PUSH VERSUS PULL: NUTRIENT PARTITIONING TOWARD IMMUNITY

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The Immune System: An Overview

The immune system utilizes a combination of constitutive and adaptive mechanisms that interact with one another to protect the host from microorganisms and infectious disease. Constitutive defenses of the innate immune system consist of effector cells, such as monocytes/macrophages and neutrophils, along with mucosal and epithelial barriers, secretions and plasma acute phase proteins. The innate immune system is capable of recognizing foreign invaders (i.e. non-self) and functions as the first line of defense against these potential disease causing microorganisms, or pathogens. The adaptive arm of the immune system is capable of selectively recognizing and eliminating foreign microorganisms or molecules. These functions are mediated by immunoglobulin secretion by B lymphocytes and various cellular functions mediated by T lymphocytes, collectively referred to as humoral and cell mediated immunity, respectively. Both humoral and cell mediated immunity have tremendous diversity in their recognition molecules and their response to non-self results in immunological memory. Consequently, animal health and disease resistance is achieved through the collective actions of innate, humoral and cell mediated immunity.

The Immune System: Nutrient Use

Nutritional status and nutrient availability influence the development, maintenance and function of innate, humoral and cell mediated immunity (Humphrey et al., 2002). An alteration in resource availability triggers metabolic adaptation and reallocation of existing nutrient stores to support different life-traits. Consequently, understanding how nutrients are utilized by the immune system has important implications for animal health. Unfortunately, the nutrient requirements of the immune system are not known, and this prohibits precise formulation of diets that are optimal for immunity. When considering diet modifications to help feed the immune system and optimize animal health, it is important to consider the concept of push versus pull in regards to nutrient partitioning to the immune system.

The "Push" Approach

A common approach aimed to increase activity of the immune system is to include more of a particular nutrient that is suspected to be in limited supply in the diet or during a particular physiological state. This "push" approach to feeding the immune system assumes that more is better and is fundamentally based upon the idea of tissue competition for nutrients, such as skeletal muscle versus immune tissue. Differences in a tissue’s ability to compete for nutrients creates a nutrient priority framework that Hammond originally proposed to explain nutrient partitioning between tissues (Hammond, 1944). According to this nutrient partitioning scheme, if more nutrients were provided in the diet, then
more of those nutrients would be utilized by lower priority tissues, such as adipose (Figure 1). We have speculated the position of the immune system within this nutrient priority scheme and suspect that the immune system's priority for nutrients is likely to differ between activated and inactivated states (Humphrey et al., 2002). Nonetheless, the "push" approach to feeding the immune system assumes that providing more nutrients in the diet will increase their utilization by the immune system. However, simply increasing the supply of a particular nutrient does not necessarily directly translate to increased utilization. For example, increased plasma fatty acid levels will result in increased passive diffusion into tissues, yet this does not translate into increased fatty acid esterification or oxidation. These events are also regulated by signaling systems that act independent of the nutrient supply per se. Consequently, the "push" approach to feeding the immune system is based upon nutrient supply alone and does not consider the controls that couple nutrient supply with nutrient demand. In regards to feeding the immune system, it is important to understand how these cell types and tissues coordinate nutrient utilization, as well as to understand the periods when nutrient utilization is of critical importance to the immune system.
The "Pull" Approach

The "pull" approach to feeding the immune system involves modulating the coordinated adaptations that direct nutrient partitioning toward immune function. These adaptations are complex and regulated by signals, often times cytokines, to help coordinate nutrient supply with nutrient demand across all tissues and within the animal (Humphrey et al., 2004). The coordinated adaptations of nutrient utilization throughout the body by signals from the immune system help to ensure that nutrient supply meets the nutritional demands associated with immune function. To ensure nutrient acquisition during these times, immune cells can utilize several strategies to partition more nutrients toward immunity.

