

Human lysozyme has fungicidal activity against nasal fungi

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ABSTRACT

Background: The cationic antimicrobial peptide lysozyme is the most prevalent innate immune protein in nasal secretions but there is a paucity of research regarding its role in paranasal sinus disease. Lysozyme is generally regarded as an antibacterial agent; however, some data suggest activity toward yeast. This study was designed to determine if lysozyme displays fungicidal activity toward fungi commonly identified in patients with chronic rhinosinusitis (CRS) or fungal sinusitis.

Methods: Using a colony-forming unit assay the fungicidal activity of lysozyme (0, 0.5, 5, and 50 micromolar; 0- to 7-hour treatment) was tested against strains of *Aspergillus fumigatus*, the yeast *Candida albicans*, and other fungi commonly identified in mucin of patients with CRS. Fungi cultured directly from the mucin of two CRS patients were also tested to determine if they were resistant to the fungicidal activity of lysozyme.

Results: The fungicidal effect of lysozyme was both concentration and time dependent. After 7-hour treatment lysozyme (5 micromolar) had >80% fungicidal activity against *A. fumigatus*, *Penicillium* sp., *Acremonium* sp., *C. albicans*, and *Candida parapsilosis*. The fungicidal activity of lysozyme toward *Alternaria alternata* could not be determined. Lysozyme was also fungicidal toward the clinical isolates *A. fumigatus* and *Aspergillus terreus* cultured from the mucin of CRS patients.

Conclusion: Lysozyme displays fungicidal activity toward many fungi commonly identified in patients with CRS, as well as clinical fungi isolates cultured from the mucin of CRS patients. Additional studies are required to determine the regulation of lysozyme in CRS.

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Although fungi are established as the cause of invasive fungal rhinosinusitis,¹ the etiology of chronic rhinosinusitis (CRS) remains unknown and is an active area of research. Both bacteria and fungi have been implicated in the pathogenesis of this disease; however, the role of fungi in the development of CRS remains controversial.^{2–8} A large number of fungal species have been cultured from nasal mucous and thought to be associated with CRS. However, similar species have been identified in normal control specimens^{9,10} and may represent commensal fungi trapped in nasal mucous but unrelated to any disease process.¹¹ The most frequent fungi identified from eosinophilic mucin collected during endoscopic sinus surgery for CRS have been identified as *Aspergillus*, *Alternaria*, and *Penicillium* species.¹²

Nasal secretions are an important innate immunity component because they form the first line of defense against inhaled antigens and micro-organisms.¹³ Paranasal sinus secretions contain a number of innate immune defense proteins, including lysozyme, lactoferrin, human β -defensin, and secretory leukocyte protease inhibitor, secreted by the epithelia, local immune cells, goblet cells, and serous glands of the nasal mucosa. The host defense mechanisms and innate immunity in the nose and sinuses has been reviewed extensively.^{13–15} However, relatively few studies have focused on the interactions between filamentous fungi and innate cationic peptides.^{16–19}

Lysozyme is abundant in nasal secretions and its bactericidal activity has been well characterized.²⁰ It causes cell lysis by catalyzing hydrolysis of a polysaccharide component in the cell wall of

Gram-positive bacteria. Lysozyme is generally not regarded as fungicidal because its enzymic muramidase-dependent activity is inhibited by chitin oligosaccharides present in the fungal cell wall.²¹ However, lysozyme is a cationic protein and the bactericidal action is not solely dependent on its enzymic activity.^{21–23} Ionic interactions between the cationic peptide and anionic structures in the microbial cell wall may not directly cause cell lysis but result in damage to the cell wall so that a subsequent challenge (e.g., exposure to salt, detergent, and osmotic pressure) results in cell lysis. Therefore, we hypothesized that lysozyme has potential fungicidal activity through its cationic activity.

There is some evidence that lysozyme has activity toward the yeast *Candida albicans*,^{24–26} with the mechanism of action thought to be mediated through the enzymic hydrolysis of *N*-glycosidic bonds in the cell wall and injury to the cytoplasmic membrane via cationic-protein interactions.²⁴ Interestingly, a progressive resistance to lysozyme, but not lactoferrin, was observed in a few yeast isolates obtained from AIDS patients with oral thrush.²⁵ We hypothesize that fungi may have developed mechanisms to evade the host innate immune defenses in the paranasal sinuses in CRS. The aim of our investigations was to determine (1) if lysozyme has fungicidal activity toward filamentous fungal species often associated with CRS and (2) if fungal isolates obtained from patients with CRS were resistant to the fungicidal effects of lysozyme.

