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Toward an Understanding of the Pathophysiology of Chronic Laryngitis

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Abstract

Chronic laryngitis, characterized by inflammation of the laryngeal tissues, is the most commonly diagnosed organic voice disorder, yet treatments targeting suspected etiologic factors have demonstrated limited efficacy. A major barrier to the development of improved medical therapies for chronic laryngitis is a fundamental gap in knowledge related to the pathophysiology of laryngeal inflammation. This article provides a review of the literature specific to laryngeal immunity in health and disease.

Chronic laryngitis is the most commonly diagnosed organic voice disorder, with nearly 10% of all dysphonia cases classified as such (Cohen, Kim, Roy, Asche, & Courey, 2012). Laryngitis refers to inflammation of the tissues of the larynx due to infectious (e.g., viral, bacterial, fungal) and mechanical agents (for a complete glossary of terms, see Appendix A). Chronic laryngitis develops gradually with waxing and waning underlying signs and symptoms over very long periods of time. The disease is characterized by a variety of symptoms including hoarse voice, effortful speaking, sore throat, throat clearing, and cough. Laryngeal examinations are heterogeneous, but commonly reveal diffuse supraglottal and glottal erythema and edema in addition to excessive thick mucus. Ulcerative changes, granulation, and scar may be noted, as well as benign vocal fold pathology such as polypoid changes or Reinke's edema.

Treatment for chronic laryngitis is prescribed empirically based on assumptions about etiologic factors (e.g., laryngopharyngeal reflux (LPR) and tobacco inhalation) that result from subjective judgments of videostroboscopic laryngeal examinations (Belafsky, Postma, & Koufman, 2001; Hickson, Simpson, & Falcon, 2001). While inflammatory clinical signs are commonly ascribed to reflux, multiple investigators have documented endoscopic clinical reflux findings in normal, healthy volunteers with no symptomatic history of reflux (Hicks, Ours, Abelson, Vaezi, & Richter, 2002; Milstein et al., 2005). A study examining correlations between Reflux Findings Score ratings and measures from multichannel intraluminal impedance combined with pH monitoring found no clear correlation between the two assessments (Jetté, Gaumnitz, Birchall, Welham, & Thibeault, 2014). Medical treatment for chronic laryngitis using proton pump inhibitors has been shown to have limited efficacy (Vaezi et al., 2006), though in spite of a lack of efficacy data it is estimated that

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46.2% of patients diagnosed with chronic laryngitis receive medication (Cohen, Kim, Roy, & Courey, 2013). An incomplete understanding of the pathophysiology of laryngeal inflammation is a major barrier to the development of improved medical therapies for chronic laryngitis.

As an emerging scientist in the area of voice disorders research, my aim is to expand our understanding of the physiologic mechanisms that lead to laryngeal inflammation and voice problems. My ultimate goal is to contribute to innovations in treatment for chronic laryngitis. This article will outline what is currently known about the pathophysiology of laryngeal inflammation with an emphasis on mucosal immunity.

Immune Regulation in the Larynx

The immune response is a series of biological processes by which the body responds to pathogens and other danger signals. These processes include innate and adaptive immune responses. Innate immunity is the first line of defense and provides an initial barrier to invading pathogens, whereas adaptive immunity incites specific and specialized responses to a variety of antigens, thereby adding efficiency to the immune response.

The human mucosal immune system is a major defense system of the human body. It developed not only to defend the human host against pathogens and to react to tissue damage, but also to accommodate host colonization by symbiotic microorganisms and to maintain host-microbe homeostasis (Cerf-Bensussan & Gaboriau-Routhiau, 2010). Mucosal immunity is governed by the interplay among the epithelium, communities of bacteria inhabiting the tissue, and resident immune cells. The term *tolerance* describes the physiologic process by which we are protected from immune reactivity against self-antigens. Mucosal tolerance is regulated by a set of signals provided by innate immune cells that shape the adaptive immune responses. To avoid targeting its own body tissues, the immune system must differentiate between the host's resident components and foreign agents, controlling the activity of its numerous constituents in a very precise way. Unregulated or misdirected adaptive immune responses against self or resident components can cause chronic inflammatory disorders such as allergy and autoimmune diseases and chronic inflammation of the gut and respiratory mucosae (Prescott, 2009; Tulic et al., 2011). The mucosal epithelium is primarily responsible for immune regulation via its function as a barrier, cell contact-mediated signals, and the production of cytokines. Specific mechanisms by which the epithelial barrier regulates the immune response are not completely understood.

