

Fat meets the cholinergic antiinflammatory pathway

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The cholinergic antiinflammatory pathway is a neural mechanism that is controlled by the vagus nerve and inhibits local cytokine release, thereby preventing the damaging effects of cytokine overproduction. A new study now shows that dietary fat can activate this pathway, a finding that may help explain the immune system's failure to react to food antigens and commensal bacteria. Here we discuss this new data and its potential implications for dietary intervention in the treatment of inflammatory diseases.

The cytokine theory of disease

Cytokines underlie the clinical manifestations of many pathological and clinical syndromes ranging from shock and severe sepsis to arthritis and inflammatory bowel disease. The "cytokine theory of disease" originated in the 1970s and 80s and stemmed from basic biological and physiological studies of body weight, temperature regulation, and blood pressure (1). Early investigators established that the proinflammatory cytokine tumor necrosis factor (TNF) was both necessary and sufficient to cause the pathophysiological response to acute bacterial infection, and that administration of monoclonal anti-TNF antibodies prevented shock and lethal tissue damage. This prompted a shift in scientific focus from developing therapies that directly target pathogens to those that target products of the immune system, such as TNF. The effectiveness of these therapies in the clinic validated the cytokine theory of disease in humans and proved that it is possible to rationally control the cytokine response to clinical advantage.

The cytokine response to infection or injury is a well-orchestrated and tightly controlled process (2). When functioning properly, cytokines successfully eradicate pathogenic invaders and then restore homeostasis. Sometimes cytokines are overproduced, however, either because the magnitude of the invasive stimulus is overwhelming or because

the counterregulatory mechanisms that normally restrain cytokine release break down. For instance, high levels of bacterial endotoxin in the blood, as occur in patients with fulminant meningococemia, activate the immune system to release large amounts of TNF, which in turn causes a lethal septic shock syndrome (3). Not all infections or injuries cause this burst of TNF production; some are associated with a different cytokine response pattern. For instance, patients with intraperitoneal infection with *Escherichia coli* can develop epithelial cell dysfunction and lethal organ damage caused by excessive production of the inflammatory nuclear protein high mobility group box-1 (HMGB1), a syndrome termed severe sepsis (4). Thus, in order to effectively exploit our mechanistic and therapeutic knowledge of cytokines it is important to recognize the fundamental differences in the pathological and biological activity of different cytokines in distinct clinical syndromes. For example, anti-TNF antibodies might be effective in preventing acute septic shock syndrome but ineffective in preventing severe sepsis (5); anti-HMGB1 antibodies might effectively prevent the organ damage and lethality of severe sepsis but would not be expected to be useful in cases of acute septic shock (1, 4, 6). These examples highlight two important concepts. First, health is dependent on a controlled cytokine response, and second, knowledge about the mechanisms that normally restrain the cytokine response can be exploited to develop new therapeutics.

Keeping cytokines in check

Redundant antiinflammatory mechanisms normally restrain the cytokine response. Glucocorticoids, antiinflammatory cytokines (such as interleukin [IL]-10 and transforming growth factor [TGF]- β), hormones (such as melanocyte-stimulating hormone), and other metabolic products (such as spermine) all inhibit cytokine release. Antiinflammatory cytokines are released by macrophages, lymphocytes, and other cells of the immune system during the earliest stages of infection or injury and are delivered to the local site of inflammation by diffusion through tissues or by way of the bloodstream (1, 2). Their activities reduce the likelihood that a local inflammatory response will spill over into the blood and impair distant organ function. A major limitation of this humoral antiinflammatory system, however, is that it can cause immunosuppression. Thus, an infected wound in the palm of one hand may increase circulating levels of glucocorticoids and IL-10 to immunosuppressive levels that render the patient susceptible to other infections. Recently, my colleagues and I discovered that the central nervous system is hard wired to control the cytokine response independently of the humoral antiinflammatory response (7). This mechanism inhibits cytokine release locally in tissues, without causing systemic immunosuppression.