METHODS

The protocol for this study was approved by the Flinders Clinical Research Ethics Committee. Patients with CRS as defined by the American Academy of Otolaryngology Head and Neck Surgery²⁷ who failed maximal medical treatment and consented to undergo endoscopic sinus surgery were recruited for the study.

Fungal Isolates

Fungal species commonly associated with eosinophilic mucin of patients with CRS¹² were chosen for investigation in this study. Fungal and yeast isolates were cultured by the SA Pathology Microbiology Laboratory at Flinders Medical Center. The following fungal and yeast laboratory isolates were used: *Aspergillus fumigatus*, *Acremonium* sp., *Penicillium* sp., *Alternaria alternata*, *C. albicans*, and *Candida parapsilosis*.

In addition to the aforementioned fungal and yeast species that are commonly associated with CRS, we wanted to test the susceptibility of

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The protocol for this study was approved by the Flinders Clinical Research Ethics Committee.

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isolates cultured directly from the mucin of patients with CRS to the fungicidal activity of lysozyme. Between August 2008 and February 2010 sinus mucin was collected intraoperatively from 39 consenting patients undergoing endoscopic sinus surgery for CRS at Flinders Medical Center and Flinders Private Hospital. Mucin was sent to SA Pathology for fungal culture and identification. Confirmation of fungi presence is notoriously variable between laboratories depending on fungal culture methods.²⁸ In an attempt to maximize positive fungal culture results, we used multiple fungal media methods as previously published.¹²

Antifungal Susceptibility Testing of Filamentous Fungi

In 2008 the Clinical and Laboratory Standards Institute published an updated standard "Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi,"²⁹ which describes a method for testing the susceptibility of filamentous fungi that cause fungal infections to antifungal agents. Preliminary tests using this method failed to detect the fungicidal activity of lysozyme.

Colony-Forming Unit Assay. The colony-forming unit (CFU) assay was modified from Tomee *et al.*¹⁸ Primary cultures were prepared from freezer stocks and subcultured twice to ensure viability. Fungal conidia were collected from fresh, mature (7–10 days old) cultures grown at 35°C on Sabouraud's dextrose (SAB) agar slopes (Oxoid, Adelaide, Australia). The fungi colonies were covered with 1 mL of sterile water containing 0.25% Tween80 (Laboratory Supply, Sydney, NSW, Australia) and agitated gently for a couple of minutes before settling. The supernatant containing the conidia was removed and diluted in SAB broth (Oxoid) to 10⁶ conidia/mL. Conidia were allowed to germinate for 19 hours at 28°C (100% air; model BB16, Heraeus Instruments, Hanau, Germany). Germinated conidia were diluted 1000-fold with sodium phosphate buffer (10 mM, pH 7.4) and used in the assay, except for *Acremonium* sp., which was diluted 10,000-fold.

Fresh yeast subcultures were prepared as mentioned previously with the following modifications. Using a 24-hour-old culture one colony was selected and suspended in 1 mL of sterile water. Germination of 10⁶ cells/mL occurred as mentioned previously. After the germination period the cells were resuspended at 10⁶ cells/mL, and then diluted 1000-fold as previously mentioned.

Germinated fungal conidia and yeast were treated with recombinant human lysozyme (code L1667; Sigma Aldrich, St. Louis, MO) at concentrations of 0.5, 5, or 50 μ M in a final volume of 6 mL and incubated at 35°C (100% air; model BB16; Heraeus Instruments, Hanau, Germany). The lysozyme was first dissolved in freshly prepared 0.01% acetic acid to obtain a stock concentration of 100 μ M or 1 mM. The negative control treatment (0 μ M lysozyme) consisted of an equivalent volume of 0.01% acetic acid added to the germinated conidia. At hourly intervals (0–7 hours) 200 μ L of solution was plated in triplicate onto Yeast Malt extract agar plates (Difco code 271210; prepared by Micromedia Laboratories, Melbourne, Australia). After ~24-hour incubation at 35°C the CFUs were enumerated visually. Note that although these are filamentous fungi, individual colonies could easily be enumerated after incubation for ~24 hours (Fig. 1). To confirm susceptibility to lysozyme, the assay was duplicated for each fungal isolate.

The fungicidal activity of lysozyme was calculated by the reduction of CFU using the equation, $[(N_{0h} - N_{th})/N_{0h}] \times 100$, where N_{th} and N_{0h} represent the number of CFU obtained after incubation in the presence of lysozyme after 0 hours (N_{0h}) or treatment for 1–7 hours (N_{th}). Therefore, a fungicidal activity of 100% represents no growth of fungi after treatment with lysozyme (*i.e.*, all fungi are dead). A fungicidal activity of 0% represents growth equivalent to that observed at time 0 (*i.e.*, no active growth/division of fungi). A negative fungicidal activity represents active growth of the fungi during the treatment period (*i.e.*, fungi are not killed during treatment and continue to grow). For comparison, the same calculation was applied to the control (acetic acid)-treated fungi.

