

Production of Eicosanoids and Other Oxylipins by Pathogenic Eukaryotic Microbes

Mairi C. Noverr,¹ John R. Erb-Downward,^{1,2} and Gary B. Huffnagle^{1,2*}

Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine,¹ and Department of Microbiology and Immunology,² University of Michigan Medical School, Ann Arbor, Michigan 48109-0642

INTRODUCTION.....	517
BIOCHEMISTRY	517
Mammals	517
Protozoa, Helminths, and Fungi.....	519
EFFECTS ON EUKARYOTIC MICROBES.....	523
Protozoa	523
Helminths.....	524
Fungi.....	525
EFFECTS IN INFECTION.....	526
Eicosanoids and Immunomodulation.....	526
Protozoal Infections.....	527
Helminth Infections.....	527
Fungal Infections.....	529
NEW PERSPECTIVES ON FUNGAL PATHOGENESIS.....	530
Why Is the Prostate a Target for Persistent <i>Cryptococcus neoformans</i> Infection?	530
OXYLIPIN PRODUCTION AS A TARGET OF INHIBITION	530
FUTURE PERSPECTIVES.....	530
ACKNOWLEDGMENTS	531
REFERENCES	531

INTRODUCTION

Oxygenated lipids are collectively known as oxylipins. One of the most biologically important groups of oxylipins in mammals is the eicosanoids, which include prostaglandins and leukotrienes. These eicosanoids are potent modulators of immune responses in addition to playing a role in numerous basic host physiologic processes (40). Eicosanoids act via specific receptors, with some having multiple receptors (Tables 1 and 2) (40, 50). Host cells are one source of eicosanoids and oxylipins during infection; however, another potential source of eicosanoids is the pathogen itself. Descriptions of eicosanoid and oxylipin production by eukaryotic microbes have been scattered through the literature for decades, but they have begun to receive significant attention in the last few years following reports of prostaglandins and prostaglandin-like molecules being produced by pathogenic helminths, protozoa, and fungi.

Why do these organisms produce oxylipins? Eicosanoids in eukaryotic microbes appear to play a dual role, metabolism or maturation of the organism and communication with the host on a cellular basis. Accumulating data suggest that control of phase change and differentiation in these organisms is controlled by oxylipins, including prostaglandins and lipoxygenase products. This also opens up the possibility that enzymes in this

pathway may be targets for future pharmaceuticals. The precise role of pathogen-derived eicosanoids in pathogenesis remains to be determined, but the potential link between pathogen eicosanoids and the development of Th2 responses or modulation of immunity in the host is intriguing.

The role of host eicosanoids in modulating immunity is well documented. The study of microbial oxylipins in disease pathogenesis is just beginning. This review will focus on the potential of oxylipins as secreted virulence factors not in the same context as microbial toxins but rather as factors required for virulence that augment other virulence mechanisms of pathogenic eukaryotic microbes. Using diverse sources to present a model of why these microbes produce oxylipins, we will also present evidence that these microbial oxylipins may play a significant role in a host-pathogen “cross-talk” that contributes to chronic infection.

BIOCHEMISTRY

Eicosanoid compounds have been found in almost every eukaryotic organism, providing a number of functions ranging from regulation of reproduction in invertebrates to formation of innate defense mechanisms in plants (118, 129). However, most of what is known about eicosanoids stems from investigation of mammalian biology, most often in the context of immune system regulation.

Mammals

Polyunsaturated fatty acids compose a large variety of lipids, differing in hydrocarbon length and degree of unsaturation

* Corresponding author. Mailing address: Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan Medical School, 1150 W. Medical Ctr. Drive, 6301 MSRB III, Ann Arbor, MI 48109-0642. Phone: (734) 936-9368. Fax: (734) 764-4556. E-mail: ghuff@umich.edu.

TABLE 1. Common mammalian cyclooxygenase products and their functions^a

Name	Receptor	Cellular Target	Function
PGD ₂	DP ₁ DP ₂	Lung epithelial cell CD4 ⁺ T cell	Mediates allergic asthma Polarizes cell towards Th2 phenotype, initiates chemotaxis
PGE ₂	EP ₁ EP ₂ EP ₃ EP ₄ EP ₂ , EP ₄	Spinal neurons Smooth muscle Ovarian cells Placenta Neurons Stomach Osteoclast Lung cells	Pain response Contraction of muscle Maturation for ovulation Relaxation of muscle Fever generation Inhibition of gastric acid secretion Bone resorption Polarization of T helper cells towards Th2 phenotype, downregulation of macrophage activity, regulation of inflammation, antiproliferative effects
PGF _{2α}	FP	Uterine smooth muscle	Contraction, parturition
PGI ₂	IP	Vascular smooth muscle Platelet	Vasodilation Declumping
15-Deoxy-Δ-12,14-PGJ ₂	PPARγ	Macrophage	Inhibition of activation and effector function and antiproliferative effects
TXA ₂	TP _β TP _α	Vascular smooth muscle cell Platelet	Vasoconstriction Aggregation

^a Adapted from Funk et al., 2001 (40, 87).

(113). For most eukaryotic organisms, the precursors to these lipids are essential and cannot be synthesized *de novo* but must be obtained from plant products in the diet (87). Once taken up by cells, precursor fatty acids are acted upon by a number of different elongases and desaturases to form a large family of compounds that not only aid in membrane fluidity but, more importantly, play a role in intercellular communication (113). Normally, polyunsaturated fatty acids are found esterified in

the phospholipids that make up cell membranes and are released by phospholipases (specifically phospholipase A₂), which are under the control of environmental signals (87).

Eicosanoids are biologically active lipids derived from di-homo-γ-linolenic acid, arachidonic acid, and eicosanopentanoic acid (C₂₀ polyunsaturated fatty acids which differ only in the number of *cis* double bonds) (Fig. 1 and 2). The most prevalent eicosanoids in mammals are derived from arachi-

TABLE 2. Common mammalian lipoxygenase products and their functions

Name	Receptor	Cellular target	Function
LTB ₄	B-LT1 B-LT2	Neutrophils Leukocytes Many cell types	Lysosomal enzyme release, superoxide production, chemotaxis, chemokine production Aggregation and chemotaxis ?
LTC ₄	?	Lung cells	Mediator of asthma and allergic responses; promotes anaphylaxis, smooth muscle contraction, bronchoconstriction, vascular permeability, hypersensitivity.
LTD ₄	Cys-LT1 Cys-LT2	Lung smooth muscle, endothelial cells Heart, adrenal, brain, spleen cells	Mediator of asthma and allergic responses; promotes anaphylaxis, smooth muscle contraction, bronchoconstriction, vascular permeability, hypersensitivity ?
LTE ₄	?	Lung cells	Mediator of asthma and allergic responses; promotes anaphylaxis, smooth muscle contraction, bronchoconstriction, vascular permeability, hypersensitivity, chemoattractant for eosinophils
5-HETE	?	Granulocytes	Proliferative and chemotactic effects
12-HETE	?	Tumor cells	Enhances tumor cell adhesion
15-HETE	?	?	Produced during allergic rhinitis

^a Adapted from Funk et al. (40, 87).

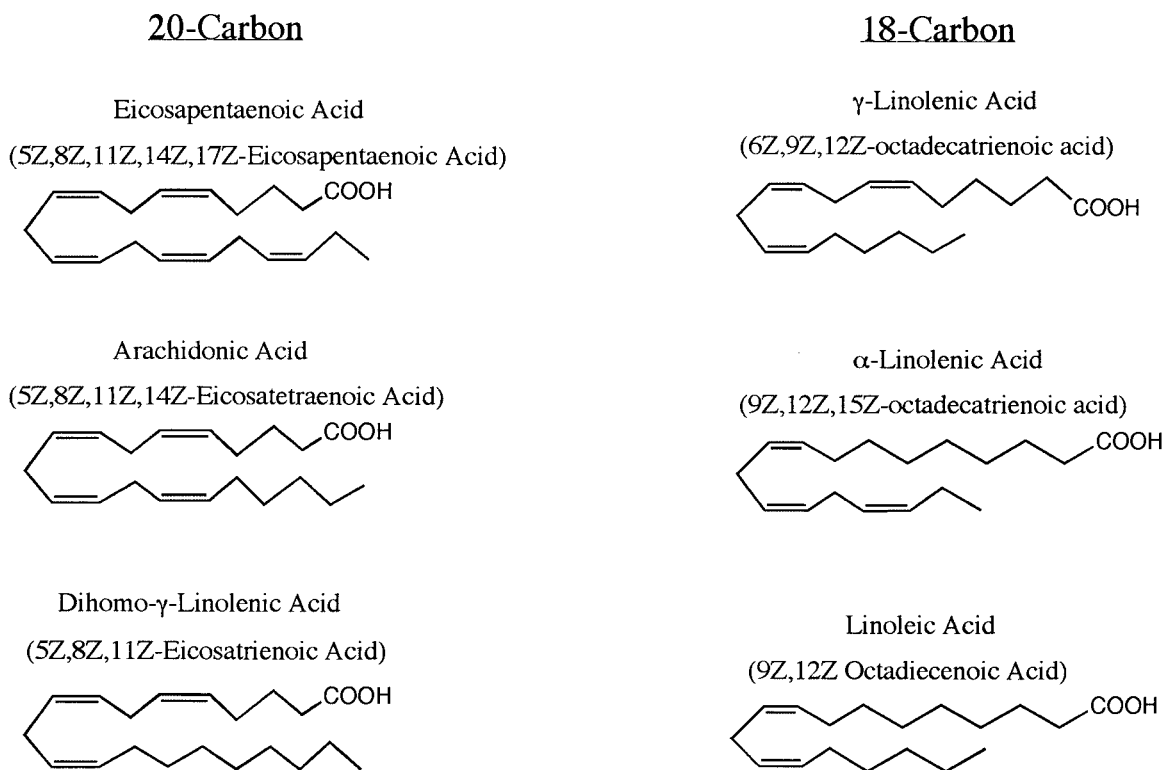


FIG. 1. Chemical structures of the 18- and 20-carbon fatty acid molecules that serve as the precursors for eicosanoid and oxylipin synthesis.

onic acid (87). Eicosanoids can be further divided into many subfamilies, the main ones being the prostanoids, which are products of a cyclooxygenase, and the leukotrienes and hydroxyeicosatetraenoic acid (HETE), which are products of lipoxygenase enzymes (Fig. 3).

Prostanoids are characterized by an enzymatically generated ring structure and include the prostaglandins and thromboxanes. The different classes of prostanoids are denoted by a letter, representing the oxygen substitution on the ring structure, and by a number, representing the number of *cis* double bonds in the lipid (113). For example, a prostaglandin of the E class derived from arachidonic acid will be denoted prostaglandin E₂ (PGE₂). The initial step of prostaglandin biosynthesis is catalyzed by cyclooxygenase (also known as prostaglandin H synthase), which produces a common precursor, prostaglandin H (PGH) (Fig. 4). This serves as the substrate for a number of different specific hydrolases and synthases that produce the downstream classes of prostaglandins and thromboxanes. In mammals, there are two cyclooxygenase enzymes, cyclooxygenase-1 being the constitutive form and cyclooxygenase-2 being the inducible form (87). These enzymes are inhibited by a class of drugs known as nonsteroidal anti-inflammatory drugs, which include aspirin, indomethacin, ibuprofen, and others. Novel drugs in this class selectively target the cyclooxygenase-2 enzyme, which is most often associated with asthma and allergy (137).

Leukotrienes are another subfamily of eicosanoids composed of linear lipids. The system of leukotriene nomenclature is similar to that of prostaglandins, with the letter representing the class and the number representing the number of *cis* double bonds. The initial step of leukotriene synthesis is catalyzed

by 5-lipoxygenase, which inserts a hydroperoxy group onto arachidonic acid, creating 5-hydroperoxyeicosatetraenoic acid (113). This species is either reduced nonspecifically to yield 5-HETE, leaving a stable hydroxyl group in place of the hydroperoxy group, or acted upon again by 5-lipoxygenase, which forms leukotriene A₄ (LTA₄), the first in the series of leukotrienes. LTA₄ then serves as the substrate for LTA₄ hydrolase, which adds a water molecule to form LTB₄. LTA₄ can also serve as the substrate for LTC₄ synthase, which adds a Gly-Cys-Glu tripeptide, creating LTC₄, which is the first in a series known as the cysteinyl leukotrienes. Subsequent removal of the glutamate residue from LTC₄ by gamma-glutamyl transferase creates LTD₄. Glycine is then cleaved from the cysteine moiety by dipeptidase, creating LTE₄. LTC₄, LTD₄, and LTE₄ are collectively known as the cysteinyl leukotrienes (113). Several other lipoxygenases exist which produce biologically active HETE compounds. 12-Lipoxygenase and 15-lipoxygenase are responsible for the production of 12-HETE, 15-HETE, and the lipoxins (113).