The cholinergic antiinflammatory pathway

The vagus nerve, a paired structure that arises in the brainstem and traverses the neck, thorax, and abdomen to innervate visceral organs, is named for its wandering and meandering course. Its fibers contain sensory and motor components that control organ functions as varied as heart rate and digestion. In addition to controlling these classic physiological functions, we recently

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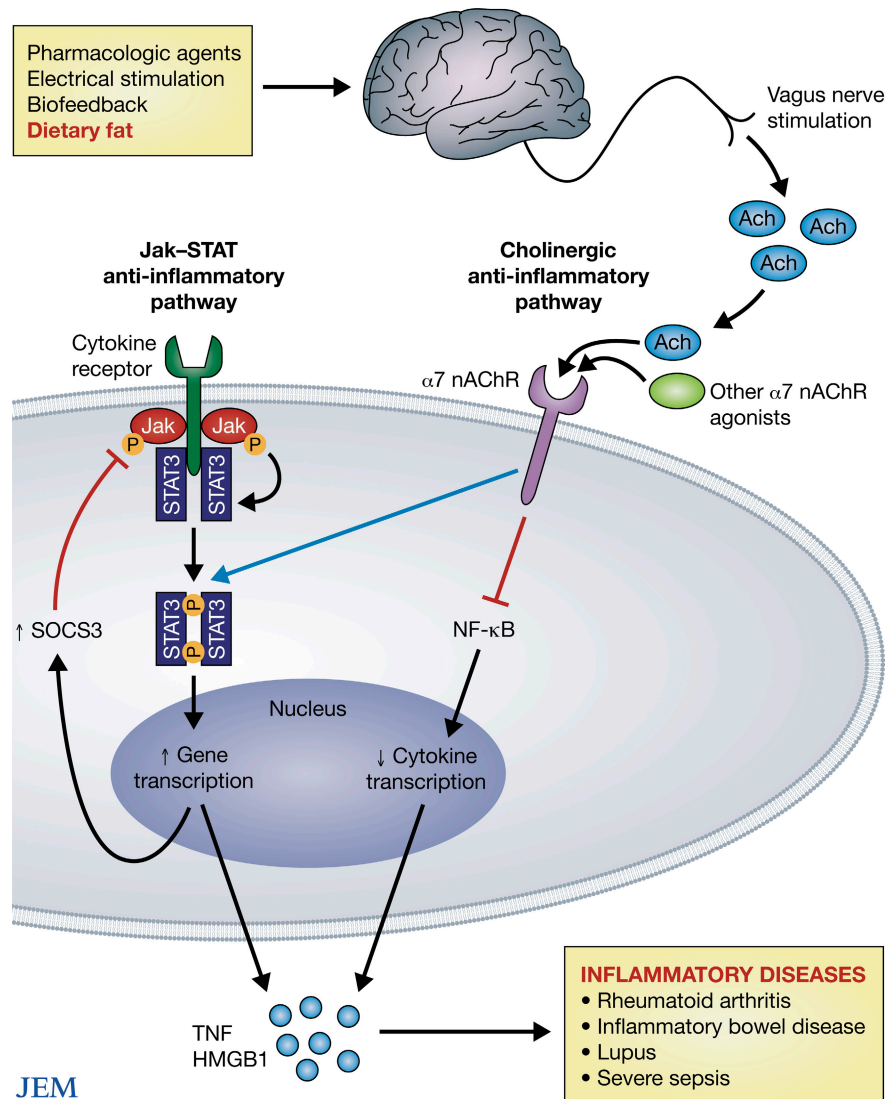
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discovered that the vagus nerve prevents the release of TNF, HMGB1, IL-1, and other proinflammatory cytokines (7). As the activity of this pathway is controlled by neural signals, it provides a way for the brain to regulate the cytokine response in a localized, controlled, and organ-specific manner.

The neurotransmitter acetylcholine is released by vagus nerve endings and binds to nicotinic $\alpha 7$ receptors on macrophages and other cytokine-producing cells in organs such as the spleen, liver, and heart (8). Ligation of nicotinic receptors by acetylcholine inhibits cytokine synthesis and release by preventing the activation and nuclear translocation of NF- κ B, and by stimulating the antiinflammatory JAK3-SOCS3 pathway (Fig. 1) (8–10). Expression of nicotinic $\alpha 7$ receptors is required for the vagus nerve to interface with the immune system, as elimination of the gene that encodes this receptor in mice abolishes the vagus nerve control of the cytokine response (8). This vagus nerve-controlled counterregulatory system, termed the “cholinergic antiinflammatory pathway,” controls cytokine synthesis by resident macrophages in the reticuloendothelial system. This pathway suppresses cytokine activity locally in specific sites, thereby preventing the release of disease-inducing cytokines into blood.

