Sampling and Immunological Studies of *Candida albicans* and *Candida tropicalis* in the Intestinal Mucosa

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Courtesy of MDJ

Abstract

Candida albicans and *Candida tropicalis* are both commensal and opportunistic pathogens of the gut that are of increasing medical significance and that have been implicated in human Crohn's Disease [2,3]. We were interested in understanding how the gut-associated lymphoid tissues (GALT) sample these two fungi, and what the consequences of this might be for the intestinal mucosa. Peyer's Patches (PPs) are responsible for sampling of microbes that pass through the gut. Our research utilized three approaches.

i) Our first question was, are fungi sample by PPs? To answer this question, BALB/c mice were infected by gavage with 10^8 cells, and the PPs were cryosectioned and immunofluorescently stained at 24 hours. To quantify the sampling of fungi, BALB/c mice were infected by gavage with 10^8 cells, and the PP, mesenteric lymph nodes (MLN), and spleens were examined by plating on Sabouraud Dextrose Agar for colony forming units (CFU) at 4 and 24 hours. Our results revealed significantly higher CFU of *C. albicans* from PP compared to *C. tropicalis* within PP. However fungal organisms were undetected in spleen and MLN. In contrast, fluorescent microscopy showed that C. *tropicalis* was more abundant in PPs. Therefore, it appears that PP do sample fungi, but in a rather selective way. The reason for this is not yet entirely unknown. There may be a different dynamic with *C. tropicalis* because it is a commensal organism to mice, while *C.albicans* is not.

ii) To determine whether trafficking of fungi from PP depended upon DCs, an additional mouse model was employed. In BALB/c mice, a subset of DC cells ($CD11b^-/CD8\alpha^-$), but positive for Langerin, a signaling receptor, were compared with the transgenic mouse strain, $CCR7^-$ which *lacks* the chemokine receptor CCR7 that is necessary for fungi to emigrate from PP. When fluorescent microscopy of tissues from WT BALB/c with the subset of DC receptors that permit emigration were compared with the transgenic $CCR7^-$ mouse tissues lacking the receptor, the data confirmed that migration from PP was DC-dependent. That is, only WT BALB/c showed emigration of fungi from the PP.

iii) To determine whether these two fungi compromise the immune system, five groups of five BALB/c mice each were gavaged with cholera toxin alone (a strong adjuvant), *C. albicans* or *C. tropicalis* plus cholera toxin, cholera toxin plus cyclophosphamide (positive control for immune suppression), or PBS only (negative control). We used an Indirect ELISA to measure fungal antibodies in the serum and feces, mice were examined on days 7, 14, and 21. Both serum and fecal data clearly showed that mice gavaged with fungi have significantly reduced antibody (Ab) IgG, IgA, IgM titers when compared with cholera toxin alone.

Using these three approaches, we now have a better understanding of how fungal pathogens interact with the gut mucosa. This may help determine new leads for better treatments of human intestinal disease.

Questions to be Addressed

i) Are fungal organisms sampled by Peyer's patches?
ii) Is trafficking of fungi dependent on dendritic cells?
iii) Do the fungi weaken an immune response?

Introduction

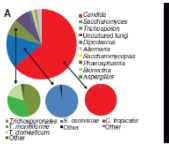


Figure 1 – Genetic analysis of C57BL/6J murine gut mycobiome. *C. tropicalis* is a commensal organism to mice. Iliyan D. Science . 2012.

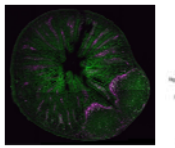


Figure 2 – Cross-section of murine PP, immuno-fluorescently stained to show the DCs(pink) within the SED of the PP. PPs are aggregates of lymphoid tissue that act as the immune surveillance system of the gut. Courtesy of MDJ.

Figure 3 – PPs are located along the anti-mesenteric side of the small intestine. This section is a segment of the proximal small intestine. Courtesy of MDJ.

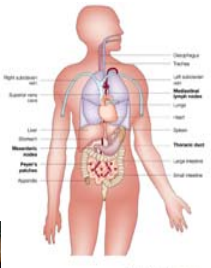


Figure 4 – PPs are located along the anti-mesenteric side of the small intestine. Nagler-Anderson C. Man the barrier! Strategic defences in the intestinal mucosa. Nat Rev Immunol. 2001. Oct;1 (1):59-67

Results

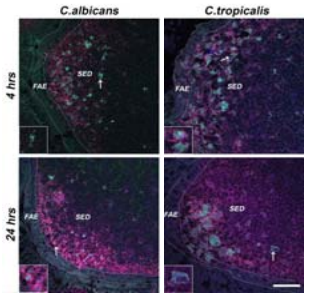


Figure 5 - Sampling of *C. albicans* and *C. tropicalis* by PPs. Fungi were FITC stained prior to gavage to fluoresce green. PP cross-sections were stained with anti-Langerin mAb (blue) and anti-CD11c (red).

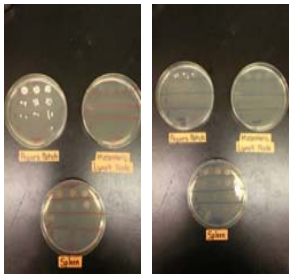
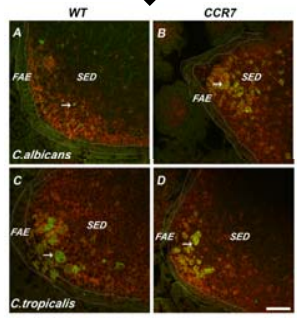


Figure 6B - Representative CFUs on Sabouraud Dextrose Agar plates with antibiotic cocktail. Suspensions on these plates were taken from mice euthanized 4 h after gavage. Left: result of mouse infected with 10^8 *C. albicans*. Right: result of mouse infected with *C. tropicalis*. Each line on the plates represents a tenfold serial dilution of suspension starting from 10^8 at the top.