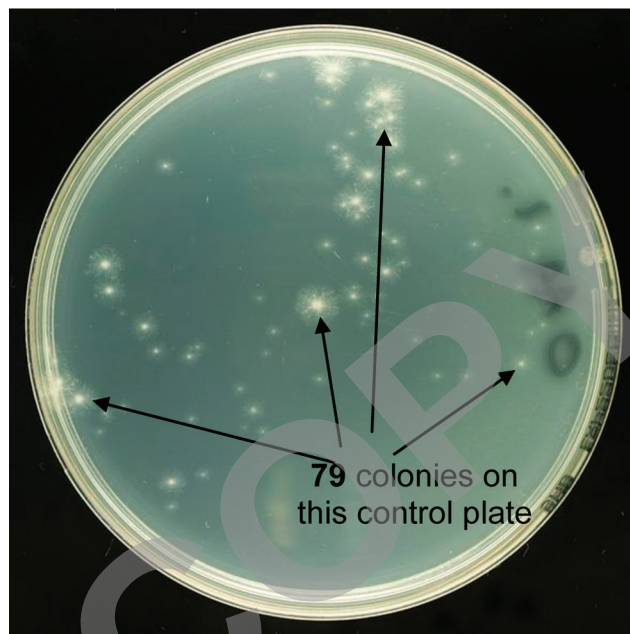


Figure 1. Colony-forming units after 24-hour incubation. *Aspergillus* sp. form discrete colonies that can be easily counted after 24-hour incubation at 35°C. Seventy-nine colonies were visible on this control plate (0 μ M of lysozyme, *i.e.*, acetic acid control).

RESULTS

Lysozyme showed fungicidal activity against fungi commonly found in patients with CRS. The fungicidal effect of lysozyme was time dependent (Fig. 2). After 7 hours of treatment lysozyme (5 μ M) had fungicidal activity of >80% against *A. fumigatus*, a *Penicillium* sp., and an *Acremonium* sp. (Fig. 3). A subtle effect of lysozyme concentration was observed, predominantly during the first 3 hours of treatment. The time-dependent fungicidal effect of lysozyme varied for each of the fungi species tested. During the 7-hour treatment protocol active growth of the fungi occurred in the control-treated germinated conidia of some species, represented graphically as a negative fungicidal activity.

The fungicidal activity of lysozyme was also tested against common yeast species found in patients with CRS. Similar to the fungi species mentioned previously, the fungicidal effect of lysozyme on yeast was also time dependent. After 7 hours of treatment, lysozyme (5 μ M) had fungicidal activity >80% against the yeasts *C. albicans* and *C. parapsilosis* (Fig. 4). For *C. albicans* an effect of lysozyme concentration was evident, with 0.5 μ M of lysozyme not displaying any fungicidal activity. Untreated *C. parapsilosis* grew rapidly during the 7-hour treatment period, represented graphically as a negative fungicidal activity.

We also cultured two fungal species from mucin specimens collected from patients undergoing functional endoscopic sinus surgery for CRS symptoms. These isolates were subcultured onto SAB slopes and the conidia were collected as described previously. The germinated conidia of these clinical CRS isolates, *A. fumigatus* and *Aspergillus terreus*, were not resistant to the fungicidal effects of lysozyme (Fig. 5), with fungicidal activities of >80 and 60%, respectively (5 μ M; 7-hour treatment).

The fungicidal activity of lysozyme toward *A. alternata* could not be clearly determined. It was observed that the germinated conidia in the control treatment started dying after ~2–3 hours, as evidenced by an increase in fungicidal activity (results not shown).