It has been hypothesized that the larynx is an important organ for immunological decision-making in the airway (Barker, Murison, et al., 2006). Situated at the junction between the respiratory and gastrointestinal tracts, the larynx represents a major site of exposure to pathogens and irritants. There is evidence that the larynx has a distinct immunological blueprint designed to address these challenges (Tang & Bluestone, 2006). However, the specifics of its composition and function are currently unknown. Both animal and human studies have revealed larynx-associated lymphoid tissue (LALT), a type of mucosa-associated lymphoid tissue (MALT) specific to the larynx (Gorti, Birchall, Haverson,

Macchiarini, & Bailey, 1999; Hiller et al., 1997; Hiller, Tschernig, Kleemann, & Pabst, 1998; Jecker, Ptok, Pabst, & Westermann, 1996). Immune cells including dendritic cells, T cells, and macrophages have been detected in histologic sections procured from human fetal laryngeal tissue (Dietrich, Jecker, Tschernig, & Mann, 2004). Macrophages and dendritic cells are involved in both innate and adaptive immunity. Dendritic cells capture pathogens, travel to lymph nodes, and present pathogens to other cells for destruction; whereas macrophages engulf and digest pathogens and then present antigens to the pathogen to other cells in the adaptive immune system. T cells are white blood cells that mature in the thymus and have receptors on their cell surface, allowing them to stimulate a defense against a specific pathogen. The functions of T cells vary based on the molecules present on their surfaces (i.e., CD4, CD8, CD25, FOXP3). In a pig model, Barker et al. (2006) found histologic evidence of T cells distributed diffusely both within and beneath the epithelium, including CD4+ and CD8+ T cells in the epithelium and subglottis. The CD4 marker denotes helper T cells, a subset of T cells that assist other white blood cells in the immune response, whereas the CD8 marker represents cytotoxic T cells, which, when activated, defend against intracellular pathogens. Double-positive CD4+CD8+ effector T cells made up less than 2% of the T cells observed in the laryngeal mucosa, with more observed in the subglottis than in the supraglottis. It has been suggested that CD4+CD8+ T cells participate in the immune response in viral infections (Nascimbeni, Shin, Chiriboga, Kleiner, & Rehermann, 2004).

Etiology and Pathophysiology of Chronic Laryngitis

Chronic laryngitis is a term used to describe a variety of specific and nonspecific inflammatory changes observed in patients with an array of symptoms. These inflammatory changes have been attributed to a variety of etiologic factors including vocal overuse, smoking, reflux, environmental factors (e.g., climate, chemical irritants, fumes, allergens; Baletic, Jakovljevic, Marmut, Petrovic, & Paunovic, 2005; Jackson-Menaldi, Dzul, & Holland, 2002; Kambic, Radsel, & Gale, 1989), orally inhaled medications, and *Helicobacter pylori* infection (Borkowski et al., 1997).

Smoking

It is hypothesized that cigarette smoke leads to laryngeal inflammation via multiple mechanisms. Cigarettes contain thousands of chemical components including nitric oxide, carbon monoxide, nicotine, formaldehyde, acetone, ammonia, and acrolein, among many others (Sopori, 2002; Yoshida & Tuder, 2007). Following inhalation of cigarette smoke, these by-products trigger inflammation and erythema of the laryngeal mucosa (Hardaker et al., 2010; van der Vaart et al., 2005). Further, persons who smoke often have an associated cough (Ebihara, Ebihara, Okazaki, & Sasaki, 2005) potentially leading to mechanical damage via repeated stress and strain of the laryngeal tissues. Finally, inhaled pollutants have hygroscopic effects (Flinn, 1935) that dry the laryngeal mucosa resulting in a decrease in mucosal viscoelasticity (Hemler, Wieneke, van Riel, Lebacq, & Dejonckere, 2001). Loss of mucosal viscoelasticity yields elevated subglottal pressure requirements for initiation of vocal fold vibration, also known as phonation threshold pressure (Chan & Titze, 2006). A

patient may perceive a change in viscoelasticity as increased vocal effort and fatigue, difficulty producing a loud or soft voice, and discomfort or pain with phonation.

It has been demonstrated that cigarette smoking induces lower airway inflammation in smokers, with increased T lymphocytes (mainly CD8+) and macrophages within the bronchial wall (Di Stefano et al., 2001), higher neutrophil counts within bronchial secretions, and infiltration of peripheral lung with mononuclear cells and macrophages (Saetta & Turato, 2001). In addition, the peripheral lung sections from smokers have been reported to have increased numbers of eosinophils infiltrating the submucosa compared with nonsmokers (Lams, Sousa, Rees, & Lee, 1998). Eosinophils have multiple immunologic functions, though they are most commonly implicated in asthma. A study of the immunologic consequences of tobacco smoke on the laryngeal mucosa revealed increased numbers of CD4+ T cells in smokers as well as an association between increased CD4+ T cells and older age (Rees et al., 2006).

