

## Regulation of Eicosanoid Pathways: A Pathway to Health and Development

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There are few biological pathways that are more central to overall human and animal health than the eicosanoid pathway. The eicosanoids are central not only to the inflammatory pathway, but metabolic diseases such as obesity, arteriosclerosis, autoimmunity, cancer, growth, pain, and development. Tools for the regulation of this pathway have always been with us, but have been neglected and ignored. In this short summary, a description of the eicosanoid pathway, diseases related to the pathway, and a method to manipulate the pathway will be presented. Manipulation of the eicosanoid pathway provides a potential mechanism for the replacement of antibiotics in animal feeds for improving growth and feed efficiency.

**Eicosanoid Pathway:** Eicosanoids are a family of lipid mediators regulating numerous physiological processes (Hwang, 1989). These lipid mediators are derived from the fatty acid, linoleic acid (18:2, cis 9, cis 12), which is an essential nutrient for all animals. Linoleic acid is inserted into the sn 2 position of phospholipids that make up cell membranes. It is modified by desaturase and elongase enzymes which convert it to arachidonic acid (AA) (20:4). Under the action of numerous stimuli (such as endotoxin, cytokines, hormones, and other cellular stimuli), an enzyme, known as phospholipase A<sub>2</sub>, cleaves arachidonic acid from the sn 2 position of phospholipids in the cell membrane. The released AA is converted, depending on cell type, to prostaglandins (PGs) (via cyclooxygenase, or COX) or leukotrienes (LTs) (via lipoxygenase, or LOX). The PGs and LTs signal autocrine, paracrine, and perhaps even endocrine metabolic processes in the host. For example, inhibition of COX is well known to prevent pain, and is the basis for acetylsalicylic acid (aspirin). The discovery of two COX isoforms, COX-1 and COX-2, has shown that basal levels of COX lipid mediators (COX-1 products) are essential to normal health, whereas the products of the COX-2 (which is an inducible enzyme) may have adverse health implications. Recent data on COX-2 knockout mice has shown that many inflammatory processes, such as arthritis, are greatly reduced by specifically inhibiting the COX-2 pathway (Myers *et al.*, 2000). Hence, there has been an intensive effort by pharmaceutical companies to design specific drugs that inhibit the COX-2 pathway, without having adverse effects on the constitutive and maintenance pathway of COX-1 (Riendeau *et al.*, 2001).

One of the problems of selective inhibition of inducible COX products is that AA cleavage by PLA<sub>2</sub> results in substrate availability for the LOX pathway (Van Wauwe and Goossens, 1983). It is now well recognized that inhibition of COX results in the over production of LOX products (LTs). One of the classical disease situations in which this occurs is aspirin-induced asthma. By the inhibition of the COX pathway, substrate is available for the synthesis of LOX products that are well known to enhance the inflammatory process of the disease. In the case of asthma, an LT antagonist has been developed to inhibit the adverse effects of LTs in the inflammatory process. Hence, regulation of the eicosanoid pathway must consider both COX and LOX regulation.

**Eicosanoids in Disease:** There is clear evidence that the eicosanoid pathway is involved in many metabolic diseases of animals and humans. Some forms of cancer appear to evade the immune defenses by the over production of prostaglandins, which in turn inhibit immune defense against malignant cell development (PGs are well known immune suppressants) (Marnett, 1992; Thun *et al.*, 1993; Hansen Petrik *et al.*, 2000; Liu *et al.*, 2002). The role of PGs

in cardiovascular disease is best shown by the benefits of the use of COX inhibitors. Central in the pathway of fat accretion and metabolism are both the leukotrienes and prostaglandins (Forman *et al.*, 1995; Sessler and Ntambi, 1998). Inhibition of COX products is known to prolong the life and improve the quality of life of patients with arthritic and lupus autoimmune disorders (Yang *et al.*, 2000), and there is limited evidence that inhibition of the general eicosanoid pathway may improve animal growth and feed efficiency.

If the eicosanoid pathway is important in the regulation of normal physiological processes, what are the consequences of regulating it, and how should it be regulated? Is there the possibility that modern animal and human conditions have put abnormal pressure on the eicosanoid pathway? If one were to regulate the eicosanoid pathway, how would they do it and what would be the unintended consequences and benefits?

A great deal can be written about the consequence and benefit of this pathway. A few comments will be made with regard to select animal health issues.

There are considerable costs in maintaining the interface between the microbial world and the animal. To put it simply, the microbial/animal interface cost, in an animal production context, over \$1 billion in production per year, in the absence of overt disease (Cook, 2001; Lev and Forbes, 1959). This loss is mainly in the form of reduced growth and poorer feed efficiency. The loss of productivity (growth and feed efficiency) is not due to the microbial world, but due to the animals' immune defense against the microbial interface. During an immune challenge, the immune cells release a plethora of cytokines, two most notably, Interleukine 1 (IL-1) and tumor necrosis factor (TNF), which directly causes decreased weight gain and decreased feed efficiency (Cook and Pariza, 1998). The adverse effects are due to the eicosanoid pathway (Cook *et al.*, 1993). Cytokines such as IL-1 and TNF stimulate cells to produce lipid mediators (eicosanoids) that in turn cause the collateral damage associated with the immune reaction. The consequence of immune stimulation is, hence, decreased growth, weight loss, and decreased appetite (cachexia). The eicosanoid products are well known to suppress immune function (Cook *et al.*, 1993). The success of aberrant tumor cells, in some cases, is via the over production of select lipid mediators. By the over production of select prostaglandins, tumor cells can suppress immunological rejection of the mutated tissues and thus thrive in the host.

On occasion, the immune system turns against the host. As with any defense system, the host is not always protected from the collateral damage of its defense. One of the major vehicles of collateral damage during autoimmunity are lipid mediators, or eicosanoids. For example, during the autoimmune disease, lupus, where the immune system begins to make antibodies against one's own DNA, immune complexes form that filtrate on the basement membrane of the kidney. Complement is activated, inflammatory eicosanoids are released, attracting inflammatory white blood cells, which in an attempt to eliminate the stimulant, cause damage to the basement membrane. The basement membrane becomes damaged and begins to leak plasma protein in the urine (proteinuria) and the animal eventually dies from kidney failure.

There is evidence that the eicosanoid pathway plays an intimate role in growth and development. This link has been discussed in a number of reviews (Cook, 2001). Briefly, select cytokines from the macrophage cell lineage, induce muscle wasting via prostaglandins, during immune stimulation. The result of eicosanoid-induced

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weight loss is evidenced by reduced performance post vaccination, where decreases in performance are directly linked to cytokine production during immune stimulation. Hence, if one could decrease eicosanoid release during the immune response, one should increase animal growth and decrease the age to sexual maturity.

### Regulation of the Eicosanoid pathway as an alternative to antibiotics

**Conjugated Linoleic Acid (CLA):** In order for a product to successfully prevent the adverse health effects of the eicosanoid pathway, ideally it must allow for basal production of prostaglandins and leukotrienes for house keeping purposes, inhibit inducible eicosanoids, particularly during the inflammatory process, and inhibit both the lipoxygenase and cyclooxygenase pathway in a similar manner, since the inhibition of only one pathway results increased substrate flow to the other pathway. Since linoleic acid (18:2, cis 9, cis 12) is the precursor for both the LOX and COX enzymatic pathways, we believed that an analogue of linoleic acid would be potentially beneficial in the regulation of eicosanoids. CLA is also an 18-carbon fatty acid with two double bonds. Unlike linoleic acid, the double bonds in CLA are in a geometrical and positional configuration that is unlike linoleic acid. The two major isomers of CLA are cis 9, trans 11, and trans 10, cis 12. Hence, either the 12 carbon double bond of linoleic acid is move to position 11 on the carbon chain in a trans geometry, or the 9 carbon double bond is move to carbon 10, also in a trans geometry. These subtle changes in the precursor (linoleic acid) for eicosanoid biosynthesis were found to have dramatic effects on eicosanoid biosynthesis, and animal health and disease (See [www.wisc.edu/cook](http://www.wisc.edu/cook) for CLA bibliography).