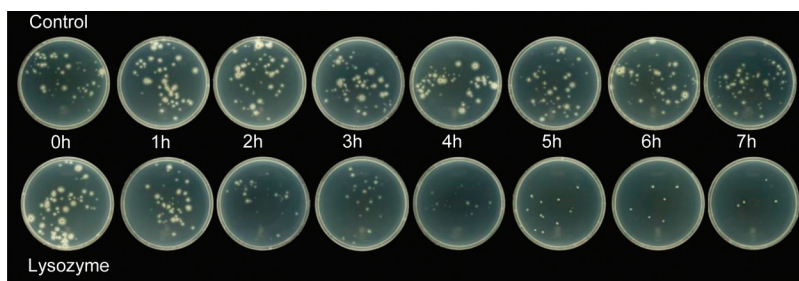


Figure 2. The time-dependent fungicidal effect of lysozyme. Photographs of representative colony-forming units (CFUs) on yeast malt agar plates visually show the time-dependent fungicidal activity of lysozyme against germinated *Aspergillus fumigatus*. The top row represents germinated conidia treated with control (acetic acid) showing no obvious change in the number of CFU over time. The bottom row represents germinated conidia treated with lysozyme (5 μ M) showing a decrease in the number and size of CFU over time.

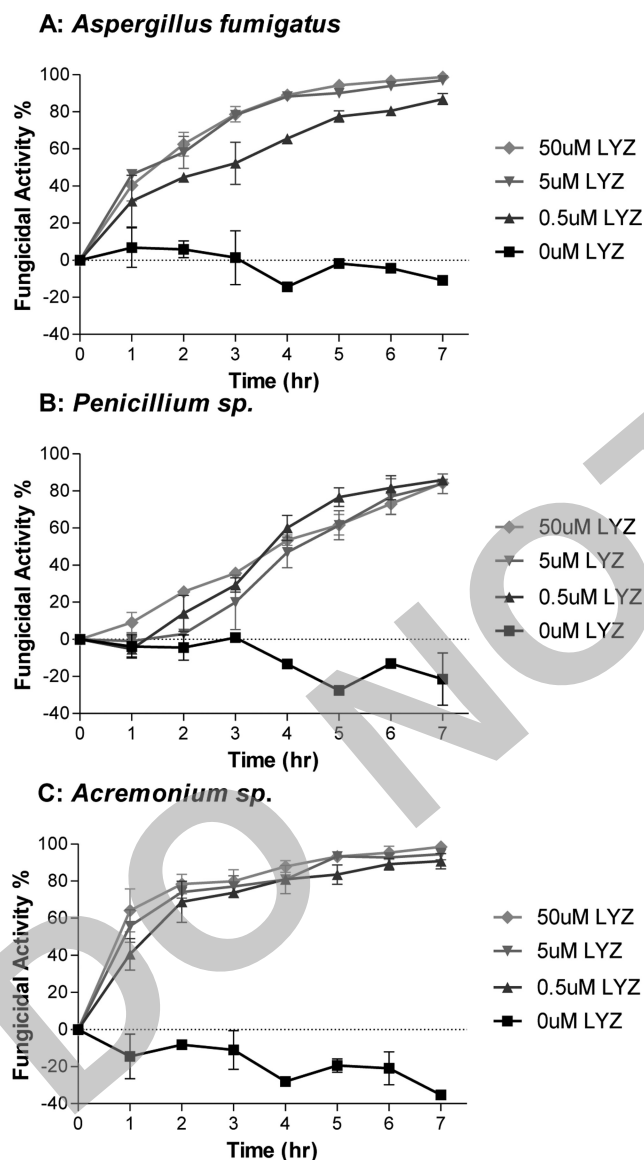


Figure 3. The fungicidal effect of lysozyme against clinical (nonchronic rhinosinusitis [non-CRS]) filamentous fungi isolates. Graphs showing the fungicidal activity of lysozyme over time for each lysozyme concentration tested (0, 0.5, 5, and 50 μ M) against (A) *Aspergillus fumigatus*, (B) *Penicillium sp.*, and (C) *Acremonium sp.* After 7-hour treatment with lysozyme, fungicidal activity was >80% for each fungi isolate.