Protozoa, Helminths, and Fungi

Compared to mammalian oxylipin production, very little is known about the biochemistry of oxylipin production in eukaryotic microbes. Comparative genomic searches for the enzymes responsible for oxylipin production in protozoa, helminths, and fungi have not yielded a single enzyme with homology to the mammalian enzymes. However, phospholipase A₂ and phospholipase B have been identified in a large number of eukaryotic microbes, including *Toxoplasma gondii*, *Trypanosoma cruzi*, *Plasmodium falciparum*, *Lagenidium gigan-*

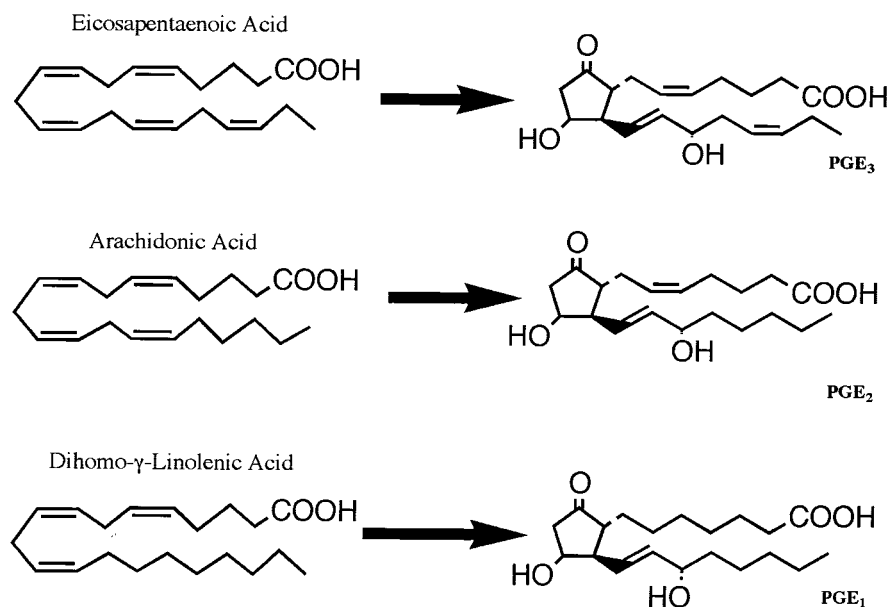


FIG. 2. Synthesis of 1, 2, and 3 series prostaglandins: different C₂₀ fatty acids are used as precursors for the 1, 2, and 3 series prostaglandins (e.g., PGE₁, PGE₂, and PGE₃). The series number corresponds to the number of double bonds in the fatty acid side chains of the prostaglandin.

teum, *Schistosoma japonicum*, *Trypanosoma brucei*, *Cryptococcus neoformans*, *Candida albicans*, and *Aspergillus fumigatus* (25a, 31a, 47, 62a, 112a, 114a, 123a, 144, 145). Phospholipases A₁, A₂, and B cleave the fatty acid side chains of phospholipids and have been implicated in virulence in a number of parasitic and fungal species, presumably via destruction of host cell membranes and subsequent lysis (47). However, cleaving polyunsaturated fatty acids from membrane phospholipids or lung surfactant also yields polyunsaturated fatty acid precursors for eicosanoid and oxylipin production. This is another potential role for secreted phospholipases A and B in the normal physiology of the organisms or in virulence.

The search for homologs of mammalian cyclooxygenase and lipoxygenase in eukaryotic microbes has proven to be difficult. Proteins that are similar to mammalian cyclooxygenase and lipoxygenases can be detected. However, the enzymes still remain to be cloned and are most likely distinct from mammalian enzymes. Proteins from the tissue nematode *Oesophagostomum dentatum* that cross-react with polyclonal antibodies directed against both mammalian cyclooxygenase-1 and cyclooxygenase-2 or cyclooxygenase-2 alone have been detected (58). However, these worm proteins cannot be detected with polyclonal antibodies against cyclooxygenase-1 alone or monoclonal antibodies against cyclooxygenase-1 or cyclooxygenase-2. In addition, proteins that cross-react with polyclonal antibodies against 12-lipoxygenase and 15-lipoxygenase but not 5-lipoxygenase have been detected.

Eicosanoids have also been detected in the blood fluke *Schistosoma mansoni* (1, 6, 42, 43, 115). Interestingly, researchers have detected soluble lipoxygenase activity but no cyclooxygenase activity (1). This enzyme produces a 15-HETE-like product from arachidonic acid as well as a 13-hydroxyoctadecanoic acid-like product from linoleic acid. The *Schistosoma mansoni* lipoxygenase is also inhibitable by mammalian and plant lipoxygenase inhibitors. Two lipoxygenase proteins have

been detected by immunoblot and by PCR with degenerate probes against plant and mammalian lipoxygenase. Overall, this suggests that the enzymes involved in the initial step of eicosanoid synthesis in eukaryotic microbes are similar to but distinct from the mammalian enzymes.

There are several lines of evidence that a cyclooxygenase may not be involved in nonmammalian eicosanoid synthesis. First, the classical inhibitors of prostaglandin synthesis do not inhibit nonclassical prostaglandin synthesis (69–71, 96, 97). Second, Blast searches of various fungal and parasite genomes do not reveal any sequences with significant homology to cyclooxygenases. Third, many eukaryotic microbes possess exceedingly small quantities of arachidonic acid or are unable to synthesize it (54). Although little is known about this “nonclassical” pathway for prostaglandin generation in eukaryotic microbes, we can draw from a large body of information about the generation of oxylipins in other eukaryotes, notably plants.

Despite the lack of evidence for the existence of a nonmammalian cyclooxygenase, other enzymes may be able to generate eicosanoids. Probably the most distinctive feature of a prostaglandin is the pentacyclic “head” region of the molecule. As shown in Fig. 4, half of the cyclooxygenase reaction adds a hydroperoxide group at carbon 15 and then reduces that hydroperoxide to a more stable hydroxyl group (87). While cyclooxygenase can generate this structure, other enzymatic reactions can also generate the pentacyclic head group. One such alternative pathway has been well described in the generation of the plant danger molecule jasmonic acid (Fig. 5) (87). In this pathway, 13-hydroperoxyoctadecatrienoic acid is generated from α -linolenic acid by means of a ω 6-lipoxygenase. This unstable intermediate can then be acted on by a number of enzymes, one of which is allene oxide synthase, which catalyzes the formation of an epoxide ring without disruption of the adjacent double bond. This intermediate can in turn be acted on by allene oxide cyclase to form the pentacyclic ring structure

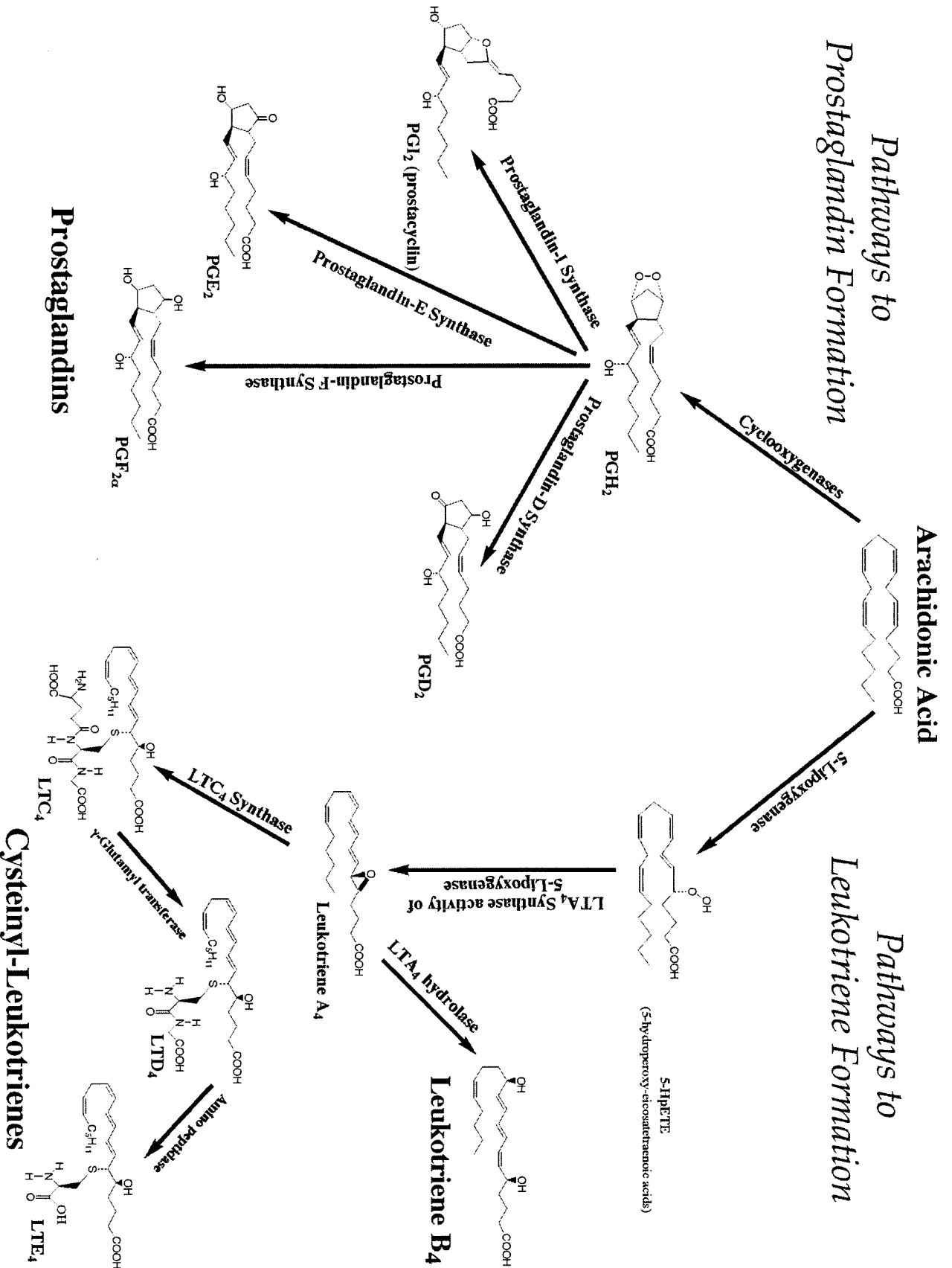


FIG. 3. Pathways to prostaglandin and leukotriene synthesis from arachidonic acid. Cysteinyl leukotrienes include LTC₄, LTD₄, and LTE₄.

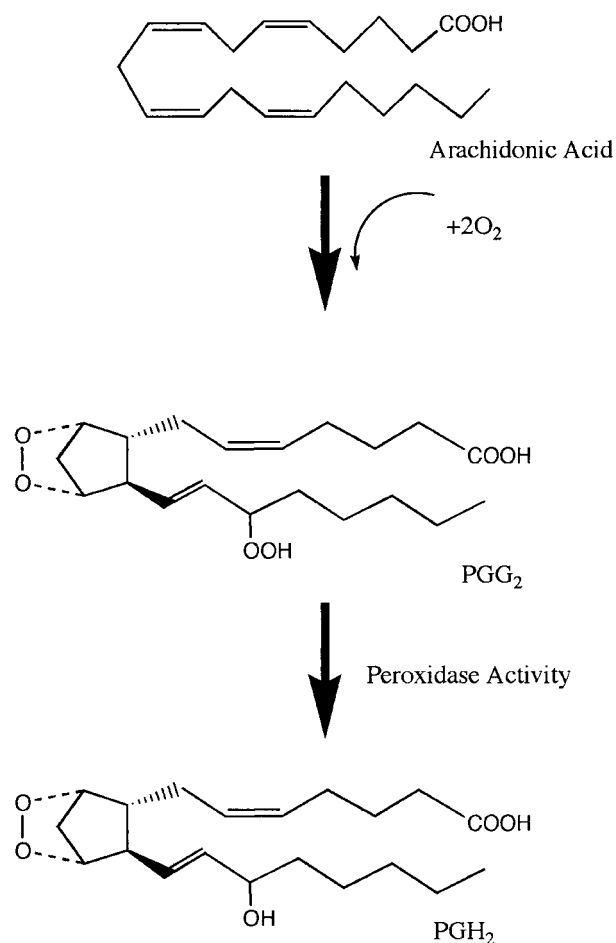


FIG. 4. Mechanism of cyclooxygenase action. The formation of PGH₂ is a two-step process beginning with the addition of two dioxygen molecules to arachidonic acid, followed by reduction of the peroxide at C-15.

found in the prostanoids. Of note, the product of this reaction, 12-oxophytodienoic acid, is structurally similar to 15-desoxy- $\Delta^{12,14}$ -PGJ₂, a prostanoid known to be a ligand for the nuclear receptor peroxisome proliferation-activated receptor gamma, which can downregulate macrophage activation (111).

Another alternative mechanism for the generation of prostaglandin-like compounds is the nonenzymatic generation of molecules called isoprostanes (93). These molecules are formed by the autooxidation of arachidonic acid by a free-radical mechanism. It is believed that these molecules are physiologically relevant signals of oxidative stress (112), as some of them possess profound vasoconstrictive properties (93). Structurally, they fall into four groups that are named according to the position at which the oxygen molecule attacks; however, only one species resembles an authentic prostaglandin.

Lipoxygenases have been described in many organisms and catalyze the addition of a dioxygen molecule to an unsaturated carbon chain, resulting in rearrangement, not destruction, of the double bonds (Fig. 6) (87). Lipoxygenases are generally named for the position into which they insert the hydroperoxide group; thus, a 12-lipoxygenase inserts the hydroperoxide at carbon 12 of its substrate. This last statement is an important

one, particularly when studying oxylipin formation in new systems. One aspect that is not described in the name is substrate preference, and while a mammalian lipoxygenase will use arachidonic acid as a preferred substrate, a fungal lipoxygenase may not. When describing a new lipoxygenase, consideration should be given to the niche that the organism occupies and the fatty acids that are available to it. Another potentially confounding issue is that lipoxygenases can use different fatty acids as substrates, although one will be preferred (87).

A series of oxygenases have been purified from the fungal

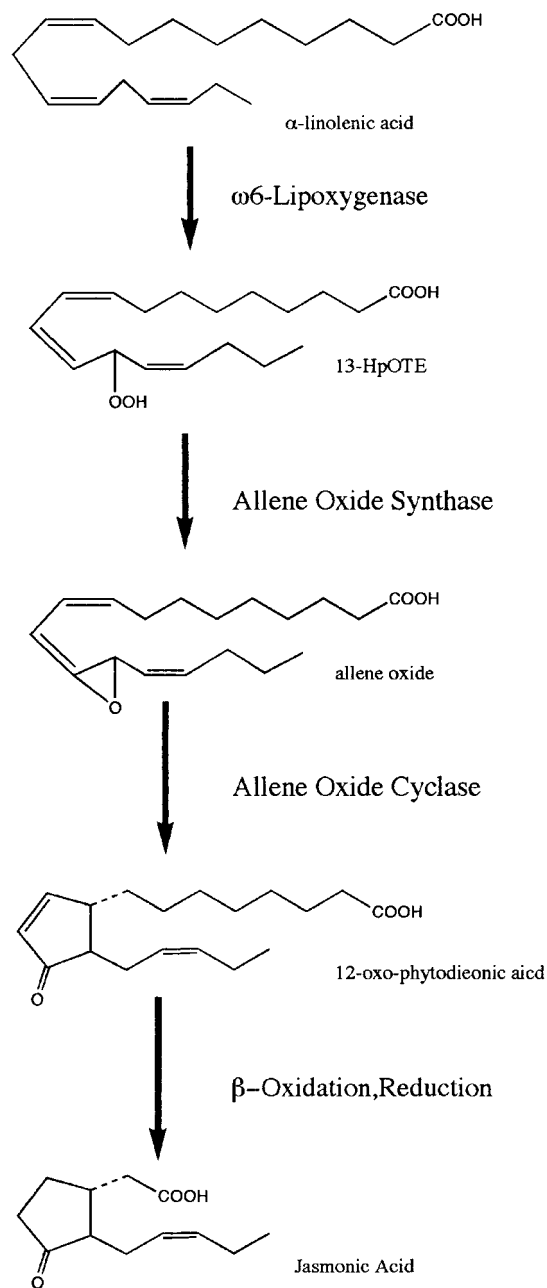


FIG. 5. Jasmonic acid synthesis. The jasmonic acid cascade is utilized by plants and represents an alternative method for the formation of prostanoid-like molecules without a cyclooxygenase. 13-HpOTE, 13-hydroperoxyoctadecatrienoic acid.