CLA was shown to have potent inhibitory effect on antigen-induced eicosanoid release from several smooth muscles without inhibiting basal eicosanoid production (Whigham *et al.*, 2001; Whigham *et al.*, 2002). Both leukotrienes and prostaglandins were similar in trachea, bladder and lung from antigen sensitized guinea pigs fed either a control or CLA supplemented diet prior to tissue challenge with antigen. Following antigen challenge of the tissues, *ex vivo*, LTs and PGs increased in tissues from control fed animals, but release was inhibited in CLA fed animal.

The regulation of eicosanoid biosynthesis is believed to be the central pathway by which CLA has its effects on a wide range of biological activities. CLA was shown to reduce lipopolysaccharide and autoimmune-induced cachexia (Cook *et al.*, 1993; Miller *et al.*, 1994; Yang *et al.*, 2000). CLA was also shown to be a potent inhibitor of cancer of the prostate (Visonneau *et al.*, 1997), colon (Liew *et al.*, 1995), breast (Ip *et al.*, 1991, 1994, 1995) as well as cancer in several other tissues (Pariza *et al.*, 1999). CLA was shown to extend the longevity of the lupus autoimmune mouse model 1.5 to 1.7 fold (Yang *et al.*, 2000), and enhance immune function (Cook *et al.*, 1993; Miller *et al.*, 1994; Sugano *et al.*, 1998). Several unexpected discoveries were that CLA enhanced animal growth and improved feed efficiency (Chin *et al.*, 1994), reduced the clinical signs of arteriosclerosis (Lee *et al.*, 1994; Nicolosi *et al.*, 1997), and reduced body fat (Park *et al.*, 1997). Hence, in some animal species, CLA is a possible candidate for antibiotic replacement with added agronomical and health benefits.

**Anti- PLA<sub>2</sub>:** Another strategy we had to enhance growth and improve feed efficiency in growing livestock was to develop a method to inhibit the enzyme Phospholipase A<sub>2</sub> (PLA<sub>2</sub>), which is responsible for the release of substrate for LOX and COX enzymatic pathways. As previously mentioned, linoleic acid is elongated and desaturated into arachidonic acid in the sin 2 position of phospholipid. During select immune stimulation, PLA<sub>2</sub> cleaves AA from phospholipid, where it is in turn converted to LTs and PGs by their respective enzymes (Balsinde *et al.*, 1999). PLA<sub>2</sub> is also an enzyme that bacteria produce as an invasive factor that allows bacteria to gain entry through the intestinal mucosal interface.

The strategy employed was based on some prior work involving the synthesis of egg yolk antibody. We had previously shown that antibody to a neuropeptide, cholecystokinin, was effective in improving animal growth and feed efficiency, and could be used as a replacement for antibiotics (Cook and Jerome, 1998; Cook *et al.*, 1998a; 1998b; 1999). The laying hen is very efficient in transferring antibodies into egg yolk, and was hence immunized with PLA<sub>2</sub>. Egg yolks were collected as a source of antibody, dried, and fed to chicks. In a number of trials we found that chicks fed the antibodies to PLA<sub>2</sub> had over a 5% improvement in growth and feed efficiency when compared to control fed chicks (Cook, 2001).

**Conclusion:** The regulation of inflammatory eicosanoids, both of the lipoxygenase and cyclooxygenase pathways, represents a possible mechanism by which animal growth and feed efficiency can be improved. Unlike antibiotics which target the microbial ecosystem, which induces immune stimulation, and hence the inflammatory response, targets involving how the host responds to the inflammatory response provide an alternative to antibiotics for the purpose of improved growth and feed efficiency. These new targets should focus on providing a buffer between the host and immunological reactivity. By protecting the host from the negative consequence of the immune response (Cook, 1999), we can maintain enhanced productivity without a concern of creating resistant organism, and maintain the integrity of the animal's immune defense. Regulation of the eicosanoid pathway is one mechanism by which this can be accomplished.

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