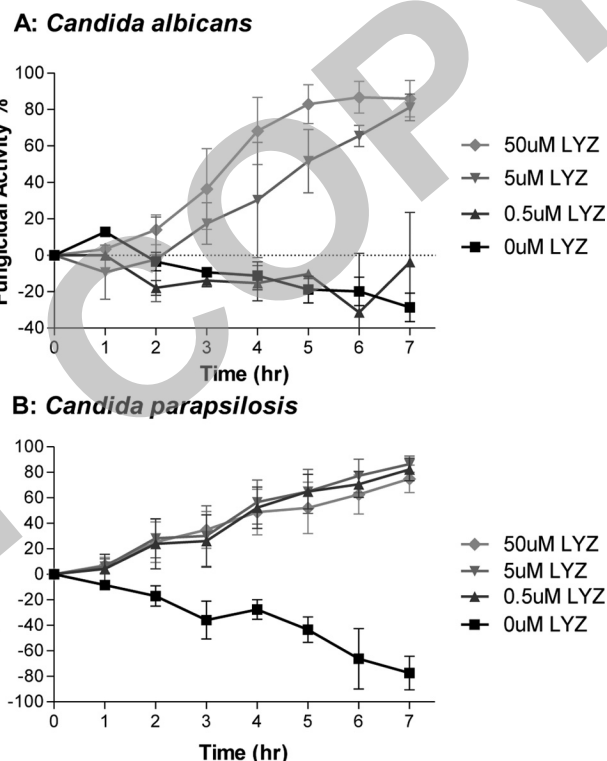


Figure 4. The fungicidal effect of lysozyme against laboratory yeast isolates. Graphs showing the fungicidal activity of lysozyme over time for each concentration tested (0, 0.5, 5, and 50 μ M) against (A) *Candida albicans* and (B) *Candida parapsilosis*. The lowest concentration of lysozyme (0.5 μ M) displayed no fungicidal effect toward *C. albicans*. After 7-hour treatment with lysozyme, fungicidal activity was >80% for each yeast isolate.

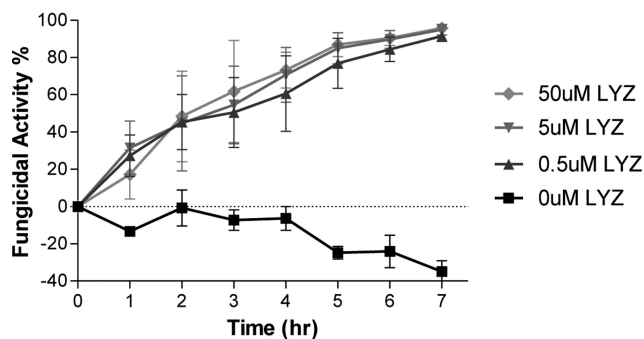
usually regarded as a bactericidal agent with well-characterized enzymic activity. However, in this study we have shown that it also has fungicidal activity toward a number of filamentous fungal species commonly found in patients with CRS. Furthermore, fungal isolates obtained directly from the mucin of patients with CRS were also sensitive to the fungicidal effects of lysozyme.

Preliminary experiments used the Clinical and Laboratory Standards Institute, "Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi,"²⁹ which describes a method for testing the susceptibility of filamentous fungi that cause fungal infections to antifungal agents. This method allows for the minimum inhibitory concentration of antifungal agents to be determined and compared. Unfortunately, preliminary tests using this method failed to detect any fungicidal activity of lysozyme. A likely explanation could involve degradation of the peptide by fungal proteases, which may be related to the use of nongerminating conidia.^{16,18,31,32} However, the most important factor appears to be the salt (NaCl) concentration of the incubation media RPMI-1640. High salt concentrations have been reported to inhibit the antimicrobial

DISCUSSION

Lysozyme is a major part of the paranasal sinus innate defenses, being the most prevalent secretory innate antimicrobial peptide.³⁰ It is

A: *Aspergillus fumigatus* (CRS+)



B: *Aspergillus terreus* (CRS+)

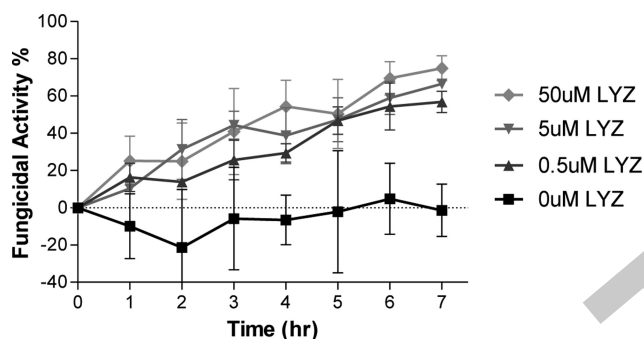


Figure 5. The fungicidal effect of lysozyme against clinical fungi isolates cultured from the mucin of patients with chronic rhinosinusitis. Graphs showing the fungicidal activity of lysozyme over time for each concentration tested (0, 0.5, 5, and 50 μ M) against clinical fungi isolates cultured from the mucin of patients with chronic rhinosinusitis (CRS), (A) *Aspergillus fumigatus* and (B) *Aspergillus terreus*. After 7-hour treatment with lysozyme, fungicidal activity was >80% for *A. fumigatus* and >60% for *A. terreus*. These isolates are not resistant to the fungicidal effects of lysozyme.

activity of other innate peptides.^{18,33,34} We believe the salt concentration interfered with the ionic interactions required for the cationic peptide lysozyme to bind to the anionic fungal cell wall, resulting in a lack of fungicidal activity. Interestingly, normal airway surface liquid (ASL) has been reported to have low concentrations of ionic salts (*i.e.*, hypotonic compared with plasma^{35–37}), which would aid the antimicrobial activity of these innate peptides.