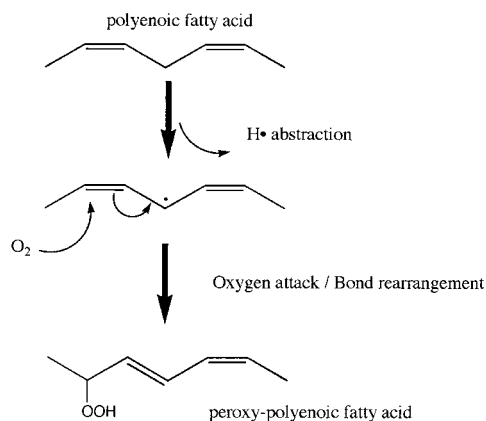


FIG. 6. Mechanism of lipoxygenase action. The first hydrogen abstraction takes place from a doubly allylic methylene (a CH_2 group flanked on either side by $-\text{CH}=\text{CH}-$), followed by the attack of a dioxygen molecule at C-2 from the radical and subsequent bond rearrangement.

wheat pathogen *Gaumannomyces graminis*, including a lipoxygenase and a linoleate diol synthase, which shows some homology to mammalian PGH synthases (cyclooxygenase) (57, 131). *G. graminis* also possesses a linoleic acid 8*R*-dioxygenase, which catalyzes the conversion of linoleic acid to 8-hydroperoxyoctadecadienoic acid, which immediately separates it from the lipoxygenase family of enzymes due to the insertion of a dioxygen molecule at a carbon not occupied by a double bond (16). Further investigation into the mechanism of this enzyme revealed that it does not utilize the bis-allylic radical, as in the lipoxygenase reaction (87); rather, the radical generated by hydrogen abstraction occurs at the same position as oxygen attack, without any shift in bond positions or orientation (16). The significance of this compound has yet to be revealed. However, the fact that none of the double bonds are altered during oxygenation raises the possibility that this compound could then be acted on by enzymes such as lipoxygenases to generate a unique oxylipin, unlike what one would expect to be possible from a dienoic fatty acid.

Recently, a 13-lipoxygenase that demonstrates a marked preference for linoleic and α -linolenic acid over arachidonic acid was purified from this fungus; however, details of this enzyme have yet to be published. A lipoxygenase was purified from *Saccharomyces cerevisiae* but was never cloned or sequenced (122). However, a bifunctional LTA_4 hydrolase from *Saccharomyces cerevisiae* has been cloned and characterized (72). This enzyme has 42% identity with mammalian LTA_4 hydrolase and produces LTB_4 from LTA_4 in vitro (73).

In summary, there are numerous biochemical differences between fungal-parasitic eicosanoid synthetic pathways and mammalian eicosanoid biosynthetic pathways. These differences have slowed the study of fungal-parasitic oxylipin production, but in turn, these differences may make fungal-parasitic oxylipin biosynthetic enzymes a viable target for the rational design of future therapeutics.

The search for other enzymes involved in the generation of specific eicosanoid products has been more successful in eukaryotic microbes. A glutathione-dependent transferase has been cloned from the nematode *Ascaridia galli* based on sequence similarity with a mammalian PGH-D isomerase (90).

The nematode enzyme is able to convert PGH to PGE class prostaglandins in vitro, making it the first PGH-E isomerase discovered in parasitic worms. This suggests that other glutathione-dependent transferase enzymes from parasitic worms may be involved in helminth eicosanoid synthesis.

Recently, a $\text{PGF}_{2\alpha}$ synthase from *Trypanosoma brucei* was purified, cloned, and expressed in *Escherichia coli* (69). Both live *Trypanosoma brucei* and lysates of the organism are readily capable of producing a number of oxylipin products that the researchers identified as prostaglandins. The production of these molecules was eliminated when the lysates were boiled, indicating enzymatic activity; however, prostaglandin production was not inhibitable by either aspirin or indomethacin. The cloned $\text{PGF}_{2\alpha}$ synthase from *Trypanosoma brucei* does not possess any sequence homology to mammalian $\text{PGF}_{2\alpha}$ synthases. Phylogenetic analyses of this protein revealed that it forms a distinct and distant phylogenetic clade from mammalian PGF synthases, with only 39 to 40% amino acid identity. Rather, the trypanosomal $\text{PGF}_{2\alpha}$ synthase is an NADPH-dependent oxidoreductase belonging to the aldol-keto reductase superfamily of enzymes. Interestingly, the $\text{PGF}_{2\alpha}$ synthase discovered in *Trypanosoma cruzi* (71) is similar not only to the $\text{PGF}_{2\alpha}$ synthase from *Trypanosoma brucei*, but also to the *Saccharomyces cerevisiae* old yellow enzyme, an oxidoreductase long known to exist in budding yeast but whose physiologic function remains a mystery (139). Blast searches of the sequence of the $\text{PGF}_{2\alpha}$ synthase from *Trypanosoma brucei* against the *Cryptococcus neoformans* and *Candida albicans* genomic sequences also revealed stretches of significant homology (M. C. Noverr, J. R. Erb-Downward, and G. B. Huffnagle, unpublished data).

Taken together, these data demonstrate the existence of a completely different system of prostaglandin synthesis in trypanosomes and likely other eukaryotic microbes, a nonclassical cyclooxygenase-dependent first step in prostaglandin synthesis and a nonclassical synthase-dependent second step.

EFFECTS ON EUKARYOTIC MICROBES

Protozoa

Eicosanoid production has been discovered in numerous protistan organisms, including parasites. Eicosanoids in these organisms are believed to play a role in life cycle control, growth stage-specific transformation, and sexual maturation. Among the protistan parasites known to produce eicosanoids and oxylipins are *Acanthamoeba* spp., *Trypanosoma brucei* (causative agent of trypanosomiasis), and *Plasmodium falciparum* (causative agent of malaria).

As eicosanoid production has been reported in various protistan parasites, it has been noted that production varies with growth stage, suggesting a role in life cycle control. *Trypanosoma brucei* produces prostaglandins endogenously or from exogenously supplied arachidonic acid, with $\text{PGF}_{2\alpha}$ being a major metabolite produced (69). The protozoan $\text{PGF}_{2\alpha}$ synthase has been cloned from *Trypanosoma brucei* (69). *Trypanosoma brucei* $\text{PGF}_{2\alpha}$ synthase mRNA expression and enzyme activity are dependent on growth stage, with expression and activity higher in the early logarithmic growth and lower in the stationary phase. *Plasmodium falciparum*, like *Trypanosoma*

brucei, produces prostaglandins both endogenously and exogenously (70). Prostaglandin production (PGE₂, PGD₂, and PGF_{2α}) is highest in the trophozoite and schizont stages in the life cycle of *Plasmodium falciparum*, two stages responsible for infection of the host.

The mechanism of production of eicosanoids and oxylipin molecules in these organisms is just beginning to be understood (69–71). However, over the years many studies have described pronounced effects of oxylipins on the growth and development of the protozoan parasites, and while these pieces of evidence have been scattered, they are compelling indicators that long-chain fatty acids and oxylipins are of critical importance to these organisms (12, 21, 30, 36, 52, 68, 69, 70, 81, 98). Some of the more striking results of these studies include inhibition of growth of *Plasmodium falciparum* in culture and *Plasmodium vinckei petteri* and *Plasmodium yoelii nigeriensis* in vivo by C₁₈ fatty acids (68), reversal of chloroquine resistance in a resistant strain of *Plasmodium berghei* when infected mice are treated with prostaglandin analogs, and even direct cytotoxicity towards *Toxoplasma gondii* by platelet-derived thromboxane A₂ and 13-hydroxyoctadecanoic acid (52, 142).

Eicosanoid production has also been detected in slime molds, which are protists that resemble fungi in life style. With the division *Oomycota*, the organisms *Lagenidium giganteum* and *Saprolegnia parasitica* have been found to produce not only traditional arachidonic acid metabolites but also novel compounds. The life cycle of *Lagenidium giganteum*, a parasite of mosquito larvae, is regulated by endogenous oxidative lipid metabolism (63). Inhibitors of lipoxygenase and cyclooxygenase prevent maturation of the sexual stage, the oospore (62). This inhibition could be overcome by addition of partially purified culture supernatants of oospore stage-specific *Lagenidium giganteum*. This indicates that lipoxygenase and/or cyclooxygenase products are responsible for regulating life cycle progression in this organism.

As in *Lagenidium giganteum*, cyclooxygenase inhibitors (indomethacin and aspirin) prevent sexual maturation in *Saprolegnia parasitica*, a fish parasite (55). In addition, treatment with these inhibitors results in asterisk-shaped colony morphology owing to shortened branching patterns of the slime mold. This abnormal colony morphology can be overcome by addition of PGE_{1α} to the growth medium. Addition of PGE_{1α} does not, however, overcome inhibition of sexual maturation. Overall, this indicates that different endogenous prostaglandins or prostaglandin-like products are involved in regulation of growth and sexual maturation.

Helminths

Parasitic worms contain a variety of polyunsaturated fatty acids in their cellular membranes, including arachidonic acid. Eicosanoids, both prostaglandins and leukotrienes, have been detected in many species of helminths, including human pathogens. As in protistan parasites, eicosanoids and oxylipins are involved in different aspects of life cycle regulation and sexual maturation.

The first of these, *Schistosoma mansoni*, has a complex life cycle consisting of a free-swimming miracidia which infects the snail as an intermediate host, transforms itself, and is released as cercariae (100). The cercariae, upon encountering a mam-

malian host, burrow into the skin and undergo transformation once again, this time into schistosomula. At this stage, *Schistosoma mansoni* enters the vasculature and migrates to the portal system, where the schistosomula mature into adult male and female worms (100). Sexual reproduction occurs, and the female releases eggs that leave the vein, burrowing through the tissues to enter the intestine, where they are released from the mammalian host in the feces. Eventually, the eggs are returned to a water source, where they hatch to release miracidia, completing the cycle (100). Since the only form that can infect mammals is the cercarial form (100), a great deal of attention has been given to the transition from cercariae to schistosomula.

Cercariae from *Schistosoma mansoni*, a human blood fluke, produce eicosanoids from linoleate, presumably via conversion of linoleate to arachidonic acid. The cyclooxygenase products PGE₁, PGE₂, PGD₂, and PGA₂ can be detected along with lipoxygenase products LTB₄, 5-HETE, and 12-HETE (42, 115). Treatment of *Schistosoma mansoni* cercariae with either cyclooxygenase or lipoxygenase inhibitors decreases both prostaglandin and leukotriene-HETE production (115). In 1970 it was determined that *Schistosoma mansoni* is incapable of synthesizing long-chain fatty acids and sterols de novo despite a membrane that is replete with these molecules (91). This led researchers to study the effects of various fatty acids on *Schistosoma mansoni*, which led to two important discoveries. First, agar infused with 3.3 mM linoleic acid is sufficient to induce the transformation from cercariae to schistosomula (41). Second, incubation with linoleic acid induced the release of a variety of eicosanoids and oxylipin products (42). Researchers also found that by treating the cercariae with ibuprofen and esculetin, inhibitors of cyclooxygenases and lipoxygenases, respectively, they could decrease the linoleic acid-induced transformation and decrease production of oxylipin products. However, neither inhibitor behaved as expected, for decreases were seen in both cyclooxygenase and lipoxygenase products for each of the inhibitors (43, 115).

This control of transformation can be seen mirrored in the filarial parasite *Brugia malayi*. Microfilariae from *B. malayi* have been shown to produce eicosanoids (PGE₂, PGD₂, 6-ke-to-PGF_{1α}, PGF_{2α}, and thromboxane B₂) from both endogenous and exogenous arachidonic acid in vitro. It has also been recognized that these molecules probably play a critical role in preventing platelet aggregation around the parasite as it grows in the blood vessel (79). However, the effect of oxylipin molecules on the growth and development of *B. malayi* has only recently been discovered. As with many of the parasites, culturing certain stages of *B. malayi* has proven difficult (124). In particular, a serum-free in vitro system that promotes molting to the fourth-stage larva (L4) from infectious third-stage larva (L3) remains elusive. Interestingly, addition of arachidonic or linolenic acid along with the basidiomycetous yeast *Rhodotorula minuta* to cultures of *B. malayi* can overcome this requirement for serum (124). As has been discussed previously, many fungi are known to turn various fatty acids into oxylipin products, and since the fatty acids alone did not produce significant amounts of L3 to L4 molting, the role of oxylipins in this process was strongly supported. In a follow-up study, these researchers confirmed the role of oxylipin prod-

ucts by abolishing L3 to L4 molting with the addition of various lipoxygenase inhibitors (125).

Interestingly, eicosanoid production in the cestode *Spirometra erinaceieuropaei*, a mammalian and reptilian parasite, is time and temperature dependent (44). Eicosanoid production also correlates with changes in fatty acid composition. When incubated with arachidonic acid, PGE₂, PGD₂, 6-keto-PGF_{1α}, and PGF_{2α} but not TxB₂ was differentially detected in worm supernatants (39). The differential expression of these eicosanoids could be a mechanism for adaptation to hosts with various body temperatures.

Eicosanoid products have also been detected in nematodes. *Oesophagostomum dentatum* is a nodular parasitic worm that infects the swine intestine. This arachidonic acid-containing parasite produces both prostaglandins (PGE₂, PGD₂, 6-keto-PGF_{1α}, PGF_{2α} and TxB₂) and leukotrienes (LTB₄ and cysteinyl leukotrienes) endogenously (26, 58). Inhibitors of both cyclooxygenase and lipoxygenase prevented migration and growth of the worm and, more specifically, prevented development to stage 4 larvae (26–28). These effects were reversible, and the inhibition was overcome by addition of exogenous eicosanoids. The individual prostaglandins and leukotrienes were differentially detected in worm homogenates and supernatants at different times of in vitro cultivation, indicating complex regulation of expression according to growth stage.