Nutrient transporters: Nutrient transporters mediate substrate specific uptake across the plasma membrane. Nutrient transporter families have multiple isoforms that differ in substrate affinity ($K_m$), maximum rate of transport ($V_{max}$), tissue distribution and regulation. The substrate specificity of nutrient transporters, combined with multiple isoforms differing in transport kinetics, provides a robust repertoire for tissues
to modulate their nutrient priority status. Comparing functional categories of transporters can be used to determine the priority of tissues for a particular nutrient. For example, those tissues expressing high affinity isoforms have a high priority for a particular nutrient compared to those tissues expressing a low affinity isoform. We previously examined tissue priority for lysine and arginine by comparing functional classes of cationic amino acid transporters (CAT) and found that tissue growth during a lysine deficiency was related to the levels of high affinity CAT isoforms in chickens fed a lysine adequate diet (Humphrey et al., 2006). Since thymus and pectoralis high affinity CAT levels were lowest in chickens fed a lysine adequate diet, the growth of these tissues was the least when the lysine supply was limited. In contrast, the high levels of CAT isoforms in the bursa were related to a greater ability to grow in a nutritional environment where lysine is limiting.

In addition to CATs, developing chicken lymphocytes also express nutrient transporters for glutamine, fatty acids and glucose (Rudrappa et al., 2007). These latter nutrient transporters are important for the acquisition of energy substrates to support the high rates of lymphocyte proliferation. Increased expression of glutamine and glucose transporters presumably account for increased utilization of these energy substrates by these cells. Indeed, increased glucose transporter expression is associated with increased glucose uptake in developing chicken lymphocytes (Rudrappa et al., 2007).

Macrophage activation results in increased utilization of nutrients to synthesize killing compounds (see below) and this increased demand is supported by increased expression of nutrient transporters. For example, activated macrophages increase expression of arginine transporters to supply these cells with sufficient arginine for increased nitric oxide production (Nicholson et al., 2001). In fact, mammalian macrophages cultured in vitro increase arginine transport in response to increased arginine concentrations. Increased arginine uptake results in greater nitric oxide production. However, in vivo, plasma arginine levels remain unchanged during infection (Humphrey et al., 2005), indicating that this amino acid may not be limiting for macrophage function.

**Metabolic enzymes:** In addition to nutrient acquisition, metabolic enzymes play an important role in directing nutrient flux through specific metabolic pathways. The coordinated upregulation of both nutrient transporters with specific metabolic enzymes allows the immune system to coordinate nutrient utilization. For example, developing lymphocytes require energy generation to support the high rates of cell proliferation in primary immune tissues. In chickens, developing B and T lymphocytes alter the activity of enzymes involved in the oxidation of amino acid, glucose and lipid (Rudrappa et al., 2007), and this increase is coordinated with periods of heightened proliferation.

**Altered nutrient partitioning:** Coordination of nutrient partitioning to the immune system is further illustrated by reciprocal regulation of nutrient metabolism in skeletal muscle and immune tissue during periods of infection. In general, tissues engaged in host defense require greater amounts of amino acid substrate to synthesize protective factors and to support leukocyte proliferation (Klasing et al., 1984). Those tissues not directly involved in immune defense are either unaffected or increase their rates of protein degradation (Klasing et al., 1984). Skeletal muscle
is the largest labile pool of amino acids, and infection increases protein degradation and release of amino acids into plasma (Hentges et al., 1984, Tian et al., 1989, Tian et al., 1989). The release of amino acids from skeletal muscle plays an important role in supplying amino acid substrate for the synthesis of hepatic acute phase proteins involved in host defense (Barnes et al., 2002). This coordination of altered nutrient partitioning during infection is related to changes in amino acid transporter expression, with elevated levels in the skeletal muscle to allow for amino acid liberation and elevated levels in the liver to allow for increased amino acid absorption for synthesis of acute phase proteins (Humphrey et al., 2005).

Regulating the "Pull": Coordination of nutrient partitioning to the immune system is achieved in large part through the actions of cytokines. Leukocytes display many cytokine receptors, but other cell types have a much more limited expression pattern. This difference allows cytokines to act selectively upon cells of the immune system to increase their nutrient acquisition. Furthermore, the selective action of cytokines can affect nutrient acquisition by specific leukocyte populations. For example, activated T lymphocytes produce interleukin-2 (IL-2) that acts in an autocrine and paracrine manner to increase T lymphocyte proliferation. IL-2 also increases T lymphocyte glucose transporter-1 (GLUT-1) protein to provide energy for proliferation. During lymphocyte development, IL-3 also increases lymphocyte nutrient transporters for glucose, amino acids, lipids and metals (Edinger et al., 2002) and IL-7 maintains the metabolic activity of naïve T lymphocytes (Rathmell et al., 2001).