Reflux

LPR, a disease that is estimated to affect 35% of people over the age of 40, is widely accepted as the most common etiologic factor contributing to chronic laryngitis, particularly in nonsmokers (Koufman, 1991, 2002; Reulbach, Belafsky, Blalock, Koufman, & Postma, 2001). There are two schools of thought concerning the mechanisms of laryngeal inflammation in the setting of gastric acid reflux and LPR. The first suggests a direct acid-peptic injury to the larynx and surrounding tissues, a theory that is supported by evidence from human tissue (Johnston, Dettmar, Bishwokarma, Lively, & Koufman, 2007; Johnston, Knight, Dettmar, Lively, & Koufman, 2004; Rees et al., 2008), animal studies (Gaynor, 1988; Little, Koufman, Kohut, & Marshall, 1985), and *in vitro* work (Johnston et al., 2007; Samuels & Johnston, 2010). The second hypothesis suggests that acid in the distal esophagus stimulates vagally mediated reflexes resulting in chronic throat clearing and coughing which eventually lead to laryngeal lesions and symptoms (Cherry, Siegel, Margulies, & Donner, 1970; Hallewell & Cole, 1970). It is possible that a combination of these mechanisms exist in the same patient or that other risk factors such as voice overuse or chronic throat clearing in the setting of reflux contribute to laryngeal inflammation. Gastroesophageal reflux disease (GERD) and its extraesophageal manifestations (LPR) have been implicated as comorbidities in other inflammatory diseases including asthma, sinusitis, allergic rhinitis, and nasal polyposis (Bisaccioni et al., 2009; Halstead, 1999; Harding et al., 1996; Mansfield & Stein, 1978); however, as with chronic laryngitis, the pathophysiologic contribution of reflux has not been fully ascertained across these diseases.

Inflammatory reactions in the airway have also been demonstrated to be induced by components of gastric reflux such as hydrochloric acid (HCl; Rabinovici, Vernick, Hillegas, & Neville, 1996; Van de Louw et al., 2002). Injection of HCl into the lungs of rabbits resulted in massive influxes of neutrophils and elevated IL-8, a chemokine produced by macrophages (Folkesson, Matthay, Hebert, & Broaddus, 1995). While there is limited research investigating the immunologic mechanisms specific to LPR-induced inflammation in the larynx, examinations of laryngeal tissues collected from patients with LPR have demonstrated increased CD8+ cell counts (Rees et al., 2008).

Allergens

There has been limited research demonstrating a causal link between inhaled or ingested allergens and laryngitis to date. Two forms of allergy-related laryngeal inflammation have been proposed including acute, IgE-mediated laryngitis, which is induced by anaphylactic reactions, and chronic, non-IgE mediated laryngitis (Chadwick, 2003; Corey, Gungor, & Karnell, 1998). Mucosal mast cells appear to be primarily responsible for the pathogenesis of allergic inflammation in the larynx, as they are in the upper airway in general. Mast cells proliferate mostly in the epithelium and superficial layer of connective tissue, and upon exposure to a previously sensitized antigen, they degranulate and release histamine and neuropeptides into local tissues. Late-phase inflammation is mediated by granulated leukocyte or eosinophilic cells that are recruited to the affected tissues (Ishii et al., 1997). Microscopic dissection studies in humans have demonstrated an abundance of connective tissue mast cells within the epiglottis, subglottis, and arytenoids, with relatively fewer quantities in the vocal folds (Ishida, Yoshida, Iwae, & Amatsu, 2005). In rats, neither the squamous epithelium nor associated neurons of the vocal folds contain mast cells or their derivatives, possibly explaining why researchers have been unable to provoke vocal fold inflammation in the laboratory (Lidegran, Domeij, Forsgren, & Dahlqvist, 1996; Niklasson & Dahlqvist, 2005; Reidy, Dworkin, & Krouse, 2003).

Laryngeal Microbiota

It has been well established that more microbes than human cells exist in and on the human body (The Human Microbiome Project Consortium, 2012) and that these microbes have a decisive role in the perinatal maturation of the mucosal immune system (Renz, Brandtzaeg, & Hornef, 2012). The microbiome, comprised of all microbes (commensal and pathogenic) and their genomes (metagenome), is a complex system characterized by microbe-microbe and host-microbe interactions that influence various aspects of host physiology. Normal microbes (also called commensal microbes) participate in the metabolism of food products, provide essential growth factors, protect against infections, and stimulate immune responses. Conversely, pathogenic microbes are those that cause disease via pathologic processes resulting from microbial factors such as production of toxins or the host's immune response to the organism. A variety of microbial communities exist throughout the human body, with fundamental roles in health and disease. Genetic and environmental factors such as age, diet, hormonal state, and personal hygiene can affect the microbiota predisposing the host to various diseases. For example, obesity, inflammatory bowel disease, and colon cancer are associated with changes in gut microbiome (Chung & Kasper, 2010; Ley, 2010; Round & Mazmanian, 2009).

To date, there has been a paucity of research examining the microbiota of laryngeal tissue and its role in mucosal health and disease. In 13 patients with chronic laryngitis and 5 patients with laryngeal polyps, Kinnari, Lampikoski, Hyyrynen, & Aarnisalo (2012) identified DNA corresponding to several microbes including *Staphylococcus aureus, Haemophilus influenza, Candida albicans, Moraxella nonliquefa, Propionibacter acnes, Neisseria meningitides, Streptococcus pneumonia, and Lactobacillus spp*. Gong et al. (2013) compared bacterial communities in laryngeal cancer relative to vocal fold polyp and found

15 bacterial subtypes potentially associated with laryngeal cancer, suggesting that changes to microbial communities may be a risk factor for carcinoma. In a similar study, Gong et al. (2014) demonstrated different microbial communities in the upper throat (i.e., near the epiglottis) compared to the area surrounding the glottis. In Hanshew, Jetté, & Thibeault (2014), we found highly similar bacterial communities in 44 benign vocal fold lesions, the majority of which were dominated by communities of *Streptococcus*, specifically *Streptococcus pseudopneumoniae*. Further, the communities present in vocal fold lesions were distinct from healthy throat and saliva samples.

It has been suggested that *Helicobacter pylori* colonizes the surfaces of the larynx (Aygenc, Selcuk, Celikkanat, Ozbek, & Ozdem, 2001) and that its detection is correlated with laryngeal inflammation and benign vocal fold lesions. Borkowski et al. (1997) investigated the role of *H. pylori* in the pathogenesis of chronic laryngitis in 35 patients with a 3-month or longer history of chronic laryngitis, with positive results in 15/35 patients. Rubin, Benjamin, Prior, Lavy, & Ratcliffe (2002) reported a 54% prevalence of *H. pylori* in chronic laryngitis. In one study, *Helicobacter* was detected in vocal polyps, but not in vocal nodules, in patients with hoarseness (Fang, Lee, Li, Yang, & Huang, 2008). Other researchers using alternate research methodology concluded that *H. pylori* is present in the majority of patients with vocal polyps and vocal nodules (Tiba, Fawaz, & Osman, 2010). Conversely, Akbayir, Basak, Seven, Sungun, & Erdem (2005) did not find any evidence for the presence of *H. pylori* in benign laryngeal lesions. In Hanshew et al. (2014), gene sequences identified as *Helicobacter* were present in only 5 of 44 benign lesions.

There have been few studies examining the complex interactions of the microbiota and immune cell function in the larynx. Ebenfelt and Finizia (2000) examined secretions isolated from the laryngeal mucosa in patients with chronic laryngitis and found large numbers of bacteria with few neutrophils and no evidence of phagocytosis, suggesting the bacteria were nonpathogenic colonizers. To date, the most extensive study of laryngeal immune cell function in the presence of microbes employed three groups of gnotobiotic pigs; the first group was kept germfree and the other two were colonized with standard microbiota (Schaedler flora) in addition to either *Staphylococcus* or *Bacillus* (Birchall et al., 2008). Three markers were assessed including CD4 (T cells), CD16 (monocyte activation), and MHC II (monocytic cells). At Week 3, the germfree pigs demonstrated minimal increase in any of the markers while the colonized pigs developed a significant increase in CD4+ cells. Further, the pigs colonized with *Staphylococcus* showed an increase in MHC II expression. These findings suggest that normal development of laryngeal immunity depends on local microbiota. To study leukocytic involvement in the subglottic and glottic mucosa during infection, Jecker et al. (1999) induced acute laryngitis in rats using two different pathogens including heat-killed *Moraxella catarrhalis* and *Bordatella pertussis*. Two hours after inhalation of *M. catarrhalis*, the authors found three to five times more neutrophils, dendritic cells, and T and B lymphocytes in the mucosa of the supra and subglottic regions compared to the glottic region, suggesting that physiologic features of the epithelium, specifically in the subglottis, might permit the adherence of pathogens thereby provoking a region-specific response.