Fungi

Eicosanoids were first discovered in fungi in the early 1990s through investigation of fungal fatty acid production in environmental fungi (64). Since that time, eicosanoid and oxylipin production has been observed in a number of fungal species. A survey of both pathogenic and nonpathogenic fungal species reveals that all species produce detectable amounts of both cyclooxygenase and lipoxygenase products endogenously and from arachidonic acid (97). Investigations of fungal eicosanoid products and their pathways indicate a role in sexual maturation and life cycle control as well as in pathogenesis of virulent fungi.

The arachidonic acid metabolites PGF_{2α} and PGF_{2α}-lactone have been detected in a number of environmental yeasts of the *Lipomyetaceae* family (*Dipodascopsis*, *Lipomyces*, *Zygozoma*, and *Myxozyma*), as well as *Saccharomyces cerevisiae* (64). Fungal extracts inhibit blood platelet aggregation, a well-known function of PGF_{2α}, and the production of these compounds can be inhibited by aspirin, indicating that an enzyme similar to mammalian cyclooxygenase may be involved in their production (64). A more thorough examination of the effects of aspirin on fungal eicosanoid production revealed that inhibition of prostaglandin products (3-HETE, PGE₂, and PGD₂) correlated with increases in lipoxygenase products (5-HETE, 12-HETE, and 15-HETE), suggesting not only that both pathways exist in fungi but also that arachidonic acid can be shunted from one pathway to the other (25). When *D. uninucleata* is fed exogenous arachidonic acid, a novel aspirin-inhibitable eicosanoid, 3-HETE, is formed, confirming the presence of an arachidonic acid cascade in these yeasts (136).

One of the immediate controversies to arise in relation to fungal eicosanoid production is determining whether fungi produce authentic eicosanoids de novo. Many of the tech-

niques used to measure eicosanoids exhibit cross-reactivity with chemically similar species, and polyunsaturated fatty acids more than 18 carbons long are rarely detected in many species of fungi (80). However, more reports of arachidonic acid-producing fungi arise as researchers optimize growth conditions. A species of soil fungus, *Mucor genevensis*, can convert exogenous arachidonic acid to the oxylipin 3-HDTE, which is an 18-carbon species (14, 106). In addition, this fungus also produces large quantities of eicosanoid precursors (dihomo- γ -linolenic acid, arachidonic acid, and eicosanopentaenoic acid) when grown under specific conditions. Another genus of arachidonic-producing fungus, *Mortierella*, was found to be sensitive to aspirin in its growth medium and produced PGE₂ and PGF_{2α} de novo (15, 76). Therefore, many species of fungi do possess arachidonic acid and are able to produce authentic prostaglandins de novo.

Evidence has accumulated suggesting that fungal eicosanoid production is involved in growth and maturation of the organisms. A connection exists between eicosanoid production and life cycle regulation in the family *Dipodasaceae*. Eicosanoid production in these yeasts varies depending on life cycle stage (25). The highest eicosanoid production occurs during ascosporeogenesis, which marks the transition from the sexual stage to the asexual stage. Completion of the yeast life cycle could be inhibited by indomethacin and aspirin (13). In addition, ascospore aggregation could be inhibited by aspirin (66). Novel hydroxylipins that are localized to the surface of aggregating vegetative cells have been discovered in *Saccharomyces cerevisiae*, *Saccharomycopsis malanga*, and mucoralean fungi (65, 67, 120). Similarly, the growth and conidiation of the filamentous fungus *Trichoderma viride* are inhibited by indomethacin and rapanidal. *Saccharomyces cerevisiae* was also found to be sensitive to these drugs. The nature of the growth-inhibitory effects of nonsteroidal anti-inflammatory drugs on fungi and the molecular targets of the drugs remain to be elucidated.

The potential for eicosanoid production in medically important fungi has been overlooked until recently. The pathogenic yeasts *Cryptococcus neoformans* and *Candida albicans* produce eicosanoids (both prostaglandins and leukotrienes) endogenously or from exogenous arachidonic acid (96). Treatment of these yeasts with cyclooxygenase inhibitors has proven to be toxic in vitro, suggesting a requirement for an eicosanoid product or biosynthetic pathway in growth (96). A novel eicosanoid product (3,18-diHETE [3,18-dihydroyeicosatetraenoic acid]) has been detected in *Candida albicans*, which is related to the 3-HETE product detected in the lipomyetacean fungi (33). By immunofluorescence, products that cross-react with 3-HETE have been detected in hyphae but not in yeast forms of *Candida albicans*. This suggests that fungal eicosanoids may be involved in *Candida* morphogenesis. The production of 3,18-diHETE could be inhibited by aspirin 33, which also suppresses the growth of the yeast form and prevents the yeast to hypha transition of *Candida albicans* (32). Exogenous PGE₂ from either host or fungal sources enhances germ tube formation in *Candida albicans*, implicating fungal eicosanoids as a morphogenic factor (61, 96). The yeast to hypha transition is often associated with progression of infection by *Candida albicans*, and therefore, fungal eicosanoid regulation of morphogenesis may be considered a virulence mechanism.

Another function of oxygenated fatty compounds that has

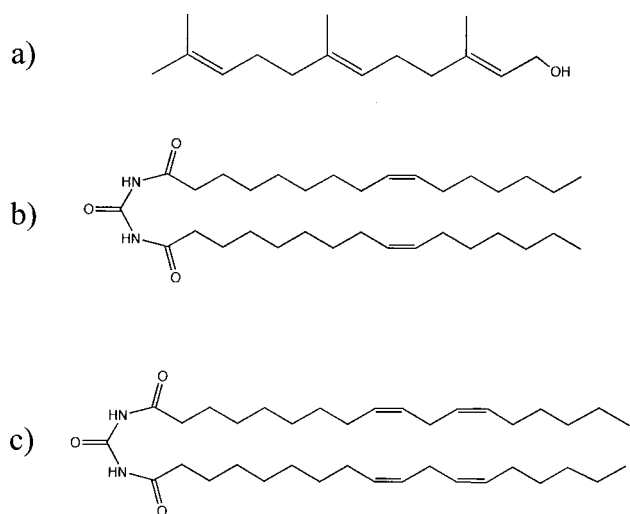


FIG. 7. Other fungal lipid signaling molecules. (a) *trans,trans* stereoisomer of farnesol ($C_{15}H_{26}O_5$), a quorum-sensing molecule in *Candida albicans*. (b and c) Diacylated urea compounds isolated from *Saccharomyces cerevisiae* that have recently been identified as chemoattractant molecules.

only recently been described is in quorum sensing. Quorum sensing is the phenomenon by which a group of single-celled organisms coordinate their behavior in a manner akin to a multicellular organism (9). The mechanisms of quorum sensing have been well studied in bacteria, where particular molecules, such as homoserine lactones (called autoinducers), are released from individual bacteria and accumulate until a particular concentration of autoinducer is achieved. Upon reaching this concentration, the organisms each respond by up-regulating factors that are beneficial to the survival of the organisms within the group (9). Recently, *Candida albicans* has been shown to demonstrate the phenomenon of quorum sensing, the first for a eukaryotic organism. The quorum-sensing molecule in *Candida albicans* has been identified as the long-chain polyunsaturated alcohol farnesol (Fig. 7a) (56). The quorum behavior that was studied was the phenomenon of germ tube formation. *Candida albicans* germ tube formation is a population density-dependent phenomenon and occurs at densities of less than 10^6 yeast cells/ml. The addition of farnesol to cells that would otherwise form germ tubes resulted in a culture of budding yeast cells. Thus, farnesol provides a signal that effectively mimics a higher concentration of cells.

Another area where farnesol has been implicated is in the proper functioning of mating factor signaling. In the yeasts *Cryptococcus neoformans* and *Ustilago maydis*, the mating factor is a peptide that is often posttranslationally modified by farnsylation. Interestingly, it has been found that in the absence of farnsylation, the mating factor is rendered up to 1,000-fold weaker than the farnylated peptide (29). While this behavior has not been directly implicated in quorum sensing, it is interesting to consider that induction of mating behaviors only makes sense if other cells are in close proximity. It will be interesting to see what the future holds for this fatty compound and others in fungal biology. In a subsequent study, farnesol was employed, with great effect, to prevent the formation of *Candida albicans* biofilms, a virulence trait that allows *Candida*

albicans to colonize medical equipment and biomaterials such as orthopedic joints (108). Studies such as this suggest new ways of potentially controlling *Candida albicans* infections, in which a drug could be targeted not to kill the organism but to prevent its transformation into a more virulent phenotype.

EFFECTS IN INFECTION

Eicosanoids and Immunomodulation

In mammals, eicosanoids regulate many systems in a manner akin to hormones. They are involved in regulation of the cardiovascular system, renal function, and reproduction and may play a role in carcinogenesis. However, they are most often associated with regulation of inflammation and allergy. To date, there are over 10,000 references pertaining to eicosanoids and the immune system in the literature. Eicosanoids regulate both proinflammatory and anti-inflammatory responses of the immune system (87). A single eicosanoid can have pleiotropic effects due to the existence of multiple receptors for each lipid species. In turn, these receptors exert different responses on different cell types (Tables 1 and 2) (40, 50, 87). Another unique characteristic of eicosanoids is their potency at very low (nanomolar) concentrations. In relation to this potency, most eicosanoids have very short half-lives, being made de novo (as opposed to stored and released) and acting near their site of synthesis (40, 87). This, combined with the overwhelming number of members of this class of regulators, makes investigation difficult. However, much progress has been made, and many of the pro- and anti-inflammatory functions and cellular targets of the eicosanoids have been determined (Tables 1 and 2).

Prostaglandins are often associated with anti-inflammatory activities such as inhibition of effector functions of inflammatory cells. These include inhibition of mediator release from macrophages, neutrophils, mast cells, basophils, and lymphocytes (59). PGE_2 and other eicosanoids can downregulate macrophage function, including phagocytosis (74, 103, 128, 130). Eicosanoids are membrane diffusible, and many are potent ligands of the intracellular receptors peroxisome proliferation-activated receptor alpha and gamma in macrophages (135). Binding to peroxisome proliferation-activated receptor alpha and gamma causes macrophage deactivation. Thus, one potential mechanism of intracellular survival of eukaryotic microbes in macrophages is deactivation of macrophages by prostaglandins and leukotrienes produced by the microbe (in phagosomes or extracellularly). Prostaglandins can also inhibit Th1-type immune responses, chemokine production, phagocytosis, and lymphocyte proliferation (11, 74, 88, 103, 121, 128, 130). Prostaglandins can promote Th2-type responses and tissue eosinophilia (31, 88, 103, 126). Prostaglandins also mediate proinflammatory activities such as vasodilation, which potentiates pain and edema, as well as leukocyte recruitment (eosinophils) (59, 92, 107). Evidence is now accumulating that prostaglandins (especially PGE_2) likely play a role in overall immune regulation (positive and negative) (102, 104); thus, production of prostaglandins by eukaryotic microbes is likely an important pathogenic mechanism for these organisms.

Leukotrienes are most often associated with proinflammatory functions, such as upregulation of adhesins on endothelial

and inflammatory cells, recruitment of leukocytes (neutrophils and eosinophils), and upregulating phagocytosis (38, 82–85) (59). 5-Lipoxygenase knockout mice are more susceptible to pulmonary bacterial infection, and phagocytosis is impaired in these mice (7, 85). LTB₄ is a potent neutrophil chemotactic factor, while the cysteinyl leukotrienes play a role in eosinophil recruitment (17). In addition, most members of this group of eicosanoids are potent bronchoconstrictors, mediating many of the symptoms of allergic asthma (94). Eosinophil infiltrates are a common feature of many chronic fungal infections, and fungi are a common cause of atopic diseases (75, 86). In addition, other eukaryotic microbes such as *Leishmania* spp. elicit an immune response characterized by a dynamic T1/T2 balance that is dependent on numerous factors (110, 114) (note that T2 responses are not characteristic of prokaryotic pathogen infections). Overall, eicosanoids provide a complex network of control in immune responses during infection and disease manifestation.

Protozoal Infections

The intracellular protistan parasite *Toxoplasma gondii* finds its niche within host macrophages during infection. Eicosanoid production is altered by parasite infection in multiple host cell types, leading to modulation of both parasite and host activities. Infection of macrophages induces altered prostaglandin and leukotriene production (134). Surprisingly, treatment of parasites with phospholipase A₂ inhibitors prevents not only eicosanoid production by macrophages but also penetration of the cells by *Toxoplasma gondii* (134). This indicates a role for parasite enzymes in host eicosanoid production and a role for host eicosanoids in parasite infectivity. In addition, treatment of macrophages with a 5-lipoxygenase inhibitor decreased gamma interferon-induced antitoxoplasma activity, suggesting that 5-lipoxygenase products may help control infection (143).

Overall in *Toxoplasma gondii* infections, elaboration of controlled proinflammatory cell-mediated responses is required to control an infection without causing excessive damage to the host (141). Induction of interleukin-12 production by host dendritic cells and subsequent gamma interferon induction is central to initiation of a successful inflammatory response (46). Dendritic cell interleukin-12 induction by *Toxoplasma gondii* soluble antigen is dependent upon CCR5 and G-protein-coupled signaling. Interestingly, CCR5 and interleukin-12 production are downregulated after primary exposure to antigen, which leads to dendritic cell paralysis (4). This unresponsiveness to a secondary antigen exposure is mediated by induction of host lipoxin A₄, which signals via a G protein receptor (Fig. 8) (5). Mice deficient in 5-lipoxygenase are more susceptible to *Toxoplasma gondii* infection, suggesting a role for lipoxin A₄ in regulation of the inflammatory response (3).

As mentioned previously, *Trypanosoma brucei* produces prostaglandins from exogenous arachidonic acid (69). A role for trypanosomal prostaglandin production in pathogenesis remains to be proven. However, prostaglandin production is known to be upregulated in the host upon trypanosomal infection (37, 101). Host prostaglandin production is believed to be involved in different aspects of pathogenesis, from elaboration of symptoms to modulation of immune responses (51). Host prostaglandin production is involved in induction of nitric ox-

ide and suppression of lymphocyte proliferation. Inhibition of prostaglandin production with cyclooxygenase inhibitors results in increased proinflammatory cytokine production (105). This indicates a role for host prostaglandins in control of disease and supports the idea that parasite-derived prostaglandins can participate in pathogenesis.