The inflammatory reflex

The cholinergic antiinflammatory pathway is the efferent arm of the inflammatory reflex (7). IL-1, endotoxin, and other inflammatory products activate afferent signals in the vagus nerve, which serves as a sensor of inflammation or injury. This information is relayed to the central nervous system via the hypothalamic-pituitary axis and triggers the release of acetylcholine from efferent vagus nerve endings. The vagus nerve is anatomically positioned to inhibit cytokine production in the organs of the reticuloendothelial system. It may be possible to exploit the inflammatory reflex to therapeutic advantage. We and others have explored the effects of stimulating the vagus



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Figure 1. Activation of the vagus nerve leads to the release of acetylcholine (ACh) that binds to $\alpha 7$ nicotinic receptors ($\alpha 7$ nAChR) on cytokine producing cells. This inhibits the activation of NF- κ B and activates STAT3 phosphorylation. Phosphorylated STAT3 triggers an antiinflammatory signal by increasing SOCS3 expression, which suppresses signaling from cytokine receptors. Together, these signals prevent the release of TNF, HMGB1, and other cytokines implicated in the pathogenesis of inflammatory disease.

nerve using pace maker-like devices that have been safely implanted in thousands of patients with seizure disorders (10–14).

Preclinical animal studies have shown that vagus nerve stimulators are extremely effective in suppressing cytokine-mediated inflammation and damage in endotoxemia, severe sepsis, hemorrhagic shock, ischemia reperfusion injury, arthritis, and postoperative ileus (7–14). These experiments

reveal a central regulatory role for vagus nerve activity in suppressing cytokine release in organs and in serum, and correspondingly in attenuating disease severity in response to a variety of inflammatory stimuli. A second experimental strategy has been to develop agonists for the $\alpha 7$ nicotinic receptor as experimental therapeutics. Agents from this class effectively inhibit HMGB1, TNF, and other proinflammatory cytokines in animals models of

endotoxemia, severe sepsis, hemorrhagic shock, ileus, and experimental diabetes (7, 9).

Dietary fat and the cholinergic antiinflammatory pathway

On page 1023 of this issue, Luyer et al. (15) present compelling evidence that consumption of fat in the diet can activate the cholinergic antiinflammatory pathway (15). They found that administration of high fat nutrition reduced circulating levels of TNF and IL-6 in rats subjected to hemorrhagic shock, a manipulation known to activate high blood cytokine levels. When these experiments were repeated in animals subjected to vagotomy, the administration of the high fat diet no longer prevented the increase in TNF and IL-6. The authors also revealed a mechanistic role for cholecystokinin (CCK), a neuropeptide released after consumption of dietary fat that triggers several digestive functions including stimulation of gall bladder contraction and exocrine pancreas secretion, and activation of afferent vagus nerve signals that induce satiety. Administration of CCK receptor antagonists to rats fed a high fat diet impaired the fat-induced suppression of TNF and IL-6 during hemorrhagic shock. Luyer et al. also explored the effects of administering a nicotinic receptor antagonist (chlorisondamine) on the dietary fat-induced regulation of cytokines (15). Nicotine receptor antagonism blocked the ability of dietary fat to suppress TNF and IL-6, which, when considered together with the vagotomy experiments, indicates that the intake of dietary fat suppresses cytokine release by activating the cholinergic antiinflammatory pathway (15). These provocative findings are important for at least two reasons that are discussed below. First, this pathway may help explain why the trillions of microorganisms that reside in the human intestines do not stimulate an exaggerated cytokine response. Second, these data open up several possibilities for new clinical studies involving nutritional manipulation of the cytokine response.

Trillions of microbes, but no inflammation

A fundamental unanswered question in immunology and physiology is why the enormous intestinal reservoir of microbes, and their associated endo- and enterotoxins fail to activate an inflammatory cytokine response. The gut is a major source of blood cytokines in humans subjected to i.v. endotoxin infusion, indicating that the enteric immune system is capable of producing a cytokine response (16). This contrasts with the absence of cytokine production or an inflammatory response in the bowel wall, which is adjacent to the endotoxin-rich gut lumen. Intact food proteins, bacterial products, and other antigens can cross the epithelium through intermittent openings in the tight junction, and interact with macrophages and other cytokine-producing cells in the lamina propria, with intraepithelial lymphocytes, and with T and B cells in the Peyer's patches (for review see reference 17). But, under normal circumstances, these interactions fail to trigger an inflammatory response. There is growing evidence that cytokine-mediated inflammation in the gut is prevented in part by regulatory T (T reg) cells. T reg cells produce antiinflammatory mediators, including TGF- β and IL-10, that inhibit cytokine synthesis (17). Luyer's results (15) now also raise the possibility that cholinergic signals from the enteric nervous system may also help suppress local inflammatory responses to commensal microbes.