We have shown and confirmed the fungicidal activity of lysozyme against *A. fumigatus*, *Acremonium* sp., *Penicillium* sp., *C. albicans*, and *C. parapsilosis*, all commonly associated with fungal CRS.^{9,12} The fungicidal effect of lysozyme occurred within the first 3 hours of the experiment for each of the isolates tested. Some variation in the time and concentration profiles was evident between repeated experiments for any particular fungal/yeast isolate. Similar intra- and interspecies variations in the susceptibility to lysozyme were also observed in studies on *Candida* sp.,^{25,38} as well as bacterial species.³⁹ These variations are likely caused by the individual growth dynamics and cell wall characteristics of a particular fungal isolate/subculture. Factors such as age of the culture and source of isolation are known to affect microbial cell wall composition. Furthermore, microbial growth in artificial culture media have been shown to alter the cell wall characteristics, making them less susceptible to challenge by cationic antimicrobial peptides,⁴⁰ also explaining the observed intraspecies variability.

The fungicidal activity of lysozyme toward *A. alternata* was not clearly determined in this study. Despite many repeated attempts at

the assay, maintained growth of the control-treated cells was not achieved for the duration of the experiment. This is likely to be because of the individual growth characteristics of this fungus (*e.g.*, repeated subcultures often failed to produce conidia) and/or an incompatibility with the culture conditions or growth media. Therefore, concise conclusions regarding the fungicidal activity of lysozyme against *A. alternata* cannot be made.

The observation of progressive resistance to the fungicidal activity of lysozyme by a few yeast isolates in AIDS patients²⁵ raises the possibility that microbes may develop mechanisms capable of evading the host's innate defenses. Many bacterial species have developed mechanisms to overcome host innate immunity,⁴¹ and therefore it is logical that fungi may develop similar strategies. CRS is also a chronic condition characterized by repeated and often ongoing fungal presence resistant to current medical therapies. We hypothesized that the fungi present in CRS may have developed a mechanism that renders them resistant to innate defenses, such as lysozyme. However, we found that the two fungal isolates obtained from the mucin of CRS patients were still susceptible to the fungicidal effects of lysozyme. Therefore, fungal resistance to lysozyme is unlikely the main mechanism involved; however, it would be desirable to test more CRS fungal isolates to confirm this. It remains to be determined if there is a defect in lysozyme expression or protein production in this population of patients, which may facilitate an ongoing fungal presence.

Nasal ASL contains a number of important innate cationic antimicrobial peptides including lysozyme, lactoferrin, secretory leukocyte protease inhibitor, and human β -defensin. Many of these peptides are broad-spectrum antimicrobials with reported activity toward Gram-positive and Gram-negative bacteria as well as fungi and some viruses. There are reports of synergistic activity when combinations of these peptides have been tested, suggesting it is the combined effects of many components that provides the ASL with its antimicrobial activity. However, there are relatively few reports specifically assessing the direct fungicidal properties of lysozyme.^{18,26,38} Our study suggests that high NaCl concentration adversely affected the fungicidal activity of lysozyme. We therefore speculate that the lack of studies reporting fungicidal activity of lysozyme may be caused by protocols using NaCl at too high a concentration in the growth media, collection of conidia in saline, degradation of peptides by conidia-produced proteases, or collection of ASL samples in saline solutions/buffers. We feel that this is an important finding and further studies of the activity of innate immune peptides at low NaCl concentrations are required.

This study raises important questions regarding the efficacy of using saline-based irrigation or nasal sprays for treatment of CRS. These solutions are isotonic or hypertonic, whereas ASL from normal people/animals is reported to have much lower concentrations of salts (*i.e.*, hypotonic compared with plasma).^{35–37} Furthermore, there are many reports, primarily arising from the cystic fibrosis literature, stating the loss of antibacterial activity of these cationic antimicrobial peptides with increasing saline concentrations. There is high level evidence that aggressive douching provides symptom relief through the physical action of removing thick eosinophilic mucin, crusts, and postoperative blood clots. However, we speculate that repeated saline irrigation inhibits the antimicrobial activity of these important innate defenses, thus facilitating microbial attachment to the nasal/sinus epithelium, thereby enabling microbial colonization. Additional laboratory and clinical studies are required to determine if a solution low in ionic strength, thereby retaining activity of the cationic antimicrobial peptides, would be more beneficial for the treatment of CRS.