Plasmodium falciparum also produces prostaglandins, both endogenously and exogenously (70). Prostaglandin production (PGE₂, PGD₂, and PGF_{2α}) is highest in the trophozoite and schizont stages in the life cycle of *Plasmodium falciparum*. The malarial fever, which is believed to be mediated in part by prostaglandins, is associated with a burst of schizont-stage parasites. This suggests that parasite-derived prostaglandins may play a direct role in elaboration of disease symptoms. In addition, in vivo production of tumor necrosis factor alpha has been shown to decrease parasite growth. PGE₂ can downregulate tumor necrosis factor alpha expression, and it is therefore tempting to speculate that PGE₂ produced by the parasite could likewise mediate this effect. It is not unreasonable to believe that immunomodulation by the parasite is a likely outcome of in vivo prostaglandin production.

Both pathogenic and nonpathogenic *Acanthamoeba* species endogenously produce a variety of prostaglandins, including PGE₂, PGD₂, and PGF_{2α} (48). However, pathogenic species produce more PGF_{2α} than nonpathogenic species. Prostaglandins can enhance penetration of tissue by parasitic worms; therefore, PGF_{2α} in protistan parasites may play a similar role (41).

Helminth Infections

As with other parasitic worms, helminth infections are chronic in nature, with parasites surviving for long periods of time in host tissues. The strategies used by the parasites to evade the immune responses are largely unknown. However, infection is associated with increases in host eicosanoid production, which regulate responses to infection and mediate symptoms of infection. The discovery that the worms also produce eicosanoids provides one mechanism through which the parasites could induce symptoms, modulate the immune response of the host, and increase infectivity.

Many of the symptoms associated with parasitic disease progression are mediated by eicosanoids. Among the most prevalent causes of lymphatic filariasis are the eicosanoid-producing species *Brugia malayi* and *Wuchereria bancrofti* (78, 79). Adult worms live in the lymphatic vessels of the host and release microfilariae into the bloodstream of infected hosts. The ability of microfilariae to survive within the bloodstream is partly explained by their ability to inhibit platelet aggregation and thromboxane release by host cells, activities known to be mediated by prostaglandins (79). In some cases, release of the microfilariae results in a severe inflammatory response characterized by pulmonary eosinophilia, which can be mediated or exacerbated by PGE₂ (99).

Likewise, *Fasciola hepatica*, a sheep liver fluke, causes an infection whose symptoms include anemia, fever, and liver damage, which can be mediated by eicosanoids. Adult *F. hepatica* worms were cultured from bile ducts of infected sheep, and PGE₂, PGI₂, TxB₂, and LTB₄ were subsequently detected in culture supernatants and parasite homogenates (2). There-

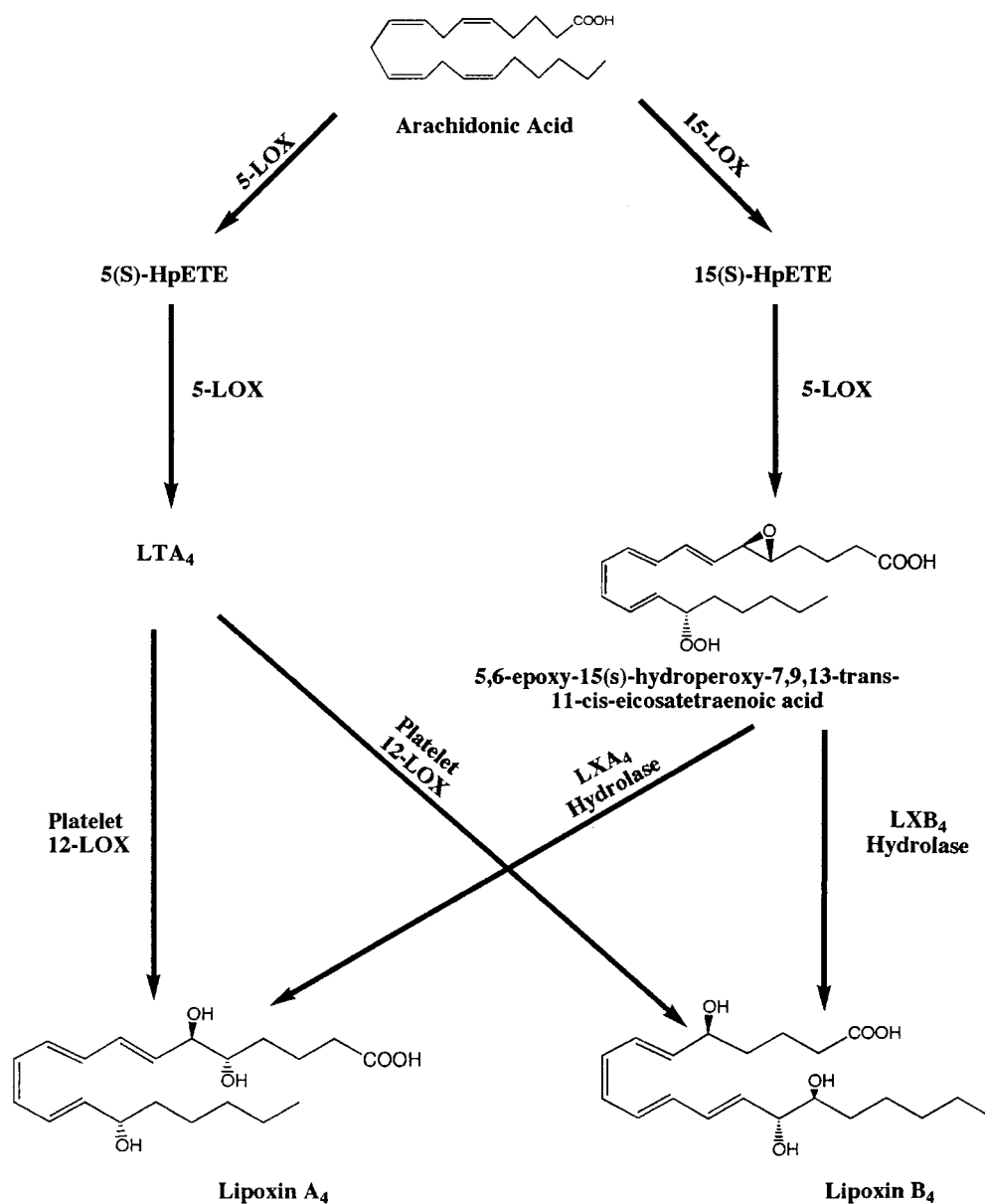


FIG. 8. Formation of lipoxins by lipoxygenases. Lipoxin A₄ is another eicosanoid derivative of lipoxygenases that has recently been implicated in the immune response to *Toxoplasma gondii*. 5-LOX, 5-lipoxygenase; 12-LOX, 12-lipoxygenase; 15-LOX, 15-lipoxygenase; HpETE, hydroperoxyeicosatetraenoic acid.

fore, the parasite itself may mediate infection symptoms via endogenous eicosanoid production.

A role for nematode eicosanoids in pathogenesis has been proposed. In vivo, infection with the heartworm parasite *Dirofilaria immitis* causes significant depression of endothelium relaxation, a symptom associated with disease. Treatment of *Dirofilaria immitis* but not host vasculature with cyclooxygenase inhibitors prior to infection prevented depression of heart endothelium relaxation (60). This indicates that parasite-derived eicosanoids are responsible for this effect on the host, which is associated with the pathogenesis of filariasis.

In addition, recent evidence suggests that the parasites use oxylipins to evade the host's immune system. A generalized immune suppression is known to exist in most parasitic infec-

tions, even though the species causing the infections are quite diverse (6, 22, 35, 49, 78, 109, 132, 133). Prostaglandins, leukotrienes, and other oxylipins are potent immunomodulatory molecules. They can act directly on leukocytes and indirectly through the induction of cytokines that control the development of inflammation, T1/T2 cellular immunity, and humoral immunity. Thus, evolutionary convergence may have resulted in parasites with eicosanoid biosynthesis to enhance or facilitate parasitism.

The best evidence of parasite-derived prostanoids in the subversion of the immune response comes from studies investigating the helminth *Schistosoma mansoni*. As has been mentioned previously, *Schistosoma mansoni* infects the host as cercariae, which burrow into the skin and undergo transformation

to schistosomula. Polyunsaturated fatty acids and eicosanoids stimulate cercarial penetration of host skin (116, 117). Production of eicosanoids correlates with both penetration and transformation, two activities required for infection. Penetration correlates with increasing leukotriene and HETE production, while transformation is correlated with increasing prostaglandin levels (41). This indicates that either the fatty acid or a parasite-derived eicosanoid is involved in control of virulence mechanisms. It has been estimated that the cercariae remain in the skin for 3 to 4 days. However, during that time the parasite does not come under attack by the innate immune system. In a more recent study, it was found that skin-stage schistosomula produce significant amounts of PGE₂ and also induce the production of PGE₂ and interleukin-10 from the host (6), both of which are known to be able to subvert a T1 to a T2 response (11, 45, 123, 126). While this study did not employ an in vivo system to study virulence directly, it is interesting that PGE₂ and interleukin-10 have also been implicated in the generation of regulatory T cells, which can produce chronic infections (89).

More recent evidence provides a role for *Schistosoma mansoni* eicosanoids in modulation of the immune response in vivo (6). It was observed that cutaneous infection by *Schistosoma mansoni* blocked local Langerhans cell migration to the lymph node. Langerhans cells are dendritic cells that reside in the skin, and their migration is a prerequisite for initiation of contact hypersensitivity. This inhibition of migration was effective not only for the inflammation caused by the cercariae infection, but also against the inflammation caused by tumor necrosis factor alpha administration. A parasite-derived lipophilic product mediated this anti-inflammatory mechanism of the parasite. Further analysis suggested that it was PGD₂ (6). When treated with a PGD₂ receptor agonist, Langerhans cell migration could be blocked in a similar manner, and when treated with a PGD₂ receptor antagonist, the migration of Langerhans cell in *Schistosoma mansoni*-infected tissues could be restored. These studies suggest a novel mechanism for parasite evasion of immune responses.

One of the more interesting aspects of many parasites is the ability to grow and reproduce in multiple hosts of widely disparate origins. To accomplish this, parasites have adopted a strategy of changing their physical forms to suit their environment. This has, of course, made the study of these organisms difficult, as frequently the only means to obtain an organism at a particular stage of development is to grow it within a host. In some cases host serum will suffice, but it is safe to say that for the most part, in vitro culturing of many parasites is difficult. While the exact nature of the signals that trigger the transformation from one form to another has been elusive, some have recently been discovered. Notably, in the helminths *Schistosoma mansoni* and *Brugia malayi*, these transformation signals are oxylipin products (1, 125).

Fungal Infections

A series of studies implicate fungal eicosanoid production in the pathogenesis of *Cryptococcus neoformans* infection. *Cryptococcus neoformans* is an opportunistic pathogenic yeast acquired via the respiratory tract, and it possesses a phospholipase (PLB1) that can degrade the phospholipid components

of cellular membranes and lung surfactant (24). Furthermore, phospholipase activity among different strains of *Cryptococcus neoformans* correlates with virulence in mice (23). PLB1 is required for virulence during infection acquired via the respiratory tract (95). Mice infected with a wild-type strain generate a nonprotective inflammatory response with subsequent eosinophilia, while mice infected with a PLB1-deficient strain generate a protective immune response that controls the infection. The PLB1-deficient strain produces lower levels of eicosanoids when incubated with arachidonoyl-phosphatidylcholine, and infected mice have lower levels of eicosanoids in lung homogenates (95).

In addition, *Cryptococcus neoformans* and *Candida albicans* produce a PGE-like compound de novo and from arachidonic acid. This fungal compound can downmodulate chemokine production, tumor necrosis factor alpha production, and splenocyte proliferation while upregulating interleukin-10 production. These are all activities previously documented for mammalian PGE₂ (123). In addition, prostaglandin levels in culture supernatants of different *Cryptococcus neoformans* strains correlate with their ability to evoke eosinophilia in mice (M. C. Noverr and G. B. Huffnagle, unpublished observations).

A PGE series cross-reactive product has been isolated from these fungi and has effects on host cells similar to those of commercially available PGE₂, including alteration of cytokine production and inhibition of cellular proliferation (96). *Candida albicans* is known to cause release of arachidonic acid from host tissues (19, 20). Interestingly, arachidonic acid stimulates the growth of *Candida albicans* when used as a sole carbon source (33).

Noneicosanoid lipids from fungi also participate in immunomodulation and pathogenesis. *Candida albicans* produces aspirin-sensitive oxylipins from arachidonic acid. Treatment of strains isolated from patients with recurrent vaginal candidiasis with aspirin inhibits not only oxylipin production but also growth (32). The oxylipins are selectively located in *Candida albicans* hyphae, as opposed to the yeast form (33). As mentioned previously, host PGE₂ can promote the yeast to hypha transition (61, 96). *Candida albicans* induces PGE₂ production by host cells, which also can exert immunosuppressive activity. This indicates that both host and fungal lipids play a role in immunomodulation and control of morphogenesis in *Candida albicans*.

A hallmark response to fungal infections by the innate host response is the recruitment and activation of neutrophils. Unusual lipid-like compounds that consist of diacylated ureas, a novel class of unstable lipoids, have been isolated from *Saccharomyces cerevisiae* (Fig. 7b and 7c) (119). These compounds induce adherence and degranulation in human neutrophils and activate leukocytes via a G-protein receptor and may represent a fungus-specific pathogen-associated molecule. Thus, while the study of fungal lipids in pathogenesis is in its infancy, it appears very likely that lipids contribute to the ability of fungi to cause infections.

Eicosanoid production (prostaglandins and leukotrienes) is almost certainly ubiquitous among pathogenic fungi. Given the biological activity of purified fungal eicosanoids, they have the potential to mediate host-pathogen cross-talk in which the pathogen can downregulate (local) host immune responses at the site of infection and the host can inadvertently augment

pathogen virulence. The result of such interactions could be an immunological stalemate, i.e., chronic low-grade infection or parasitism. The most common feature of fungal infections is their chronic persistence within tissues of otherwise healthy individuals, ranging in severity from athlete's foot to chronic vaginitis to pulmonary granulomas (86). Prostaglandins can modulate the Th1/Th2 balance of a response and promote tissue eosinophilia, a feature of some chronic fungal infections (11, 88, 103, 126). Fungi secrete or shed a variety of products that can contribute to virulence and immunomodulation. Fungal eicosanoids may be an important key to understanding the link between fungi and atopic diseases such as allergy and asthma.