Figure 7 – Egress of *C. albicans* from PPs is dependent on DCs. *C.* tropicalis is sampled and held in the PPs 24 h after sampling in the presence of CCR7. Left: Balb/c mice were gavaged with 10^8 fungi. Right: $CCR7^-$ mice were gavaged with 10^8 fungi. Cross-sections were stained with anti-CD11c(red) and fungi were FITC stained (green) prior to gavage.



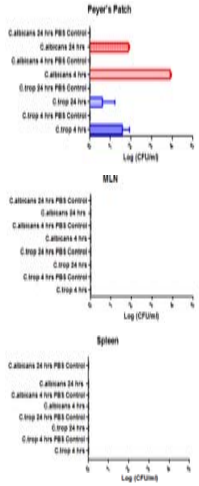
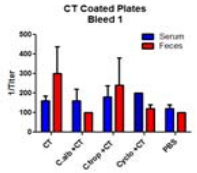
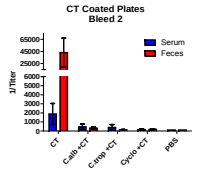
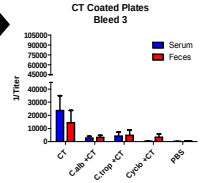


Figure 6A – Fungi are only in detectable concentrations in PPs at 4 and 24h. PPs, MLNs, and spleens were collected after 4 and 24 h. Each group contained two female Balb/c mice. Tissues were ground with a syringe pusher and suspended in PBS. After serial dilutions were performed, suspensions were placed on Sabouraud Dextrose agar plates as shown in Figure 6B. After 48 h incubation at 30°C, the log of the mean CFU/ml of suspension was calculated as shown.

Figure 8 – Fungi may cause immune suppression of the intestinal mucosa. Balb/c mice were gaveged on days 1, 8, and 15. We used an Indirect ELISA to measure fungal antibodies in the serum and feces, mice were examined on days 7, 14, and 21.







Conclusions

First, the uptake of live fungi was analyzed by cryosectioning and immunostaining. As expected, the fungi were only taken up by the subset of PP DCs which are $CD8\alpha^-/CD11b^-$, but Langerin+. The DCs in mice gavaged with *C. tropicalis* had a larger volume of fungi taken up, compared to mice gavaged with *C. albicans.* From the CFU experiment it is important to see that the abundance of *C. tropicalis* cells that are taken up are not viable, compared to smaller volume of *C.albicans* taken up, as shown by cross-sections. Also, it is important to see that no detectable level of fungi was present in the CFU experiment, even though *C. tropicalis* is a commensal organism to mice.

Using the CCR7 knockout mice, the fate of fungi after uptake was tested. The CCR7- mice were gavaged with fungi, and at the twenty-four hour time point the DCs are significantly more gorged than WT mice. Since the chemokine receptor CCR7 is required for DCs to migrate out of the PP, it is possible that the egress of fungi is dependent on DCs. If the egress of fungi was mediated by T-cells or B-cells, then the DCs would not be gorged with fungi at this time point, as seen in Balb/c mice. In the WT Balb/c mice a significant volume of fungi was cleared from the PP by the twenty-four hour time point.

To test whether the fungi cause a suppression of the intestinal mucosa, an antibody titer experiment was utilized. As expected from data in pilot study not shown, the mice did not build any Ab to the fungal organisms. In the positive control for maximum Ab production, the group which received only CT, the Ab titers went up sharply after the first boost, and then dropped after the system was overloaded by the second boost. In the positive control for immune suppression, the group which received CT in addition to cyclophosphamide injections, the Ab titers went up gradually over the three week experiment. The trend in Ab production in the two groups which were gavaged with fungal organisms followed the immune suppressed group closely. It appears that the fungi may suppress the intestinal immune system, but further testing would be required to come to a definite conclusion.

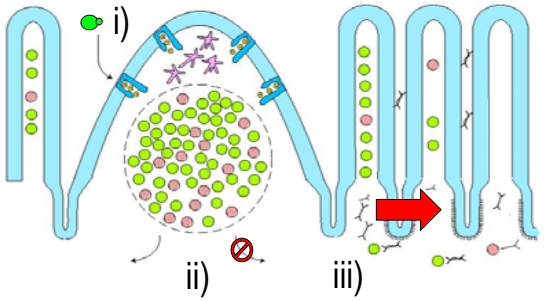


Figure 9 – Model of Conclusions

Citation

- [1] Iliyan D, et al. Interactions Between Commensal Fungi and the C-type Lectin Receptor Dectin-1 Influence Colitis. Science . 2012.
- [2] Standaert-Vitse A, et al. Am J Gastroenterol. 2009 Jul;104(7):1745-53. doi: 10.1038/ajg.2009.225. Epub 2009 May 26.
- [3] Gerald R, et al. Crit Rev Microbiol, Early Online: 1-5. Informa Healthcare USA, Inc. 2013. DOI: 10.1309/1040841X.2013.810587

Acknowledgements

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Contact Information

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