In conclusion, this study has shown that in addition to its well-characterized antibacterial activity, lysozyme also possesses fungicidal activity toward many species commonly associated with CRS. Furthermore, it appears that resistance of the fungi to lysozyme is not involved in the pathophysiology of CRS and there may be other mechanisms, such as local NaCl concentration, relating to paranasal

sinus innate defenses that may encourage the establishment of fungal colonization in CRS patients.

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REFERENCES

- Li Y, Li P, and Zhang G. Diagnosis and endoscopic surgery of chronic invasive fungal rhinosinusitis. *Am J Rhinol Allergy* 23:622–625, 2009.
- Pant H, Schembri MA, Wormald PJ, and Macardle PJ. IgE-mediated fungal allergy in allergic fungal sinusitis. *Laryngoscope* 119:1046–1052, 2009.
- Slavin RG. Sinusitis: Viral, bacterial, or fungal and what is the role of Staph? *Allergy Asthma Proc* 27:447–450, 2006.
- Lanza DC, Dhong HJ, Tantilipikorn P, et al. Fungus and chronic rhinosinusitis: From bench to clinical understanding. *Ann Otol Rhinol Laryngol Suppl* 196:27–34, 2006.
- Chakrabarti A, Das A, and Panda NK. Controversies surrounding the categorization of fungal sinusitis. *Med Mycol* 47(suppl 1):S299–S308, 2009.
- Matsuaki Y, Wada K, White TA, et al. Recognition of fungal protease activities induces cellular activation and eosinophil-derived neurotoxin release in human eosinophils. *J Immunol* 183:6708–6716, 2009.
- Hutcheson PS, Schubert MS, and Slavin RG. Distinctions between allergic fungal rhinosinusitis and chronic rhinosinusitis. *Am J Rhinol Allergy* 24:405–408, 2010.
- Foreman A, Psaltis AJ, Tan LW, and Wormald PJ. Characterization of bacterial and fungal biofilms in chronic rhinosinusitis. *Am J Rhinol Allergy* 23:556–561, 2009.
- Ponikau JU, Sherris DA, Kern EB, et al. The diagnosis and incidence of allergic fungal sinusitis. *Mayo Clin Proc* 74:877–884, 1999.
- El-Morsy SM, Khafagy YW, El-Naggar MM, and Beih AA. Allergic fungal rhinosinusitis: Detection of fungal DNA in sinus aspirate using polymerase chain reaction. *J Laryngol Otol* 124:152–160, 2010.
- Michael RC, Michael JS, Ashbee RH, and Mathews MS. Mycological profile of fungal sinusitis: An audit of specimens over a 7-year period in a tertiary care hospital in Tamil Nadu. *Indian J Pathol Microbiol* 51:493–496, 2008.
- Collins MM, Nair SB, Der-Haroutian V, et al. Effect of using multiple culture media for the diagnosis of noninvasive fungal sinusitis. *Am J Rhinol* 19:41–45, 2005.
- Ooi EH, Wormald P-J, and Tan LW. Innate immunity in the paranasal sinuses: A review of nasal host defenses. *Am J Rhinol* 22:13–19, 2008.
- Ramanathan M Jr, and Lane AP. Innate immunity of the sinonasal cavity and its role in chronic rhinosinusitis. *Otolaryngol Head Neck Surg* 136:348–356, 2007.
- Ooi E, Psaltis A, Witterick I, and Wormald P-J. Innate immunity. *Otolaryngol Clin North Am* 43:473–487, vii, 2010.
- Levitz SM, Selsted ME, Ganz T, et al. In vitro killing of spores and hyphae of *Aspergillus fumigatus* and *Rhizopus oryzae* by rabbit neutrophil cationic peptides and bronchoalveolar macrophages. *J Infect Dis* 154:483–489, 1986.
- Ooi EH, Wormald PJ, Carney AS, et al. Fungal allergens induce cathelicidin LL-37 expression in chronic rhinosinusitis patients in a nasal explant model. *Am J Rhinol* 21:367–372, 2007.
- Tomee JF, Hiemstra PS, Heinzl-Wieland R, and Kauffman HF. Antileukoprotease: An endogenous protein in the innate mucosal defense against fungi. *J Infect Dis* 176:740–747, 1997.
- Ooi EH, Wormald PJ, Carney AS, et al. Surfactant protein d expression in chronic rhinosinusitis patients and immune responses in vitro to *Aspergillus* and *Alternaria* in a nasal explant model. *Laryngoscope* 117:51–57, 2007.
- Kirby AJ. The lysozyme mechanism sorted—After 50 years. *Nat Struct Biol* 8:737–739, 2001.