NEW PERSPECTIVES ON FUNGAL PATHOGENESIS

Why Is the Prostate a Target for Persistent *Cryptococcus neoformans* Infection?

Cryptococcus neoformans is inhaled from the environment, grows in the lungs, disseminates throughout the body, grows in the brain, and persists in the prostate (cryptococcal pathogenesis, reviewed in reference 18). Why the prostate? This observation has intrigued researchers for decades (77), and there has been no model to explain the prostate tropism of *Cryptococcus neoformans*. Historically, bioactive lipid compounds that induced smooth muscle contraction were first isolated from the seminal fluid and prostrate of mammals, hence the derivation of the name prostaglandin (138). Thus, the seminal fluid and prostate are rich in phospholipids, fatty acids, and eicosanoids. So, does *Cryptococcus neoformans* persist in organs containing large amounts of these compounds? The lung contains surfactant, the central nervous system contains cerebral spinal fluid, and the prostate contains seminal fluid. All of these fluids are high in phospholipids, fatty acids, and eicosanoids.

OXYLIPIN PRODUCTION AS A TARGET OF INHIBITION

As has been discussed above, many parasites possess a unique system of oxylipin generation that is critical not only in the growth of the organism but also in the host response to infection by the organism. Clearly, this system provides a tantalizing target for new antiparasitic drugs. Unfortunately, while the evidence for the importance of oxylipins has been around for a long time, our understanding of classical eicosanoid production has actually hampered studies of other systems. Over the years, very specific inhibitors of various mammalian cyclooxygenases and lipoxygenases have been discovered. In the study of mammalian systems, the inhibitor that is employed can identify specific subtypes of these enzymes; however, the same rules do not apply to other organisms. Inhibitors of oxidative enzymes are frequently just molecules with antioxidant properties that fit nicely into the active site (140). Therefore, the fact that different cyclooxygenase inhibitors have different degrees of effectiveness on nonclassical oxylipin production is related more to the structure of the inhibitor and less to the enzyme it was originally described as inhibiting. It is possible that some of the current treatments for parasitic diseases inhibit the oxylipin synthesis enzymes, although the enzyme

inhibition may not be complete. For example, trypanosomal PGF_{2 α} synthase has been reported to specifically bind to and react with several known trypanocidal drugs (71). Thus, future studies of known and new antiparasitic drugs should analyze the effects of these drugs on parasite oxylipin synthesis.

When it comes to specific inhibitors of fungal eicosanoids, nature may have already developed such inhibitors. In response to fungal attack, plants produce a series of host defense molecules called phytoalexins (34). One class of phytoalexins includes polyphenols. Two of these, resveratrol and nordihydroguarectic acid, are also inhibitors of mammalian cyclooxygenases and lipoxygenases (10, 127). The precise mechanism by which resveratrol and nordihydroguarectic acid inhibit fungal growth is not known. Resveratrol and nordihydroguarectic acid are not as effective in inhibiting mammalian cyclooxygenases and lipoxygenases as celecoxib or zileutin. However, has nature selected (through fungal-plant interactions) these phytoalexins to be optimal for inhibiting fungal oxylipin production? Both of these compounds can inhibit fungal growth and can also inhibit oxylipin production in fungal lysates (J. R. Erb-Downward and G. B. Huffnagle, unpublished data). In summary, the biochemical differences between fungal and parasitic eicosanoid synthetic pathways and mammalian biosynthetic pathways make fungal-parasitic oxylipin production a viable target for the rational design of future therapeutics.

FUTURE PERSPECTIVES

Clearly, the study of oxylipins is an area of research that, while steeped in history, has much to answer in the future. As the production of oxylipin compounds becomes better characterized in new systems, so too will the functions of these compounds within that system need to be studied. Recently, enzyme immunoassay kits for many eicosanoids and oxylipins of interest have become widely available. And while it is important when employing such kits to remember that fats are not proteins and that a result obtained with an enzyme immunoassay kit does not constitute absolute proof, they do provide useful new tools for researchers to study both new and old systems. Through these techniques and others, parasites such as *Plasmodium falciparum* (70), and *Trypanosoma brucei* (69) have recently been demonstrated to produce prostaglandin-like compounds, as have several different genera of pathogenic fungi (96, 97). In each of these cases, the production of eicosanoids was not inhibitable by the classical inhibitors of prostaglandin synthesis, suggesting the existence of a new and possibly unique system.

It is likely that the role of eicosanoids and oxylipins in these organisms will extend from control of intrinsic metabolic functions, such as growth and maturation, to effects on the hosts, such as modulation of immune responses and enabling invasion. It remains to be seen whether microbial eicosanoids and oxylipins are virulence factors. In the case of most pathogenic microbes, it is likely that these microbial lipids participate in forming a pathogenic profile composed of various virulence factors that are all required for full virulence. As the enzymes involved in eicosanoid synthesis are discovered, genetic manipulation will allow definitive examination of the roles of non-mammalian eicosanoids in metabolism and pathogenesis.

ACKNOWLEDGMENTS

We thank Galen Toews for scholarly contributions and support.

This work was supported by a New Investigator Award in Molecular Pathogenic Mycology from the Burroughs-Wellcome Fund (G.B.H.). M.C.N. was supported by NIH NIAID training grant T32AI07528. Additional support was provided by grants RO1-HL65912 (G.B.H.) and RO1-HL63670 (G.B.H.) from the National Institutes of Health.

REFERENCES

- Abdel Baset, H., G. P. O'Neill, and A. W. Ford-Hutchinson. 1995. Characterization of arachidonic-acid-metabolizing enzymes in adult *Schistosoma mansoni*. *Mol. Biochem. Parasitol.* **73**:31–41.
- Ali, S. F., A. Joachim, and A. Dausgchies. 1999. Eicosanoid production by adult *Fasciola hepatica* and plasma eicosanoid patterns during fasciolosis in sheep. *Int. J. Parasitol.* **29**:743–748.
- Aliberti, J., C. Serhan, and A. Sher. 2002. Parasite-induced lipoxin A4 is an endogenous regulator of interleukin-12 production and immunopathology in *Toxoplasma gondii* infection. *J. Exp. Med.* **196**:1253–1262.
- Aliberti, J., and A. Sher. 2001. Positive and negative regulation of pathogen induced dendritic cell function by G-protein coupled receptors. *Mol. Immunol.* **38**:891–893.
- Aliberti, J., and A. Sher. 2002. Role of G-protein-coupled signaling in the induction and regulation of dendritic cell function by *Toxoplasma gondii*. *Microbes Infect.* **4**:991–997.
- Angeli, V., C. Faveeuw, O. Roye, J. Fontaine, E. Teissier, A. Capron, I. Wolowczuk, M. Capron, and F. Trottein. 2001. Role of the parasite-derived prostaglandin D2 in the inhibition of epidermal Langerhans cell migration during schistosomiasis infection. *J. Exp. Med.* **193**:1135–1147.
- Baillie, M. B., T. J. Standiford, L. L. Laichalk, M. J. Coffey, R. Strieter, and M. Peters-Golden. 1996. Leukotriene-deficient mice manifest enhanced lethality from *Klebsiella pneumoniae* in association with decreased alveolar macrophage phagocytic and bactericidal activities. *J. Immunol.* **157**:5221–5224.
- Reference deleted.
- Bassler, B. L. 2002. Small talk. Cell-to-cell communication in bacteria. *Cell* **109**:421–424.
- Bavareseco, L., C. Fregoni, E. Cantu, and M. Trevisan. 1999. Stilbene compounds: from the grapevine to wine. *Drugs Exp. Clin. Res.* **25**:57–63.
- Betz, M., and B. S. Fox. 1991. Prostaglandin E2 inhibits production of Th1 lymphokines but not of Th2 lymphokines. *J. Immunol.* **146**:108–113.
- Blok, W. L., M. T. Vogels, J. H. Curfs, W. M. Eling, W. A. Buurman, and J. W. van der Meer. 1992. Dietary fish-oil supplementation in experimental gram-negative infection and in cerebral malaria in mice. *J. Infect. Dis.* **165**:898–903.
- Botha, A., J. Kock, D. Coetzee, N. Linde, and M. van Dyk. 1992. Yeast eicosanoids II. The influence of non-steroidal anti-inflammatory drugs on the life cycle of *Dipodascopsis*. *Syst. Appl. Microbiol.* **15**:155–160.
- Botha, A., J. Kock, and S. Nigam. 1998. The production of eicosanoid precursors by mucoralean fungi. *Adv. Exp. Med. Biol.* **433**:227–229.
- Botha, A., I. Paul, C. Roux, J. L. Kock, D. J. Coetzee, T. Strauss, and C. Maree. 1999. An isolation procedure for arachidonic acid producing *Mortierella* species. *Antonie van Leeuwenhoek* **75**:253–256.
- Brodowsky, I. D., M. Hamberg, and E. H. Oliv. 1992. A linoleic acid (8R)-dioxygenase and hydroperoxide isomerase of the fungus *Gaeumannomyces graminis*. Biosynthesis of (8R)-hydroxylinoleic acid and (7S,8S)-dihydroxylinoleic acid from (8R)-hydroperoxylinoleic acid. *J. Biol. Chem.* **267**:14738–14745.
- Busse, W. W. 1998. Leukotrienes and inflammation. *Am. J. Respir. Crit. Care Med.* **157**:S210–S213.
- Casadevall, A., and J. R. Perfect. 1998. *Cryptococcus neoformans*. ASM Press, Washington, D.C.
- Castro, M., J. A. Bjoraker, M. S. Rohrbach, and A. H. Limper. 1996. *Candida albicans* induces the release of inflammatory mediators from human peripheral blood monocytes. *Inflammation* **20**:107–122.
- Castro, M., N. V. Ralston, T. I. Morgenthaler, M. S. Rohrbach, and A. H. Limper. 1994. *Candida albicans* stimulates arachidonic acid liberation from alveolar macrophages through alpha-mannan and beta-glucan cell wall components. *Infect. Immun.* **62**:3138–3145.
- Chandra, S., S. T. Ohnishi, and B. N. Dhawan. 1993. Reversal of chloroquine resistance in murine malaria parasites by prostaglandin derivatives. *Am. J. Trop. Med. Hyg.* **48**:645–651.
- Channon, J. Y., and L. H. Kasper. 1996. *Toxoplasma gondii*-induced immune suppression by human peripheral blood monocytes: role of gamma interferon. *Infect. Immun.* **64**:1181–1189.
- Chen, S. C., M. Muller, J. Z. Zhou, L. C. Wright, and T. C. Sorrell. 1997. Phospholipase activity in *Cryptococcus neoformans*: a new virulence factor? *J. Infect. Dis.* **175**:414–420.
- Chen, S. C., L. C. Wright, J. C. Golding, and T. C. Sorrell. 2000. Purification and characterization of secretory phospholipase B, lysophospholipase and lysophospholipase/transacylase from a virulent strain of the pathogenic fungus *Cryptococcus neoformans*. *Biochem. J.* **347**:431–439.
- Coetzee, D., J. Kock, A. Botha, M. van Dyk, E. Smit, P. Botes, and O. Augustyn. 1991. Yeast eicosanoids III. The distribution of arachidonic acid metabolites in the life-cycle of *Dipodascopsis uninucleata*. *Syst. Appl. Microbiol.* **15**:311–318.
- Connelly, M. C., and F. Kierszenbaum. 1984. Modulation of macrophage interaction with *Trypanosoma cruzi* by phospholipase A2-sensitive components of the parasite membrane. *Biochem. Biophys. Res. Commun.* **121**:931–939.
- Dausgchies, A. 1996. Investigations into the production and function of leukotrienes during histotropic development of *Oesophagostomum dentatum*. *Parasitol. Res.* **82**:416–422.
- Dausgchies, A. 1995. *Oesophagostomum dentatum*: population dynamics and synthesis of prostanoids by histotropic stages cultured *in vitro*. *Exp. Parasitol.* **81**:574–583.
- Dausgchies, A., and B. Ruttkowski. 1998. Modulation of migration of *Oesophagostomum dentatum* larvae by inhibitors and products of eicosanoid metabolism. *Int. J. Parasitol.* **28**:355–362.
- Davidson, R. C., T. D. Moore, A. R. Odum, and J. Heitman. 2000. Characterization of the MAlpha pheromone of the human fungal pathogen *Cryptococcus neoformans*. *Mol. Microbiol.* **38**:1017–1026.
- De Freitas, L. A., L. M. Mbow, M. Estay, J. A. Bleyenbergh, and R. G. Titus. 1999. Indomethacin treatment slows disease progression and enhances a Th1 response in susceptible BALB/c mice infected with *Leishmania major*. *Parasite Immunol.* **21**:273–277.
- Demeure, C. E., L.-P. Yang, C. Desjardins, P. Raynauld, and G. Delespesse. 1997. Prostaglandin E2 primes naive T cells for the production of anti-inflammatory cytokines. *Eur. J. Immunol.* **27**:3526–3531.
- Deregnacourt, C., and J. Schrevel. 2000. Bee venom phospholipase A2 induces stage-specific growth arrest of the intraerythrocytic *Plasmodium falciparum* via modifications of human serum components. *J. Biol. Chem.* **275**:39973–39980.
- Deva, R., R. Ciccoli, L. Kock, and S. Nigam. 2001. Involvement of aspirin-sensitive oxylipins in vulvovaginal candidiasis. *FEMS Microbiol. Lett.* **198**:37–43.
- Deva, R., R. Ciccoli, T. Schewe, J. L. Kock, and S. Nigam. 2000. Arachidonic acid stimulates cell growth and forms a novel oxygenated metabolite in *Candida albicans*. *Biochim. Biophys. Acta* **1486**:299–311.
- Dixon, R. A. 2001. Natural products and plant disease resistance. *Nature* **411**:843–847.
- Djurkovic-Djakovic, O. 1998. Toxoplasmosis and immunosuppression. *Srp. Arkh. Tselok. Lek.* **126**:197–203.
- Farrell, J. P., and C. E. Kirkpatrick. 1987. Experimental cutaneous leishmaniasis. II. A possible role for prostaglandins in exacerbation of disease in *Leishmania major*-infected BALB/c mice. *J. Immunol.* **138**:902–907.
- Fierer, J., J. A. Salmon, and B. A. Askonas. 1984. African trypanosomiasis alters prostaglandin production by murine peritoneal macrophages. *Clin. Exp. Immunol.* **58**:548–556.
- Ford-Hutchinson, A. W. 1990. Leukotriene B4 in inflammation. *Crit. Rev. Immunol.* **10**:1–12.
- Fukushima, T., A. Isobe, N. Hojo, K. Shiwaku, Y. Yamane, and M. Torii. 1993. The metabolism of arachidonic acid to prostaglandin E2 in plerocercoids of *Spirometra erinacei*. *Parasitol. Res.* **79**:634–638.
- Funk, C. D. 2001. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* **294**:1871–1875.
- Fusco, A. C., B. Salafsky, and K. Delbrook. 1986. *Schistosoma mansoni*: production of cercarial eicosanoids as correlates of penetration and transformation. *J. Parasitol.* **72**:397–404.
- Fusco, A. C., B. Salafsky, and M. B. Kevin. 1985. *Schistosoma mansoni*: eicosanoid production by cercariae. *Exp. Parasitol.* **59**:44–50.
- Fusco, A. C., Salafsky, B., Whitely, K., and S. Yohe. 1987. *Schistosoma mansoni*: pH dependence of cercarial eicosanoid production, penetration, and transformation. *Exp. Parasitol.* **64**:139–146.
- Gao, T., T. Fukushima, A. Isobe, N. Hojo, K. Shiwaku, and Y. Yamane. 1998. Arachidonic acid metabolism to prostaglandin E2, D2, F2 α , and I2 in the plerocercoid of *Spirometra erinacei*. *J. Parasitol.* **84**:1107–1111.
- Gasperini, S., L. Crepaldi, F. Calzetti, L. Gatto, C. Berlato, F. Bazzoni, A. Yoshimura, and M. A. Cassatella. 2002. Interleukin-10 and cAMP-elevating agents cooperate to induce suppressor of cytokine signaling-3 via a protein kinase A-independent signal. *Eur. Cytokine Netw.* **13**:47–53.
- Gazzinelli, R. T., S. Hieny, T. A. Wynn, S. Wolf, and A. Sher. 1993. Interleukin 12 is required for the T-lymphocyte-independent induction of interferon gamma by an intracellular parasite and induces resistance in T-cell deficient hosts. *Proc. Natl. Acad. Sci. USA* **90**:6115–6119.
- Ghannoum, M. A. 2000. Potential role of phospholipases in virulence and fungal pathogenesis. *Clin. Microbiol. Rev.* **13**:122–143.
- Hadas, E., and T. Mazur. 1997. Biosynthesis of prostaglandins in pathogenic and nonpathogenic strains of *Acanthamoeba* spp. *Parasitol. Res.* **83**:296–299.
- Haq, S., I. Khan, A. Haque, and L. Kasper. 1994. Impairment of the cellular immune response in acute murine toxoplasmosis: regulation of