Activation of lymphocyte antigen receptors is also a key regulatory event that signals for increased nutrient utilization (Fox et al., 2005). Antigen receptor activation triggers the lymphocyte to undergo clonal expansion, a proliferative event, to allow for the generation of antigen specific lymphocytes. Signaling pathways emanating from the antigen receptor aid in the coordination of lymphocyte metabolism to meet the energetic demands of activation (Frauwirth et al., 2004).

Preferred Energy and Amino Acid Substrates for Immunity

The immune system has specific energy and amino acid substrates that are preferred for supporting the anabolic events associated with activation. These substrates are important for fueling the proliferation of lymphocytes and the synthesis of protective factors associated with host defense.

Energy: Energy metabolism is of particular importance to lymphocytes since their development and activation involve rapid proliferation. Leukocytes primarily utilize glucose and glutamine as an energy source (Ardawi et al., 1985). Glucose is the fuel of choice for lymphocytes, and in mammals glucose is actually an essential nutrient for lymphocytes (Greiner et al., 1994). Glucose is also important for generating reducing equivalents through the pentose phosphate pathway. These reducing equivalents are essential for producing killing compounds involved in the macrophage respiratory burst (Newsholme et al., 1996). Second to glucose, glutamine is also a major energy substrate for leukocytes. Like glucose, glutamine
metabolism can also generate reducing equivalents necessary for the production of reactive oxygen species (Newsholme, 2001). Glutamine conversion to glutamate may also aid in the transport of amino acids since glutamate is a substrate for many amino acid transport exchange systems (Aledo, 2004).

**Amino acids:** Arginine can be metabolized to produce nitric oxide (NO) involved in inflammatory responses and polyamines involved in wound healing. In mammals, arginine plays an integral role in the development of B lymphocytes (de Jonge et al., 2002) and also regulates the signaling ability of T lymphocytes (Rodriguez et al., 2002). Since arginine can be synthesized in ureotelic species, immune cells, particularly macrophages, are capable of synthesizing and recycling arginine for use in nitric oxide production. In uricotelic species and strict ureotelic carnivores that are incapable of arginine synthesis, this amino acid is not only essential, but cannot be recycled (Sung et al., 1991). Consequently, this amino acid is of particular importance in these species during periods of infection since increased endogenous synthesis of arginine cannot compensate for increased utilization of this amino acid by the immune system. A considerable amount of research has been conducted in chickens examining the effect of arginine on the immune system (Kidd et al., 2001, Kwak et al., 1999, Takahashi et al., 1999), and the provision of this nutrient to the immune system appears most important for activation.

Glutamine has long been recognized as an important metabolic fuel for the immune system (Ardawi et al., 1983). Glutamine is primarily metabolized to generate energy for leukocytes, as well as reducing equivalents for synthesizing reactive oxygen intermediates (Newsholme, 2001). Glutamine can also be metabolized to arginine in murine macrophages for NO synthesis (Murphy et al., 1998). Though glutamine can be synthesized endogenously, differences in glutamine metabolism between uricotelic and ureotelic species may have implications on glutamine use by cells of the immune system between animals with these nitrogen excretion strategies.

Cysteine can be metabolized to produce glutathione (GSH). GSH is one of the major intracellular antioxidants and its production is regulated by the availability of cysteine. GSH production increases during periods of inflammation (Malmezat et al., 2000), and consequently a greater proportion of cysteine's metabolism is directed toward GSH synthesis (Malmezat et al., 1998). GSH plays an important role in leukocyte function and these cells have a strong ability to obtain cysteine (Droge et al., 1991). Increased cysteine utilization for GSH synthesis results in taurine production, and inflammatory responses result in increases in taurine levels in the liver and kidney. However, taurine levels were reduced in the gastrointestinal tract (Malmezat et al., 1998), and this reduction may have implications on bile salt formation and lipid absorption in carnivores.

"Push" versus "Pull": Applications

When formulating diets that are optimum for the immune system, it is important to consider the type of nutrients being offered to the immune system, i.e. supply, and to what specific aspect of the immune system that they are intended for, i.e. demand. Considering supply without demand can result in either no impact on immune function, or even decreased overall animal health, as evidenced by the severity of E. coli infection in iron supplemented newborn pigs (Kadis et al., 1984). Rather, nutritional
approaches to enhance immune function should focus on supplying the nutrients at the appropriate times and in the appropriate amounts that complement the "pull" associated with increased nutrient partitioning for immune function.

References:


