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LETTER TO THE EDITOR

Immunosuppressed Adult Zebrafish Model of Mucormycosis

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Mucormycosis is a life-threatening, rapidly progressing infection associated with high mortality. Current challenges include a limited arsenal of available antifungals and an incomplete understanding of disease pathogenesis (1). Therefore, new models which can act as platforms for studying disease mechanisms and therapeutic actions are of high priority to the field.

The adult zebrafish, an emerging model in the study of infectious diseases (2), offers several advantages over other vertebrate model organisms, such as high fecundity, mature immune responses, reduced housing costs, and an array of tools for genetic manipulation. While larval zebrafish models of mucormycosis have been explored (3), zebrafish larvae lack a mature adaptive immune system (4). As hosts most susceptible to mucormycosis have defects in both innate and adaptive immunity (1), we sought to develop an adult zebrafish model of mucormycosis with and without immunosuppression.

Female adult zebrafish 6 months or older (AB ZIRC catalog no. ZL1; ZFIN ID ZDB-GENO-960809-7, available from the University of Oregon [https://zfin.org/ZDB-GENO-960809-7]) and weighing 235 to 250 mg were divided into three groups: immunosuppressed via cyclophosphamide and noninfected (CP), nonimmunosuppressed and infected by *Rhizopus oryzae* (RO), and immunosuppressed and infected (CP+RO). One day prior to inoculation, animals received 10-μl subcutaneous injections adjacent to the pelvic fin of either 300 mg/kg cyclophosphamide (Sigma-Aldrich, St. Louis, MO) for CP and CP+RO animals (5) or saline for RO animals. RO and CP+RO animals were given intramuscular inoculations of 10 μl of 5×10^8 spores/ml *Rhizopus oryzae* 969 immediately caudal to the dorsal fin. All injections took place under anesthesia using 168 mg/ml tricaine methanesulfonate (Tricaine-S; Syndel USA, Fernadale, WA) as described previously (6). Following inoculation, the animals were monitored twice daily for 7 days under standard conditions prior to euthanasia. Experiments were performed in duplicate with 5 animals per group for a total of 10 animals per group. After death, the animals were subjected to histological analysis with Grocott-Gomori's methenamine silver stain. Kaplan-Meier analysis was performed to determine differences in survival rates with the log-rank test using JMP Pro software (version 11.0; SAS Institute, Cary, NC) ($\alpha = 0.05$).

CP animals experienced 20% mortality over the course of 7 days (Fig. 1A). Both RO- and CP+RO-infected groups had significantly increased mortality compared to CP animals (70% versus 20%, $P = 0.034$, and 80% versus 20%, $P = 0.012$, respectively).

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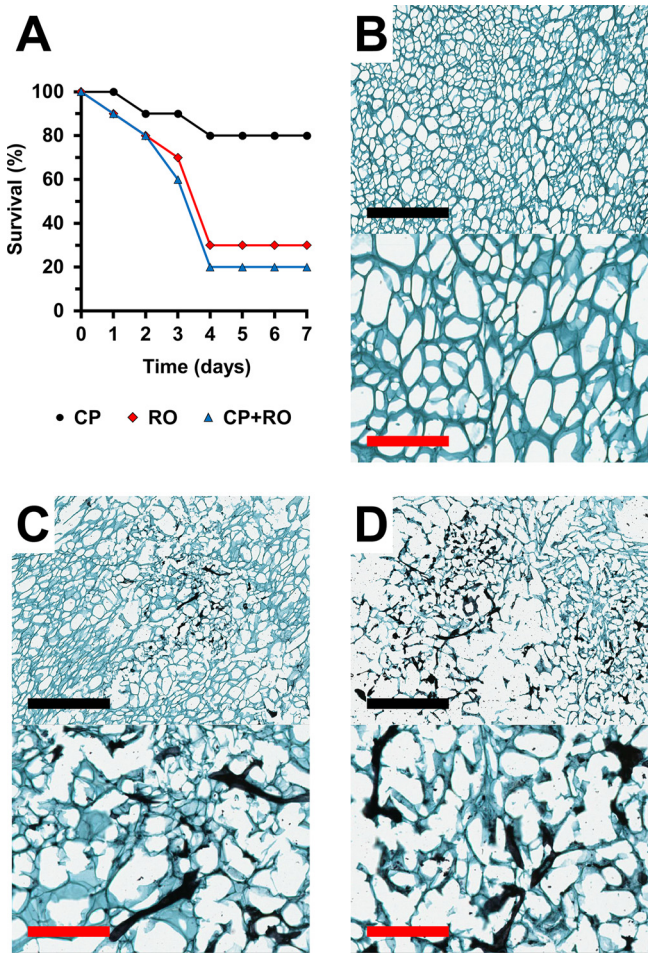

FIG 1 Zebrafish survival and representative histological images. (A) Survival curve of immunosuppressed (CP), infected (RO), and immunosuppressed and infected (CP+RO) animals. (B to D) Representative images from Grocott-Gomori's methenamine silver stain of tissue from CP animals at day 7 (B), RO animals at day 4 (C), and RO+CP animals at day 4 (D). Black scale bars, 200 μm; red scale bars, 50 μm.

There were no significant survival differences between the RO- and CP+RO-infected groups (80% versus 70%, $P = 0.6038$). The median survival times were >7, 4, and 4 days for the CP, RO, and CP+RO groups, respectively. Histological staining demonstrated extensive mycelium formation in infected animals but not in noninoculated animals (Fig. 1B to D). Hyphae were visible as early as day 2 in the RO+CP group and by day 4 in the RO group.

This preliminary work suggests that adult zebrafish experience mortality when infected via intramuscular injection of *R. oryzae*. Although immunosuppressed animals had 10% greater mortality and hyphal formation was observed earlier in these animals than in nonimmunosuppressed animals, there were no statistically significant differences in overall 7-day survival. This is consistent with the well-known ability of *Mucorales*, in contrast to other opportunistic fungi, to cause severe or even lethal disease in immunocompetent hosts (1).

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We declare no conflicts of interest.

REFERENCES

1. Farmakiotis D, Kontoyiannis DP. 2016. Mucormycoses. Infect Dis Clin North Am 30:143–163. https://doi.org/10.1016/j.idc.2015.10.011.
2. Sullivan C, Kim CH. 2008. Zebrafish as a model for infectious disease and immune function. Fish Shellfish Immunol 25:341–350. https://doi.org/10.1016/j.fsi.2008.05.005.
3. Voelz K, Gratacap RL, Wheeler RT. 2015. A zebrafish larval model reveals early tissue-specific innate immune responses to Mucor circinelloides. Dis Model Mech 8:1375–1388. https://doi.org/10.1242/dmm.019992.
4. Lam SH, Chua HL, Gong Z, Lam TJ, Sin YM. 2004. Development and maturation of the immune system in zebrafish, Danio rerio: a gene expression profiling, in situ hybridization and immunological study. Dev Comp Immunol 28:9–28. https://doi.org/10.1016/S0145-305X(03)00103-4.
5. Burgos JS, Ripoll-Gomez J, Alfaro JM, Sastre I, Valdivieso F. 2008. Zebrafish as a new model for herpes simplex virus type 1 infection. Zebrafish 5:323–333. https://doi.org/10.1089/zeb.2008.0552.
6. Phelps HA, Runft DL, Neely MN. 2009. Adult zebrafish model of streptococcal infection. Curr Protoc Microbiol 9:Unit 9D.1. https://doi.org/10.1002/9780471729259.mc09d01s13.