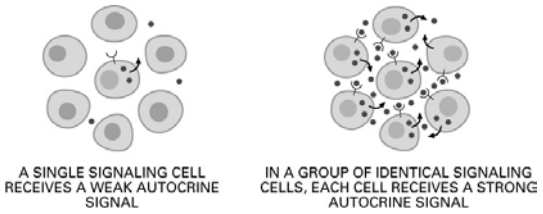
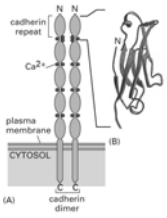


Figure 15–6. Molecular Biology of the Cell, 4th Edition.

Signals are not always diffusible but may be membrane bound or a part of the extracellular matrix



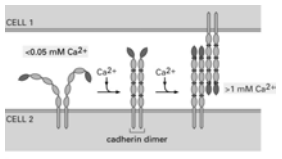


Figure 19–24 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Figure 19–24 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

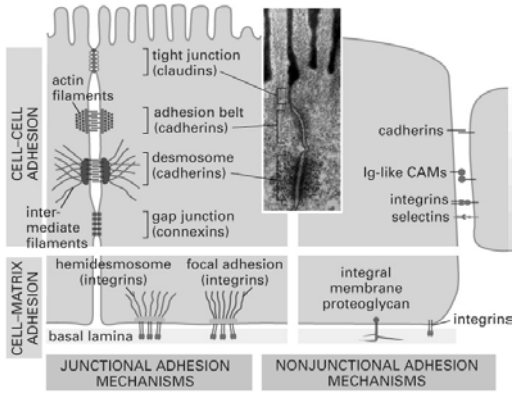


Figure 19–32. Molecular Biology of the Cell, 4th Edition.

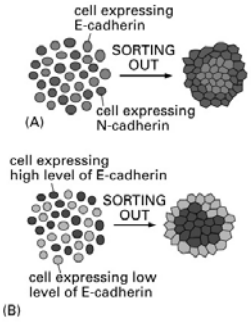


Figure 19–27. Molecular Biology of the Cell, 4th Edition

The inflammatory response

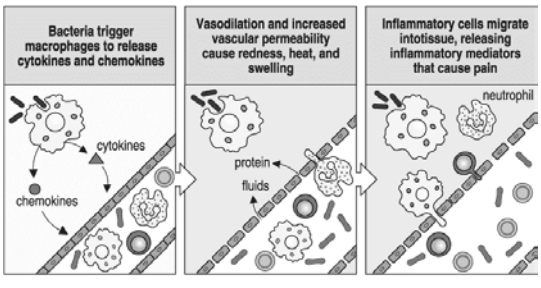


Fig 1.12 © 2001 Garland Science

Eosinophils secrete a range of inflammatory mediators

Class of product	Examples	Biological effects
Enzyme	Eosinophil peroxidase	Toxic to targets by catalyzing halogenation Triggers histamine release from mast cells
	Eosinophil collagenase	Remodels connective tissue matrix
Toxic protein	Major basic protein	Toxic to parasites and mammalian cells Triggers histamine release from mast cells
	Eosinophil cationic protein	Toxic to parasites Neurotoxin
	Eosinophil-derived neurotoxin	Neurotoxin
Cytokine	IL-3, IL-5, GM-CSF	Amplify eosinophil production by bone marrow Cause eosinophil activation
Chemokine	IL-8	Promotes influx of leukocytes
Lipid mediator	Leukotrienes C4, D4, E4	Cause smooth muscle contraction Increase vascular permeability Increase mucus secretion
	Platelet-activating factor	Attracts leukocytes Amplifies production of lipid mediators Activates neutrophils, eosinophils, and platelets

Fig 12.12 © 2001 Garland Science

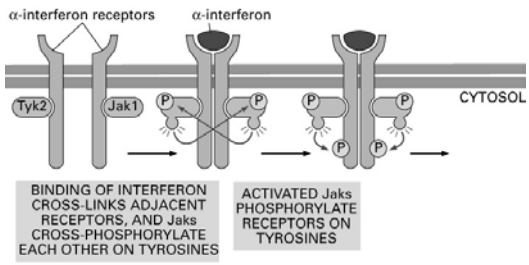


Figure 15–63 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

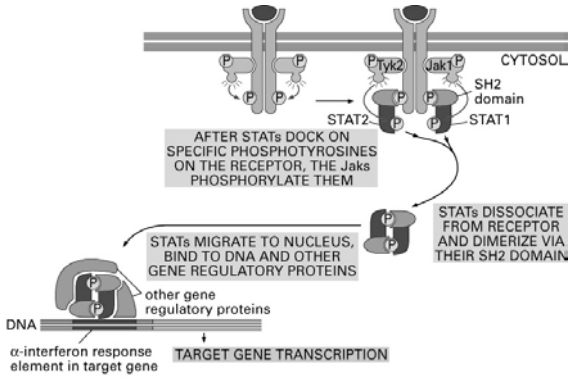


Figure 15–63 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Eicosanoids

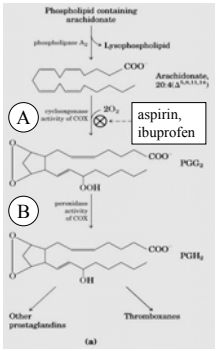
- Family of biological signaling molecules
- Act as short-range messengers
- Derived from polyunsaturated eicosanoic acids, e.g. arachidonate [20: 4; ($\Delta^{5,8,11,14}$)]
- Family includes:
  - Prostaglandins
  - Leukotrienes
  - Intermediates HPETE and HETE

  HPETE = **h**ydro-**p**eroxy-**e**icosa-**t**etra-**e**noic acid

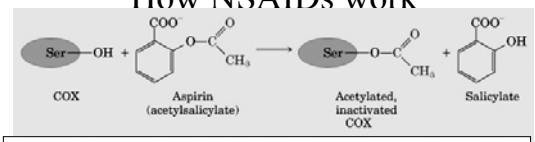
  HETE = **h**ydroxy-**e**icosa-**t**etra-**e**noic acid

Prostaglandin / thromboxane synthesis

- **"CYCLIC PATHWAY"**
- ***Phospholipase A_2*** mediated hydrolysis of arachidonate from the 2-position of a membrane phospholipid.
- Bifunctional ***prostaglandin H2 synthase* (PGHS)** aka ***cyclooxygenase (COX)***:
  - (A) **cyclooxygenase** activity
  - (B) **peroxidase** activity (requires glutathione)



How NSAIDs work



- NSAIDs = **n**on**s**teroidal **a**nti-**i**nflammatory **d**rugs (aspirin, acetomenophen, ibuprofen, naproxen)
- Aspirin (acetylsalicylate) **irreversibly** inhibits the cyclooxygenase activity of COX
- Ibuprofen inhibits the same reaction probably by mimicking the substrate.

- Aspirin controlled thromboxane synthesis
  - Thromboxanes
    - induce constriction of blood vessels
    - Are generated from PGH_2 via ***thromboxane synthase,*** which is present in platelets (thrombocytes)
- New "aspirin-derivatives" without side effects
  - COX-1 expressed in stomach, -> regulation of gastric mucin via prostaglandins
  - COX-2 (bad "COX") inducible isoform in inflammtory cells and immune cells
- Aspirin induced asthma (AIA)
  - Asthma = invasion by inflammatory cells

Leukotriene synthesis

- "LINEAR PATHWAY"
  - Not inhibited by NSAIDs
  - Lipoxygenases
  - convert arachidonate to leukotrienes
  - present in leukocytes, heart, brain, lung, spleen
  - also present in plants

Leukotriene synthesis

Q: Is lipoxygenase

a) an oxidase

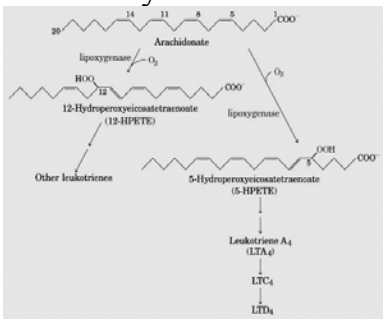
b) a monooxygenase

c) a dioxygenase

d) a cyclooxygenase

e) a dehydrogenase

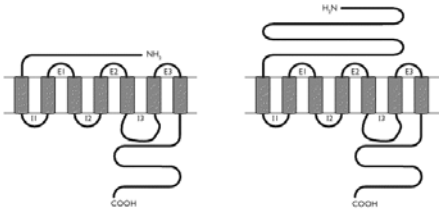
f) peroxidase



Prostanoid receptors

- Prostanoids exert their actions via membrane receptors on the surface of target cells [Narumiya et al., 1999]. We cloned a family of eight types and subtypes of the prostanoid receptors conserved in mammals from mouse to human. They are the PGD receptor (DP), four subtypes of the PGE receptor EP1, EP2, EP3, and EP4, the PGF receptor (FP), PGI receptor (IP) and TXA receptor (TP). They all are G protein-coupled rhodopsin-type receptors with seven transmembrane domains. Using homologous recombination, we have generated mice deficient in each of the prostanoid receptors individually, and subjected them to various models of human diseases.

General structure for eicosanoid receptors



Making a "knockout mouse"

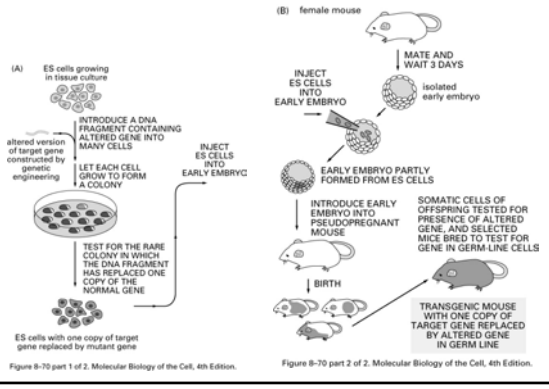


Figure 8–70 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Figure 8–70 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Figure 8–71. Molecular Biology of the Cell, 4th Edition.

Figure 8–71 Mouse with an engineered defect in fibroblast growth factor 5 (FGF5).

FGF5 is a negative regulator of hair formation. In a mouse lacking FGF5 *(right)*, the hair is long compared with its heterozygous littermate *(left)*. Transgenic mice with phenotypes that mimic aspects of a variety of human disorders, including Alzheimer's disease, atherosclerosis, diabetes, cystic fibrosis, and some type of cancers, have been generated. Their study may lead to the development of more effective treatments. (Courtesy of Gail Martin, from J.M. Hebert et al., *Cell* 78:1017–1025, 1994. © Elsevier.)

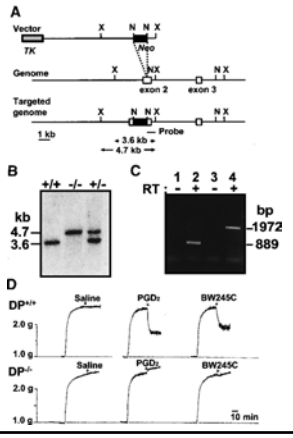


Figure 1. Disruption of the mouse DP gene. (**A**) Strategy for targeted disruption. Organization of the DP gene, construction of the targeting vector (*TK*, thymidine kinase gene; *Neo*, neomycin resistance gene), and structure of the targeted genome are shown. Restriction sites are indicated: N, Nhe I; and X, Xba I. (**B**) Southern blot analysis. Genomic DNA from newborn littermates of heterozygote intercrosses was digested with Xba I, and the resulting fragments were subjected to analysis with a Nhe I-Xba I fragment of the genomic DNA as a probe. The positions of 3.6-kb (wild-type) and 4.7-kb (mutant) hybridizing fragments are shown for mice of the indicated genotypes. (**C**) RT-PCR analysis. Polyadenylated RNA from the ileum of wild-type mice (lanes 1 and 2) and DP^{-} mice (lanes 3 and 4) was subjected to PCR amplification in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of reverse transcriptase (RT). The estimated positions of nucleotides of 1972 and 889 base pairs (bp) are indicated on the right. (**D**) Relaxation of tracheal smooth muscle by PGD_2 or BW245C. Tracheal rings from wild-type (top traces) and DP^{-} (bottom traces) mice were suspended in an organ bath and induced to contract with carbachol. Relaxation in response to 3 μM PGD_2 or 100 nM BW245C (or saline) applied at the times indicated by the arrows was assessed.

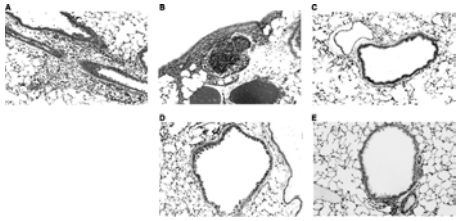


Figure 4. Histological examination of lung tissue of OVA-challenged wild-type and DP^{-} mice. (**A** to **C**) Hematoxylin and eosin staining. Accumulation of inflammatory cells (A) and formation of BALT (B) are apparent in the lungs of OVA-challenged wild-type mice, but not in those of antigen-challenged DP^{-} mice (C). (**D** and **E**) Periodic acid-Schiff staining. Mucus secretion by the airway epithelial cells is apparent in antigen-challenged wild-type mice (D) but not in OVA-challenged DP^{-} mice (E). Original magnification, ×200

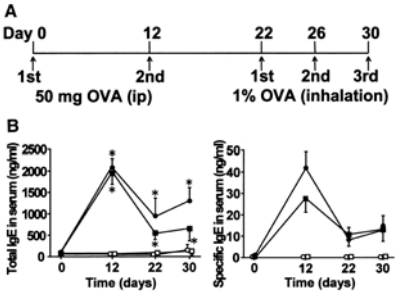


Fig. 2. Effects of DP deficiency on asthmatic responses in an OVA-induced asthma model. (**A**) Protocol for OVA immunization and challenge. (**B**) Time courses of the serum concentrations of total (left) and OVA-specific (right) IgE in wild-type and DP^{-} mice. , Wild-type mice injected with saline; , wild-type mice injected with OVA; , DP^{-} mice injected with saline; , DP^{-} mice injected with OVA

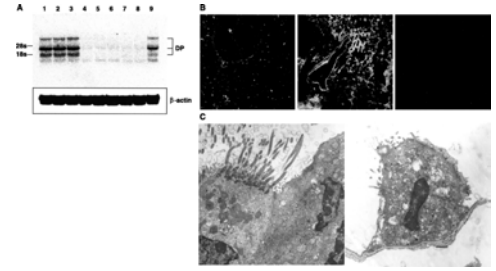


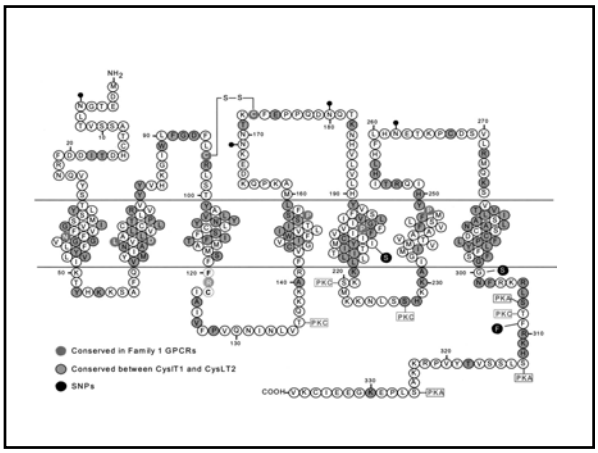
Figure 5. Expression and localization of the DP receptor in the lung. (**A**) Northern blot analysis. Expression of the DP receptor mRNA was examined in the ileum (lanes 1 to 3), the spleen (lanes 4 to 6), and the lung (lanes 7 to 9) of unimmunized (lanes 1, 4, and 7) and immunized wild-type mice before (lanes 2, 5, and 8) and after (lanes 3, 6, and 9) the OVA challenge. Positions of DP mRNA are indicated. (**B**) Immunofluorescence microscopy for DP in the lung of immunized wild-type mice before (left) and after (middle) the OVA challenge and in immunized DP^{-} mice after the challenge (right). Original magnification, ×20. (**C**) Immunoelectron microscopy for DP in the lung of immunized wild-type mice after the OVA challenge. Immunogold particles are seen on the plasma and intracellular membranes of bronchiolar (left) and alveolar (right) epithelial cells. Original magnification, ×8000.

EP4-/- and skin

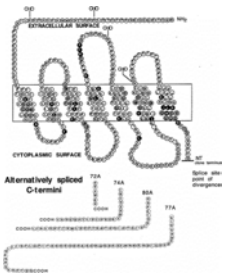
- Immune response in the skin is triggered by uptake of exposed antigens by Langerhans cells, a type of dendritic cells (DCs). Langerhans cells then migrate to draining lymph nodes, and activate naive T cells to initiate immune response. It is therefore likely that, if PGE2 in the skin works in immune response, it acts on Langerhans cells to modulate their functions. Using mice deficient in each EP subtype, we examined this issue [Kabashima et al., 2003a]. We found that, while Langerhans cells express all four PGE receptor (EP) subtypes, their migration to regional lymph nodes after antigen uptake was decreased only in EP4−/− mice. Impairment in migration was reproduced in wild type mice treated with an EP4 antagonist, whereas an EP4 agonist promoted the migration of Langerhans cells. EP4 stimulation further increased expression of costimulatory molecules in Langerhans cells, and enhanced their ability to stimulate T cells in the mixed lymphocyte reaction in vitro. These results indicate that PGE2-EP4 signaling promotes the migration and maturation of Langerhans cells and facilitates initiation of skin immune responses. Consistent with this hypothesis, contact hypersensitivity to antigen was impaired in EP4−/− mice as well as in wild-type mice treated with the EP4 antagonist during sensitization.

PGE2-EP4 signaling in inflammatory bowel disease

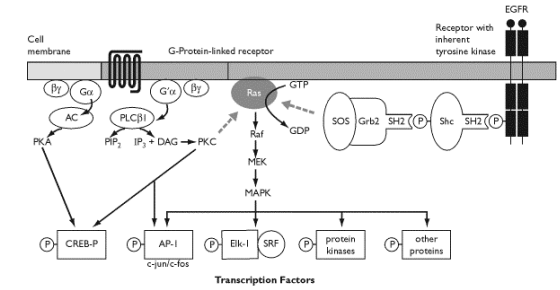
- Human inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, is a chronic, relapsing, and remitting condition of unknown origin, and is characterized by inflammation in the large and/or small intestine associated with diarrhea, occult blood, abdominal pain, weight loss, anemia, and leukocytosis [Fiocchi, 1998]. Studies in humans have implicated impaired mucosal barrier function, pronounced innate immunity, production of proinflammatory and immunoregulatory cytokines, and the activation of CD4+ T cells in the pathogenesis of IBD. It is known that PGE2 is produced abundantly in the affected intestine, and that the administration of NSAIDs often triggers and exacerbates IBD [Bjarnason et al., 1993]. These findings indicate that PGE2 works to prevent the disease initiation and progression. However, the identity of the EP receptor and mechanism of such prevention remain unknown. We subjected mice deficient in each of the eight types and subtypes of prostanoid receptors to dextran sodium sulfate (DSS)-induced colitis, an animal model of IBD, and examined this issue by comparing their susceptibility to DSS treatment [Kabashima et al., 2002]. We found that, among the eight strains of knockout mice, only EP4-deficient mice developed severe colitis with 3% DSS treatment. This susceptibility in the absence of EP4 signaling was confirmed by reproducing this phenotype in wild type mice treated with an EP4-selective antagonist. We further found that the EP4 deficiency impaired mucosal barrier function, and induced epithelial loss, crypt damage, and accumulation of neutrophils and CD4+ T cells in the colon. By DNA microarray, elevated expression of genes associated with immune response and reduced expression of genes with mucosal repair and remodeling were found in the colon of EP4-deficient mice. Thus our study revealed that EP4 maintains intestinal homeostasis by preserving mucosal integrity and down-regulating immune response.



Splice variants of EP3



Species and clone name	cDNA source	Unique sequence	Signal transduction	Expression cell line	Reference(s)
human					
III	Uterus	•EEFWGN	↓cAMP, ↑Ca^{2+}	CHO	91
E	Intestine	•EEFWGN	↓cAMP	JEG-3	82
b	Uterus	•EEFWGN	↓cAMP, ↑Ca^{2+}	BHK-12	83
III	Kidney	•EEFWGN	Not determined		147
III	Kidney	•EEFWGN	↓cAMP	COS-7/CHO	148
Rabbit 72A	Kidney	•EEFWEK	↑CRE	HEK293	9, 99
human					
d	Uterus	•MRKRRLREQEEFWEGN	↓cAMP	BHK-12	83
IV	Uterus	•MRKRRLREQEEFWEGN	↓cAMP (weak), ↑Ca^{2+}	CHO	91
IV	Kidney	•MRKRRLREQEEFWEGN	↓cAMP ↑cAMP	COS-7/CHO	148
F	Intestine	•MRKRRLREQ	Not determined		82
e	Uterus	•MRKRRLREQLICSLRTLRYRGQLHIVGKYKPIVC	↓cAMP	BHK-12	83
f	Uterus	•MRKRRLREQAPLLPTPTVIDPSRFCAQPFRWFLD	↓cAMP	BHK-12	83
		LSFPAMSSSHHPQLPLTLASFKLLREPCSVQLS			
Rabbit 74A	Kidney	•RYHTNNYASSSTSLTHQCSST	↑CRE	HEK293	9, 99
human					
I	Uterus	•RYHTNNYASSSTSLPCQCSSTLMWSDHLER	↓cAMP, ↑Ca^{2+}	CHO	91
I	Kidney	•RYHTNNYASSSTSLPCNCSSTLMWSDHLER	not determined		147
I	Kidney	•RYHTNNYASSSTSLPCQCSSTLMWSDHLER	↓cAMP ↑IP_3	COS-7/CHO	148
A	Intestine	•RYHTNNYASSSTSLPCQCSSTLMWSDHLER	↓cAMP	JEG-3	82
			MAP kinase	COS-7	149
a	Uterus	•RYHTNNYASSSTSLPCQCSSTLMWSDHLER	↓cAMP, ↑Ca^{2+}	BHK-12	83
Mouse G	P815	•RDHT-NYASSSTSLPCPGSSALMWSDQLER	↓cAMP	CHO	86



Correlation of functional data to receptor binding

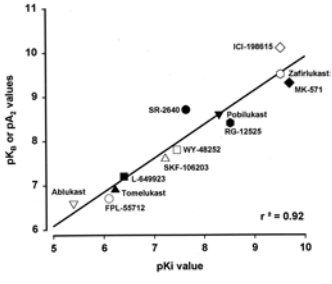
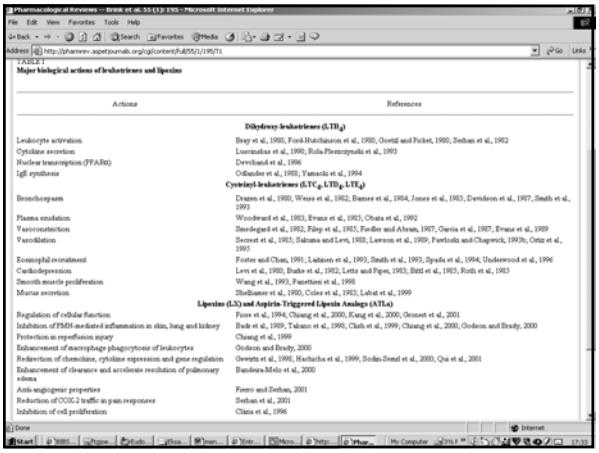
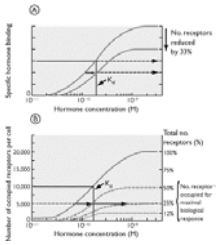


Fig. 6. Cysteinyl-leukotriene antagonists in binding versus functional assays. Data (pK_B/pA_2 values) are derived from guinea pig tissues contracted with LTD_4 and compared with results (pK_i values) obtained in $[^3H]LTD_4$ radioligand binding assays performed on membrane fractions from the same species. 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).



Major biological actions of leukotrienes and lipoxins

Actions	References
Dihydroxy-leukotrienes (LTB_4)	
Leukocyte activation	Bray et al., 1980; Ford-Hutchinson et al., 1980; Ocetti and Pickett, 1980; Serhan et al., 1982
Cytokine secretion	Luostarinen et al., 1996; Rola-Pleszczynski et al., 1993
Nuclear transcription (PPARδ)	Devchand et al., 1996
IgE synthesis	Ollerdale et al., 1988; Yamaoki et al., 1994
Cysteinyl-leukotrienes (LTC_4, LTD_4, LTE_4)	
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 Ahrens, 1987; Garcia et al., 1987; Evans et al., 1989
Vasodilation	Secrest et al., 1985; Sakuma and Levi, 1988; Lawson et al., 1989; Pawlecki and Chapnick, 1993b; Ortiz et al., 1995
Eosinophil recruitment	Foster and Chan, 1991; Lukiewn 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 (ATLs)	
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	Bade 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
Reduction of chemokine, cytokine expression and gene regulation	Gewirtz et al., 1998; Hachicha et al., 1999; Sodin-Semrl et al., 2000, Qu et al., 2001
Enhancement of clearance and accelerate resolution of pulmonary edema	Bandeira-Melo et al., 2000
Anti-angiogenic properties	Fiorro and Serhan, 2001
Reduction of CCR-2 traffic in pain responses	Serhan et al., 2001
Inhibition of cell proliferation	Chiria et al., 1996



Relationship between specific hormone binding and total number of receptors. The K_d is the concentration of hormone required to occupy 50% of the receptors. When the total number of receptors is reduced by a third, K_d remains the same but the concentration of hormone required to achieve a certain level of hormone binding is increased (arrow).

These curves demonstrate the consequences of reducing the number of receptors when only 25% occupancy is required for maximal biological response. Again K_d remains unchanged but the concentration of hormone required to achieve the occupancy of 5000 receptors per cell increases (arrows).