- Laible NJ, and Germaine GR. Bactericidal activity of human lysozyme, muramidase-inactive lysozyme, and cationic polypeptides against *Streptococcus sanguis* and *Streptococcus faecalis*: Inhibition by chitin oligosaccharides. *Infect Immun* 48:720–728, 1985.
- Ibrahim HR, Matsuzaki T, and Aoki T. Genetic evidence that antibacterial activity of lysozyme is independent of its catalytic function. *FEBS Lett* 506:27–32, 2001.
- Masschalck B, Deckers D, and Michiels CW. Lytic and nonlytic mechanism of inactivation of gram-positive bacteria by lysozyme under atmospheric and high hydrostatic pressure. *J Food Prot* 65:1916–1923, 2002.
- Marquis G, Montplaisir S, Garzon S, et al. Fungitoxicity of muramidase. Ultrastructural damage to *Candida albicans*. *Lab Invest* 46:627–636, 1982.
- Samaranayake YH, Samaranayake LP, Pow EH, et al. Antifungal effects of lysozyme and lactoferrin against genetically similar, sequential *Candida albicans* isolates from a human immunodeficiency virus-infected southern Chinese cohort. *J Clin Microbiol* 39:3296–3302, 2001.
- Samaranayake YH, Cheung BP, Parahitiyawa N, et al. Synergistic activity of lysozyme and antifungal agents against *Candida albicans* biofilms on denture acrylic surfaces. *Arch Oral Biol* 54:115–126, 2009.
- Meltzer EO, Hamilos DL, Hadley JA, et al. Rhinosinusitis: Establishing definitions for clinical research and patient care. *Otolaryngol Head Neck Surg* 131(suppl):S1–S62, 2004.
- Javer AR, Genoway KA, Gervais M, et al. Fungal cultures in patients with allergic fungal rhinosinusitis: Improving the recovery of potential fungal pathogens in the Canadian laboratory. *J Otolaryngol* 36:1–5, 2007.
- Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of filamentous fungi: Approved standard, 2nd ed. CLSI document M38–A2 (ISBN 1-56238-668-9). Wayne, PA: Clinical and Laboratory Standards Institute, 2008.
- Cole AM, Liao HI, Stuchlik O, et al. Cationic polypeptides are required for antibacterial activity of human airway fluid. *J Immunol* 169:6985–6991, 2002.
- DeLucca AJ, Bland JM, Jacks TJ, et al. Fungicidal activity of cecropin A. *Antimicrob Agents Chemother* 41:481–483, 1997.
- Manavathu EK, Cutright J, and Chandrasekar PH. Comparative study of susceptibilities of germinated and ungerminated conidia of *Aspergillus fumigatus* to various antifungal agents. *J Clin Microbiol* 37:858–861, 1999.
- Singh PK, Tack BF, McCray PB Jr, and Welsh MJ. Synergistic and additive killing by antimicrobial factors found in human airway surface liquid. *Am J Physiol Lung Cell Mol Physiol* 279:L799–L805, 2000.
- Travis SM, Conway BA, Zabner J, et al. Activity of abundant antimicrobials of the human airway. *Am J Respir Cell Mol Biol* 20:872–879, 1999.
- Widdicombe JH. Regulation of the depth and composition of airway surface liquid. *J Anat* 201:313–318, 2002.
- Joris L, Dab I, and Quinton PM. Elemental composition of human airway surface fluid in healthy and diseased airways. *Am Rev Respir Dis* 148:1633–1637, 1993.
- Govindaraju K, Cowley EA, Eidelman DH, and Lloyd DK. Microanalysis of lung airway surface fluid by capillary electrophoresis with conductivity detection. *Anal Chem* 69:2793–2797, 1997.
- Tobgi RS, Samaranayake LP, and MacFarlane TW. In vitro susceptibility of *Candida* species to lysozyme. *Oral Microbiol Immunol* 3:35–39, 1988.
- Iacono VJ, MacKay BJ, DiRienzo S, and Pollock JJ. Selective antibacterial properties of lysozyme for oral microorganisms. *Infect Immun* 29:623–632, 1980.
- Dorschner RA, Lopez-Garcia B, Peschel A, et al. The mammalian ionic environment dictates microbial susceptibility to antimicrobial defense peptides. *FASEB J* 20:35–42, 2006.
- Hornef MW, Wick MJ, Rhen M, and Normark S. Bacterial strategies for overcoming host innate and adaptive immune responses. *Nat Immunol* 3:1033–1040, 2002. □

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