Future Directions

The overarching aim of my developing line of research is to elucidate the pathophysiology of laryngeal inflammation by examining host factors such as local microbiota, immune cell responses, and epithelial barrier composition. In the context of a translational research paradigm, my goal is to understand the specific physiologic mechanisms underlying laryngeal inflammation, thereby paving the way to develop novel pharmacologic agents for treating chronic laryngeal inflammation and associated voice problems in humans. Two studies in collaboration with Dr. Susan Thibeault and other researchers at University of Wisconsin-Madison will examine microbes and T regulatory cells in human laryngeal tissue, and my ongoing research at University of Colorado Denver will address the role of neurogenic inflammation and its interplay with mucosal immunity in both human and animal models.

Summary

Chronic laryngitis, characterized by inflammation of the laryngeal tissues, is the most commonly diagnosed organic voice disorder, yet treatments targeting suspected etiologic factors have demonstrated limited efficacy. A major barrier to the development of improved medical therapies for chronic laryngitis is a fundamental gap in knowledge related to the pathophysiology of laryngeal inflammation. Studies in cells, animals, and humans have identified various immune cells that may contribute to inflammation, though the specific biological events and signaling pathways not been described. Similarly, the role of resident and pathogenic bacteria in shaping laryngeal immune responses has not been identified. Foundational studies investigating the specific biological mechanisms underlying laryngeal inflammation are much needed to improve treatment for chronic laryngitis.

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Appendix A.: Glossary of Terms (adapted from National Institute of Allergy and Infectious Diseases)

antigen—a substance or molecule that is recognized by the immune system. The antigen can be from foreign material such as bacteria or viruses

B cell or B lymphocyte—a small white blood cell crucial to the immune defenses. B cells come from bone marrow and develop into blood cells called plasma cells, which are the source of antibodies.

bacteria—very small, simple prokaryotic microorganisms that are found almost everywhere. Some bacteria cause disease in humans and animals, while others aid natural bodily functions and are beneficial to health.

chemokine—a small protein molecule that activates immune cells, stimulates their migration, and helps direct immune cell traffic throughout the body.

cytokines—powerful chemical substances secreted by cells that enable the body's cells to communicate with one another. Cytokines include lymphokines produced by lymphocytes and monokines produced by monocytes and macrophages.

cytotoxic T lymphocyte—a subtype of T cells that carries the CD8 marker and can destroy body cells infected by viruses or transformed by cancer.

dendritic cell—an immune cell with highly branched extensions that occurs in lymphoid tissues, engulfs microbes, and stimulates T cells by displaying the foreign antigens of the microbes on their surfaces.

eosinophil—a white blood cell containing granules filled with chemicals damaging to parasites and enzymes that affect inflammatory reactions.

helper T cells—a subset of T cells that carry the CD4 surface marker and are essential for turning on antibody production, activating cytotoxic T cells, and initiating many other immune functions.

inflammation—an immune system reaction to "foreign" invaders such as microbes or allergens. Signs include redness, swelling, pain, or heat.

innate—an immune system function that is inborn and provides an all-purpose defense against invasion by microbes.

lymphocyte—a small white blood cell produced in the lymphoid organs and essential to immune defenses. B cells, T cells, and NK T cells are lymphocytes.

macrophage—a large and versatile immune cell that devours invading pathogens and other intruders. Macrophages stimulate other immune cells by presenting them with small pieces of the invaders.

major histocompatibility complex (MHC)—a group of genes that controls several aspects of the immune response. MHC genes code for "self " markers on all body cells.

mast cell—a granulocyte found in tissue. The contents of mast cells, along with those of basophils, are responsible for the symptoms of allergy.

microbe or microorganism—a microscopic living organism. Examples include bacteria, protozoa, and some fungi and parasites. Viruses are also called microbes.

microbiome—all of the microorganisms (i.e., bacteria viruses, fungi and protozoa) and their physical interactions (pathogenic, commensal and mutualistic) in a particular environment.

microbiota—the ecological community of commensal, symbiotic and pathogenic microorganisms that literally share our body space

molecule—the smallest amount of a specific chemical substance. Large molecules such as proteins, fats, carbohydrates, and nucleic acids are the building blocks of a cell, and a gene determines how each molecule is produced.

monocyte—a large phagocytic white blood cell which, when entering tissue, develops into a macrophage.

pathogen—a disease-causing organism or virus.

T cell or T lymphocyte—a small white blood cell that recognizes antigen fragments bound to cell surfaces by specialized antibody-like receptors. “T” stands for the thymus gland, where T cells develop and acquire their receptors.

tolerance—a state of immune nonresponsiveness to a particular antigen or group of antigens.

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