- interleukin-2 production and macrophage-mediated inhibitory effects. *Infect. Immun.* **62**:2908–2916.
50. Hatae, N., Y. Sugimoto, and A. Ichikawa. 2002. Prostaglandin receptors: advances in the study of EP3 receptor signaling. *J. Biochem. (Tokyo)* **131**:781–784.
 51. Hayaishi, O. 2000. Molecular mechanisms of sleep-wake regulation: a role of prostaglandin D2. *Phil. Trans. R. Soc. Lond. B Biol. Sci.* **355**:275–280.
 52. Henderson, W. R., and E. Y. Chi. 1992. Cytotoxic activity of 13-hydroxyoctadecadienoic acid against *Toxoplasma gondii*. *Parasitology* **105**(Pt 3):343–347.
 53. Henderson, W. R., Jr., M. Rashed, E. C. Yong, T. R. Fritsche, and G. K. Chiang. 1992. *Toxoplasma gondii* stimulates the release of 13- and 9-hydroxyoctadecadienoic acids by human platelets. *Biochemistry* **31**:5356–5362.
 54. Herman, R. P. 1998. Oxylipin production and action in fungi and related organisms, p. 115–132. *In* A. F. Rowley, H. Kuhn, and T. Schewe (ed.), *Eicosanoids and related compounds in plants and animals*. Princeton University Press, Princeton, N.J.
 55. Herman, R. P., and C. A. Herman. 1985. Prostaglandins or prostaglandin like substances are implicated in normal growth and development in oomycetes. *Prostaglandin* **29**:819–831.
 56. Hornby, J. M., E. C. Jensen, A. D. Lisee, J. J. Tasto, B. Jahnke, R. Shoemaker, P. Dussault, and K. W. Nickerson. 2001. Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. *Appl. Environ. Microbiol.* **67**:2982–2992.
 57. Hornsten, L., C. Su, A. E. Osbourn, P. Garosi, U. Hellman, C. Wernstedt, and E. H. Oliv. 1999. Cloning of linoleate diol synthase reveals homology with prostaglandin H synthases. *J. Biol. Chem.* **274**:28219–28224.
 58. Joachim, A., B. Rutkowski, and A. Dausgschies. 2001. *Oesophagostomum dentatum*: expression patterns of enzymes involved in eicosanoid production. *Parasitol. Int.* **50**:211–215.
 59. Jonsson, E. W., and S. Dahlen. 1999. The role of eicosanoids in inflammation and allergy, p. 233–272. *In* F. Marks and G. Furstenberger (ed.), *Prostaglandins, leukotrienes, and other eicosanoids*. Wiley-VCH, Weinheim, Germany.
 60. Kaiser, L., V. L. Lamb, P. K. Tithof, D. A. Gage, B. A. Chamberlin, J. T. Watson, and J. F. Williams. 1992. *Dirofilaria immitis*: do filarial cyclooxygenase products depress endothelium-dependent relaxation in the in vitro rat aorta? *Exp. Parasitol.* **75**:159–167.
 61. Kalo-Klein, A., and S. Witkin. 1990. Prostaglandin E2 enhances and gamma Interferon inhibits germ tube formation in *Candida albicans*. *Infect. Immun.* **58**:260–262.
 62. Kerwin, J. L., C. A. Simmons, and R. K. Washino. 1986. Eicosanoid regulation of oosporogenesis by *Lagenidium giganteum*. *Prostaglandins Leukot. Med.* **23**:173–178.
 - 62a. Kerwin, J. L., J. K. MacKichan, M. J. Semon, A. M. Wiens, C. C. DeRose, and J. J. Torvik. 1996. Sterol and steryl ester regulation of phospholipase A2 from the mosquito parasite *Lagenidium giganteum*. *Lipids* **31**:1179–1188.
 63. Kerwin, J. L., and R. K. Washino. 1984. Cyclin nucleotide regulation of oosporogenesis by *Lagenidium giganteum* and related fungi. *Exp. Mycol.* **8**:215–220.
 64. Kock, J., D. Coetzee, M. van Dyk, M. Truscott, P. Cloete, V. van Wyk, and O. Augustyn. 1991. Evidence for pharmacologically active prostaglandins in yeasts. *S. Afr. J. Sci.* **87**:73–76.
 65. Kock, J. L., T. Strauss, C. H. Pohl, D. P. Smith, P. J. Botes, E. E. Pretorius, T. Tepeny, Sebolai, A. Botha, and S. Nigam. 2001. Bioprospecting for novel oxylipins in fungi: the presence of 3-hydroxy oxylipins in *Pilobolus*. *Antonie van Leeuwenhoek* **80**:93–99.
 66. Kock, J. L., P. W. van Wyk, P. Venter, D. J. Coetzee, D. P. Smith, B. C. Viljoen, and S. Nigam. 1999. An acetylsalicylic acid-sensitive aggregation phenomenon in *Dipodascopsis uninucleata*. *Antonie van Leeuwenhoek* **75**: 261–266.
 67. Kock, J. L., P. Venter, D. P. Smith, P. W. Van Wyk, P. J. Botes, D. J. Coetzee, C. H. Pohl, A. Botha, K. H. Riedel, and S. Nigam. 2000. A novel oxylipin-associated 'hosting' phenomenon in yeast flocculation. *Antonie van Leeuwenhoek* **77**:401–406.
 68. Krugliak, M., E. Deharo, G. Shalmiev, M. Sauvain, C. Moretti, and H. Ginsburg. 1995. Antimalarial effects of C₁₈ fatty acids on *Plasmodium falciparum* in culture and on *Plasmodium vinckei petteri* and *Plasmodium yoelii nigeriensis* in vivo. *Exp. Parasitol.* **81**:97–105.
 69. Kubata, B. K., M. Duzsenko, Z. Kabututu, M. Rawer, A. Szallies, K. Fujimori, T. Inui, T. Nozaki, K. Yamashita, T. Horii, Y. Urade, and O. Hayaishi. 2000. Identification of a novel prostaglandin f(2 α) synthase in *Trypanosoma brucei*. *J. Exp. Med.* **192**:1327–1338.
 70. Kubata, B. K., N. Eguchi, Y. Urade, K. Yamashita, T. Mitamura, K. Tai, O. Hayashi, and T. Horii. 1998. *Plasmodium falciparum* produces prostaglandins that are pyrogenic, somnogenic, and immunosuppressive substances in humans. *J. Exp. Med.* **188**:1197–1202.
 71. Kubata, B. K., Z. Kabututu, T. Nozaki, C. J. Munday, S. Fukuzumi, K. Ohkubo, M. Lazarus, T. Maruyama, S. K. Martin, M. Duzsenko, and Y. Urade. 2002. A key role for old yellow enzyme in the metabolism of drugs by *Trypanosoma cruzi*. *J. Exp. Med.* **196**:1241–1251.
 72. Kull, F., E. Ohlson, and J. Z. Haeggstrom. 1999. Cloning and characterization of a bifunctional leukotriene A₄ hydrolase from *Saccharomyces cerevisiae*. *J. Biol. Chem.* **274**:34683–34690.
 73. Kull, F., E. Ohlson, B. Lind, and J. Z. Haeggstrom. 2001. *Saccharomyces cerevisiae* leukotriene A₄ hydrolase: formation of leukotriene B₄ and identification of catalytic residues. *Biochemistry* **40**:12695–12703.
 74. Kunkel, S. L., M. Spengler, M. A. May, R. Spengler, J. Larrick, and D. Remick. 1988. Prostaglandin E₂ regulates macrophage-derived tumor necrosis factor gene expression. *J. Biol. Chem.* **263**:5380–5384.
 75. Kwon-Chung, K. J. 1992. *Medical mycology*. Lea & Febiger, Philadelphia, Pa.
 76. Lamacka, M., and J. Sajbidor. 1998. The content of prostaglandins and their precursors in *Mortierella* and *Cunninghamella* species. *Lett. Appl. Microbiol.* **26**:224–226.
 77. Larsen, R. A., S. Bozzette, J. A. McCutchan, J. Chiu, M. A. Leal, and D. D. Richman. 1989. Persistent *Cryptococcus neoformans* infection of the prostate after successful treatment of meningitis. California Collaborative Treatment Group. *Ann. Intern. Med.* **111**:125–128.
 78. Liu, L. X., J. E. Buhlmann, and P. F. Weller. 1992. Release of prostaglandin E2 by microfilariae of *Wuchereria bancrofti* and *Brugia malayi*. *Am. J. Trop. Med. Hyg.* **46**:520–523.
 79. Liu, L. X., and P. F. Weller. 1992. Intravascular filarial parasites inhibit platelet aggregation. Role of parasite-derived prostanoids. *J. Clin. Investig.* **89**:1113–1120.
 80. Lodewyk, J., J. Kock, P. Venter, A. Botha, D. Coetzee, P. Botes, and S. Nigam. 1998. The production of biologically active eicosanoids by yeasts. *Adv. Exp. Med. Biol.* **433**:217–219.
 81. Lonardoni, M. V., M. Russo, and S. Jancar. 2000. Essential role of platelet-activating factor in control of *Leishmania amazonensis* infection. *Infect. Immun.* **68**:6355–6361.
 82. Mancuso, P., R. W. McNish, M. Peters-Golden, and T. G. Brock. 2001. Evaluation of phagocytosis and arachidonate metabolism by alveolar macrophages and recruited neutrophils from F344xBN rats of different ages. *Mech. Ageing Dev.* **122**:1899–1913.
 83. Mancuso, P., P. Nana-Sinkam, and M. Peters-Golden. 2001. Leukotriene B₄ augments neutrophil phagocytosis of *Klebsiella pneumoniae*. *Infect. Immun.* **69**:2011–2016.
 84. Mancuso, P., and M. Peters-Golden. 2000. Modulation of alveolar macrophage phagocytosis by leukotrienes is Fc receptor-mediated and protein kinase C-dependent. *Am. J. Respir. Cell. Mol. Biol.* **23**:727–733.
 85. Mancuso, P., T. J. Standiford, T. Marshall, and M. Peters-Golden. 1998. 5-Lipoxygenase reaction products modulate alveolar macrophage phagocytosis of *Klebsiella pneumoniae*. *Infect. Immun.* **66**:5140–5146.
 86. Mandell, G. L., J. E. Bennett, and R. Dolin (ed.). 1995. *Mandell, Douglas, and Bennett's principles and practice of infectious diseases*, 4th ed. Churchill Livingstone, New York, N.Y.
 87. Marks, F. 1999. Arachidonic acid and companions: an abundant source of biological signals, p. 1–46. *In* F. Marks and G. Furstenberger (ed.), *Prostaglandins, leukotrienes, and other eicosanoids*. Wiley-VCH, Weinheim, Germany.
 88. Matsuoka, T., M. Hirata, H. Tanaka, Y. Takahashi, T. Murata, K. Kabashima, Y. Sugimoto, T. Kobayashi, F. Ushikubi, Y. Aze, N. Eguchi, Y. Urade, N. Yoshida, K. Kimura, A. Mizoguchi, Y. Honda, H. Nagai, and S. Narumiya. 2000. Prostaglandin D2 as a mediator of allergic asthma. *Science* **287**:2013–2017.
 89. McGuirk, P., and K. H. Mills. 2002. Pathogen-specific regulatory T cells provoke a shift in the Th1/Th2 paradigm in immunity to infectious diseases. *Trends Immunol.* **23**:450–455.
 90. Meyer, D. J., R. Muimo, M. Thomas, D. Coates, and R. E. Isaac. 1996. Purification and characterization of prostaglandin-H E-isomerase, a sigma-class glutathione S-transferase, from *Ascaridia galli*. *Biochem. J.* **313**:223–227.
 91. Meyer, F., H. Meyer, and E. Bueding. 1970. Lipid Metabolism in the parasitic and free-living worms *Schistosoma mansoni* and *Dugesia dorotocephala*. *Biochim. Biophys. Acta* **210**:256–266.
 92. Monneret, G., S. Gravel, M. Diamond, J. Rokach, and W. S. Powell. 2001. Prostaglandin D2 is a potent chemoattractant for human eosinophils that acts via a novel DP receptor. *Blood* **98**:1942–1948.
 93. Morrow, J. D., and L. J. Roberts, 2nd. 1996. The isoprostanes. Current knowledge and directions for future research. *Biochem. Pharmacol.* **51**:1–9.
 94. Nicosia, S., V. Capra, and G. E. Rovati. 2001. Leukotrienes as mediators of asthma. *Pulm. Pharmacol. Ther.* **14**:3–19.
 95. Noverr, M. C., G. M. Cox, J. Perfect, and G. B. Huffnagle. 2003. The role of PLB1 in pulmonary inflammation and cryptococcal eicosanoid production. *Infect. Immun.* **71**:1538–1547.
 96. Noverr, M. C., S. M. Phare, G. B. Toews, M. J. Coffey, and G. B. Huffnagle. 2001. Pathogenic yeasts *Cryptococcus neoformans* and *Candida albicans* produce immunomodulatory prostaglandins. *Infect. Immun.* **69**:2957–2963.
 97. Noverr, M. C., D. D. Williams, G. B. Toews, and G. B. Huffnagle. 2002. Production of prostaglandins and leukotrienes by pathogenic fungi. *Infect. Immun.* **70**:400–402.
 98. Ohnishi, S. T., N. Ohnishi, Y. Oda, and M. Katsuo. 1989. Prostaglandin derivatives inhibit the growth of malarial parasites in mice. *Cell. Biochem. Funct.* **7**:105–109.
 99. Peacock, C. D., N. L. Misso, D. N. Watkins, and P. J. Thompson. 1999. PGE