The localized release of acetylcholine from the enteric nervous system will likely suppress cytokine production by macrophages and dendritic cells in response to commensal bacteria. Dysfunction of this pathway would be expected to trigger the development of inflammatory bowel disease or other inflammatory diseases of the gut. This model is supported by data showing that nicotine is therapeutic for some patients with ulcerative colitis (18), a disease characterized by excessive colonic cytokine production. This effect is likely due to nicotine binding to receptors on gut immune cells, thus

mimicking the efferent cholinergic antiinflammatory pathway and suppressing cytokine production. Nicotine is not effective, however, in Crohn's disease, an inflammatory bowel disease that is responsive to anti-TNF therapy. A possible explanation for disparate effects of nicotine on ulcerative colitis and Crohn's disease is that the former could be caused by an acquired defect in acetylcholine production; such a defect could be reversed by exogenous nicotine. In contrast, Crohn's disease might not result from a problem in acetylcholine production, but rather from a defect in the nicotinic receptor or a signaling abnormality that interferes with the ability of cytokine-producing cells to respond to acetylcholine. In this setting, nicotine would be useless. Consistent with this idea, clinical studies have shown that administration of fish oil is therapeutic in patients with ulcerative colitis, but not in those with Crohn's disease (19). The study by Luyer et al. suggests the possibility that these protective effects are dependent on fat-induced activation of the cholinergic antiinflammatory pathway (15).

There are many unknowns in this pathway that remain to be addressed. One unknown is whether different types of dietary fat vary in their ability to activate the cholinergic antiinflammatory pathway. It is also possible that diet-induced activation of the cholinergic antiinflammatory pathway underlies the beneficial effects of early enteral feeding in trauma and surgery patients, which significantly reduces mortality from sepsis and organ damage (20).

Clinical correlates

Advances in molecular biology and animal physiology have helped us to study human inflammatory diseases with the hope of applying this knowledge to the development of new therapies. For this approach to succeed, it will be important to identify surrogate markers and end points for monitoring the activity of the cholinergic pathway in the clinic. A generally accepted method of measuring vagus nerve activity, or "tone," is to calculate the in-

stantaneous heart rate variability using software that measures the variations in time between individual heart beats, as detected by electrocardiographic recording. Although it is not currently known whether vagus nerve activity in the heart correlates with the activation of the cholinergic antiinflammatory pathway in humans, there have been reports of decreased vagus nerve activity in diseases associated with exaggerated cytokine responses. Patients with severe sepsis, rheumatoid arthritis, lupus, and inflammatory bowel disease have all been shown to have significant decreases in their instantaneous heart rate variability (21–24). An underlying defect in the activity of the cholinergic antiinflammatory pathway might trigger an overproduction of cytokines in response to an otherwise innocuous immunological stimulus and thus contribute to disease pathogenesis.

Animal studies indicate that the threshold of vagus nerve activity that activates the cholinergic antiinflammatory pathway is significantly lower than that required to activate a change in instantaneous heart rate variability. If this proves to be the case in humans, then patients with decreased instantaneous heart rate variability might be predicted to be at increased risk for developing cytokine-mediated diseases. We need to learn more about these potential relationships, which theoretically could be studied in the context of clinical trials of dietary fat supplementation in patients with inflammatory diseases, an approach that has already shown significant therapeutic effects in some studies (25–29). For example, Holguin and colleagues recently reported that supplementing the diet of elderly patients with either fish oil, or soy oil, significantly increased instantaneous heart rate variability (25). Their study did not address the relationship between these changes in vagus nerve activity and inflammatory markers, but the results indicate the feasibility of approaching this question in the future. Studies could also be performed to reveal whether different types of dietary fat are more potent activators of the

cholinergic antiinflammatory pathway, and thus more effective for the treatment of patients with rheumatoid arthritis or inflammatory bowel disease, two cytokine-driven diseases may respond to ingestion of dietary fat.

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