- 2 and dibutyl cyclic adenosine monophosphate prolong eosinophil survival in vitro. *J. Allergy Clin. Immunol.* **104**:153–162.
100. **Pearce, E. J., and A. J. Macdonald.** 2002. The immunobiology of schistosomiasis. *Nature* **2**:499–511.
101. **Pentreath, V. W., K. Rees, O. A. Owolabi, K. A. Philip, and F. Doua.** 1990. The somnogenic T lymphocyte suppressor prostaglandin D2 is selectively elevated in cerebrospinal fluid of advanced sleeping sickness patients. *Trans. R. Soc. Trop. Med. Hyg.* **84**:795–799.
102. **Peters-Golden, M.** 2002. 'Good' lipids for asthma. *Nat. Med.* **8**:931–932.
103. **Peters-Golden, M.** 1997. Lipid mediator synthesis by lung macrophages. *Lung Biol. Health Dis.* **102**:151–182.
104. **Peters-Golden, M.** 2002. Open mind, open airways: broadening the paradigm of prostaglandins and allergic airway inflammation. *Am. J. Respir. Crit. Care Med.* **165**:1035–1036.
105. **Pinge-Fihlo, P., Tadokoro, C. E., and I. A. Abrahamsohn.** 1999. Prostaglandins mediate suppression of lymphocyte proliferation and cytokine synthesis in acute *Trypanosoma cruzi* infection. *Cell. Immunol.* **193**:90–98.
106. **Pohl, C., A. Botha, J. Kock, D. Coetzee, P. Botes, T. Schewe, and S. Nigam.** 1998. Oxylin formation in fungi: biotransformation of arachidonic acid to 3-hydroxy-5,8-tetradecadienoic acid by *Mucor genevensis*. *Biochem. Biophys. Res. Commun.* **253**:703–706.
107. **Raible, D. G., E. S. Schulman, J. DiMuzio, R. Cardillo, and T. J. Post.** 1992. Mast cell mediators prostaglandin-D2 and histamine activate human eosinophils. *J. Immunol.* **148**:3536–3542.
108. **Ramage, G., S. P. Saville, B. L. Wickes, and J. L. Lopez-Ribot.** 2002. Inhibition of *Candida albicans* biofilm formation by farnesol, a quorum-sensing molecule. *Appl. Environ. Microbiol.* **68**:5459–5463.
109. **Ramaswamy, K., P. Kumar, and Y. X. He.** 2000. A role for parasite-induced PGE2 in interleukin-10-mediated host immunoregulation by skin stage schistosomula of *Schistosoma mansoni*. *J. Immunol.* **165**:4567–4574.
110. **Reiner, S. L., and R. M. Locksley.** 1995. The regulation of immunity to *Leishmania major*. *Annu. Rev. Immunol.* **13**:151–177.
111. **Ricote, M., A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass.** 1998. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* **391**:79–82.
112. **Roberts, L. J., 2nd, and J. D. Morrow.** 1994. Isoprostanes. Novel markers of endogenous lipid peroxidation and potential mediators of oxidant injury. *Ann. N. Y. Acad. Sci.* **744**:237–242.
- 112a. **Rogers, M. V., K. J. Henkle, V. Herrmann, D. J. McLaren, and G. F. Mitchell.** 1991. Evidence that a 16-kilodalton integral membrane protein antigen from *Schistosoma japonicum* adult worms is a type A2 phospholipase. *Infect. Immun.* **59**:1442–1447.
113. **Rowley, A. F., H. Kuhn, and T. Schewe.** 1998. Enzymes and factors involved in the biosynthesis of eicosanoids. Princeton University Press, Princeton, N.J.
114. **Sacks, D., and N. Noben-Trauth.** 2002. The immunology of susceptibility and resistance to *Leishmania major* in mice. *Nat. Rev. Immunol.* **2**:845–858.
- 114a. **Saffer, L. D., and J. D. Schwartzman.** 1991. A soluble phospholipase of *Toxoplasma gondii* associated with host cell penetration. *J. Protozool.* **38**:454–460.
115. **Salafsky, B., and A. C. Fusco.** 1985. *Schistosoma mansoni*: cercarial eicosanoid production and penetration response inhibited by esuletin and ibuprofen. *Exp. Parasitol.* **60**:73–81.
116. **Salafsky, B., Y. S. Wang, A. C. Fusco, and J. Antonacci.** 1984. The role of essential fatty acids and prostaglandins in cercarial penetration (*Schistosoma mansoni*). *J. Parasitol.* **70**:656–660.
117. **Salafsky, B., Y. S. Wang, M. B. Kevin, H. Hill, and A. C. Fusco.** 1984. The role of prostaglandins in cercarial (*Schistosoma mansoni*) response to free fatty acids. *J. Parasitol.* **70**:584–591.
118. **Schewe, T.** 1998. Basic function of lipoxygenases and their products in higher plants, p. 133–150. *In* A. F. Rowley, H. Kuhn, and T. Schewe (ed.), *Eicosanoids and related compounds in plants and animals*. Princeton University Press, Princeton, N.J.
119. **Schroder, J. M., R. Hasler, J. Grabowsky, B. Kahlke, and A. I. Mallet.** 2002. Identification of diacylated ureas as a novel family of fungus-specific leukocyte-activating pathogen-associated molecules. *J. Biol. Chem.* **277**:27887–27895.
120. **Sebolai, O., J. L. Kock, C. H. Pohl, D. P. Smith, P. J. Botes, E. E. Pretorius, P. W. Van Wyk, and S. Nigam.** 2001. Bioprospecting for novel hydroxyoxylipins in fungi: presence of 3-hydroxy palmitic acid in *Saccharomycopsis malanga*. *Antonie van Leeuwenhoek* **80**:311–315.
121. **Sergeeva, M. G., M. V. Gonchar, A. T. Mevkh, and S. D. Varfolomeyev.** 1997. Prostaglandin E2 biphasic control of lymphocyte proliferation inhibition by picomolar concentrations. *FEBS Lett.* **418**:235–238.
122. **Shechter, G., and S. Grossman.** 1983. Lipoxygenase from baker's yeast: purification and properties. *Int. J. Biochem.* **15**:1295–1304.
123. **Shinomiya, S., H. Naraba, A. Ueno, I. Utsunomiya, T. Maruyama, S. Ohuchida, F. Ushikubi, K. Yuki, S. Narumiya, Y. Sugimoto, A. Ichikawa, and S. Oh-ishi.** 2001. Regulation of tumor necrosis factor alpha and interleukin-10 production by prostaglandins I2 and E2: studies with prostaglandin receptor-deficient mice and prostaglandin E-receptor subtype-selective synthetic agonists. *Biochem. Pharmacol.* **61**:1153–1160.
- 123a. **Shuaib, M. N., H. Kanbara, T. Yanagi, D. A. Ameh, J. J. Bonire, and A. J. Nok.** 2001. Phospholipase A2 from *Trypanosoma brucei gambiense* and *Trypanosoma brucei brucei*: inhibition by organotin. *J. Enzyme Inhib.* **16**:433–441.
124. **Smith, H. L., N. Paciorek, S. Babu, and T. V. Rajan.** 2000. Development of a serum-free system for the in vitro cultivation of *Brugia malayi* infective-stage larvae. *Exp. Parasitol.* **95**:253–264.
125. **Smith, H. L., and T. V. Rajan.** 2001. Inhibitors of the lipoxygenase pathway block development of *Brugia malayi* L3 in vitro. *J. Parasitol.* **87**:242–249.
126. **Snijdewint, F. G., P. Kalinski, E. A. Wierenga, J. D. Bos, and M. L. Kapsenberg.** 1993. Prostaglandin E2 differentially modulates cytokine secretion profiles of human T helper lymphocytes. *J. Immunol.* **150**:5321–5329.
127. **Soleas, G. J., E. P. Diamandis, and D. M. Goldberg.** 1997. Resveratrol: a molecule whose time has come? And gone? *Clin. Biochem.* **30**:91–113.
128. **Standiford, T. J., S. L. Kunkel, M. W. Rolfe, H. L. Evanoff, R. M. Allen, and R. M. Strieter.** 1992. Regulation of human alveolar macrophage- and blood monocyte-derived interleukin-8 by prostaglandin E2 and dexamethasone. *Am. J. Respir. Cell Mol. Biol.* **6**:75–81.
129. **Stanley, D. W., and J. S. Miller.** 1998. Eicosanoids in animal reproduction: what can we learn from invertebrates?, p. 183–196. *In* A. F. Rowley, H. Kuhn, and T. Schewe (ed.), *Eicosanoids and related compounds in plants and animals*. Princeton University Press, Princeton, N.J.
130. **Strassmann, G., V. Patil-Koota, F. Finkelman, M. Fong, and T. Kambayashi.** 1994. Evidence for the involvement of interleukin 10 in the differential deactivation of murine peritoneal macrophages by prostaglandin E2. *J. Exp. Med.* **180**:2365–2370.
131. **Su, C., M. Sahlin, and E. H. Oliw.** 2000. Kinetics of manganese lipoxygenase with a catalytic mononuclear redox center. *J. Biol. Chem.* **275**:18830–18835.
132. **Tarleton, R. L., and R. E. Kuhn.** 1985. Measurement of parasite-specific immune responses in vitro: evidence for suppression of the antibody response to *Trypanosoma cruzi*. *Eur. J. Immunol.* **15**:845–850.
133. **Terrazas, L. I., R. Bojalil, M. Rodriguez-Sosa, T. Govezensky, and C. Larralde.** 1999. *Taenia crassiceps* cysticercosis: a role for prostaglandin E2 in susceptibility. *Parasitol. Res.* **85**:1025–1031.
134. **Thardin, J. F., C. M'Rini, M. Beraud, J. Vandaele, M. F. Frisach, M. H. Bessieres, J. P. Seguela, and B. Pipy.** 1993. Eicosanoid production by mouse peritoneal macrophages during *Toxoplasma gondii* penetration: role of parasite and host cell phospholipases. *Infect. Immun.* **61**:1432–1441.
135. **Vamecq, J., and N. Latruffe.** 1999. Medical significance of peroxisome proliferator-activated receptors. *Lancet* **354**:141–148.
136. **van Dyk, M., J. Kock, D. Coetzee, O. Augustyn, and S. Nigam.** 1991. Isolation of a novel arachidonic acid metabolite 3-hydroxy-5,8,11,14-eicosantetraenoic acid from the yeast *Dipodascopsis uninucleata* UOFs-Y18. *FEBS Lett.* **283**:195–198.
137. **Vane, J. R., and R. M. Botting.** 1998. Mechanism of action of antiinflammatory cells. *Lung Biol. Health Dis.* **114**:1–24.
138. **von Euler, U. S.** 1983. History and development of prostaglandins. *Gen. Pharmacol.* **14**:3–6.
139. **Warburg, O., and W. Christian.** 1933. Über das Gelbe Ferment und Seine Wirkungen. *Biochem. Z.* **266**:377–411.
140. **Whitman, S., M. Gezzinci, B. N. Timmermann, and T. R. Holman.** 2002. Structure-activity relationship studies of nordihydroguaiaretic acid inhibitors toward soybean, 12-human, and 15-human lipoxygenase. *J. Med. Chem.* **45**:2659–2661.
141. **Yap, G. S., and A. Sher.** 1999. Cell-mediated immunity to *Toxoplasma gondii*: initiation, regulation and effector function. *Immunobiology* **201**:240–247.
142. **Yong, E. C., E. Y. Chi, T. R. Fritsche, and W. R. Henderson, Jr.** 1991. Human platelet-mediated cytotoxicity against *Toxoplasma gondii*: role of thromboxane. *J. Exp. Med.* **173**:65–78.
143. **Yong, E. C., E. Y. Chi, and W. R. Henderson, Jr.** 1994. *Toxoplasma gondii* alters eicosanoid release by human mononuclear phagocytes: role of leukotrienes in interferon gamma-induced antitoxoplasma activity. *J. Exp. Med.* **180**:1637–1648.
144. **Zidovetzki, R., I. W. Sherman, and L. O'Brien.** 1993. Inhibition of *Plasmodium falciparum* phospholipase A2 by chloroquine, quinine, and arteether. *J. Parasitol.* **79**:565–570.
145. **Zieler, H., D. B. Keister, J. A. Dvorak, and J. M. Ribeiro.** 2001. A snake venom phospholipase A(2) blocks malaria parasite development in the mosquito midgut by inhibiting ookinete association with the midgut surface. *J. Exp. Biol.* **204**:4157–4167.