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Activity of the fumagillin production by *Aspergillus fumigatus* in experimental infections

Xabier Guruceaga[1], Uxue Perez-Cuesta[1], Oskar Gonzalez[2], Rosa María Alonso[2], Aize Pellon[3], Juan Anguita[3], Fernando Luis Hernando[1], Andoni Ramirez-Garcia[1] & Aitor Rementeria[1]

[1]Fungal and Bacterial Biomics Research Group, Department of Immunology, Microbiology and Parasitology, University of the Basque Country (UPV/EHU); [2]FARMARTEM group, Department of Analytical Chemistry, UPV/EHU; [3]Inflammation and Macrophage Plasticity laboratory, CICbioGUNE, Derio, Spain. E-mail: xabier.guruceaga@ehu.eus/aitor.rementeria@ehu.eus

Background. Our group studies the saprophytic fungus *Aspergillus fumigatus* in order to better comprehend the host-pathogen interaction using the combination of transcriptomic technology and different strategies to perform mutant strains (DJ-PCR and CRISPR-Cas9). In previous studies, we have demonstrated that most of the *A. fumigatus* genes engaged in the biosynthetic cluster of fumagillin formation were overexpressed during an intranasal murine infection.

Goal. The aim of this work was to understand the role of this mycotoxin during the infection process.

Methods. First, we performed some *in vitro* assays using commercial fumagillin and three different cell lines (macrophages RAW 264.7, B16 melanoma and lung epithelial cells A549) to develop a detection method using UHPLC-PDA and describe the effect of the mycotoxin over them. To do that, different concentrations of fumagillin (0.5, 1 and 2 μg/ml) were used. Then, employing one mutant non-fumagillin producer strain (*ΔfmaA*), its wild type ($\Delta akuB^{ku80}$) and the complemented strain ($fma^{compl.}$), we developed co-incubation assays with the macrophages RAW 264.7 and murine bone marrow macrophages (BMMs). Finally, these three strains were used to perform an intranasal infection in groups of 10 mice immunossuppresed intraperitoneally with 100 mg/kg of cyclophosphamide every 72 h. All the experiments were carried out at least by triplicate.

Results. We determined that the *A. fumigatus* WT strain is able to secrete fumagillin (1.31 μg/ml) growing in RPMI during 24 h at 37 °C. Furthermore, when we incubated the cell lines in the presence of 1 μg/ml of commercial toxin for 24 h, 80% of the toxin disappeared from the media of A549 cultures, 58% in the case of B16 melanoma and only 4% in cultures with RAW 264.7 cells. However, the viability of the A549 cells was not affected, while B16 and RAW 264.7 cells showed a significant reduction in it. In addition, no differences in germination were observed during the co-incubation of conidia with BMMs, but Δ*fmaA* showed a significant increase in the percentage of double-branched conidia compared to the other two strains. Indeed, around 25% of the germinated Δ*fmaA* conidia showed two branches after 8 h of co-incubation. However, the *ΔfmaA* was significantly more susceptible to phagocytosis by the BMMs than the complemented or Wt control strains. The *in vivo* study did not show significant differences in mice survival between the groups infected with the *ΔfmaA* and Wt strains, but mice infected with the complemented strain had higher mortality rate, probably due to a secretion of fumagillin greater than Wt, as it was proved *in vitro*.

Conclusions. Fumagillin produced by *A. fumigatus*: (1) Can penetrate inside cells causing their death, (2) Could act as fungal protective factor against phagocytosis, and (3) Plays an important role in *Aspergillus* pulmonary infection.

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Relación de *Candida albicans* con el hospedador durante la colonización gastrointestinal

Daniel Prieto, Elvira Román, Rebeca Alonso-Monge, Susana Hidalgo-Vico y Jesús Pla

Departamento de Microbiología y Parasitología. Facultad de Farmacia. Universidad Complutense de Madrid. IRyCIS, Madrid, España. E-mail Daniel Prieto: adprieto@ucm.es

Antecedentes. El patógeno oportunista *Candida albicans* se encuentra habitualmente en humanos formando parte de la microbiota de la mucosa digestiva y vaginal sin producir daño. La colonización de estos nichos requiere una adaptación adecuada que le permita sobrevivir. En el caso del ambiente gastrointestinal se ha propuesto la existencia de un programa genético específico que favorece el establecimiento comensal.

Objetivos. Determinar la importancia de elementos clave de *C. albicans* para establecer una adaptación al hospedador durante la colonización gastrointestinal, como son la ruta de señalización medida por Hog1 y circuitos genéticos relacionados con Wor1.

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Métodos. Distintas cepas de *C. albicans* han sido generadas por modificaciones genéticas que incluyen deleción y sobreexpresión de genes de interés, así como marcaje fluorescente. Estas variantes se han ensayado en un modelo de colonización gastrointestinal comensal en ratón, en un sistema de adhesión *ex vivo* a mucosa intestinal y en cultivo en distintas condiciones de interés.
Resultados. Al ensayar la colonización gastrointestinal por parte de *C. albicans* en nuestro modelo, se pueden diferenciar al menos dos fases: una inicial de establecimiento y otra de mantenimiento a largo plazo. La adaptación del hongo al nicho medida por la MAPK Hog1 es especialmente crítica para la primera de estas fases, ya que mutantes de dicho elemento están muy dificultados para establecer la colonización. Por otro lado, Wor1 media un programa genético que favorece el mantenimiento comensal a largo plazo. Curiosamente, a pesar de que la sobreexpresión de *WOR1* afecta negativamente al establecimiento inicial, es capaz de compensar los defectos de colonización derivados de la falta de *HOG1*, tanto comparando con un mutante *hog1Δ* como con una cepa silvestre. Cabe destacar, que los comportamientos observados se correlacionan, además, con alteraciones en la capacidad de adhesión específicamente a la mucosa intestinal y en la sensibilidad a sales biliares.
Conclusiones. Tanto Hog1 como Wor1 son elementos necesarios para *C. albicans* para establecerse y mantenerse en el tracto gastrointestinal del hospedador en una relación de comensalismo, afectando a funciones tan relevantes como la adhesión a mucosa y la resistencia a sales biliares.

Metabolic regulation of immune responses to *Candida albicans* in oral epithelial cells

Aize Pellon, Shervin Dokht Sadeghi Nasab and David L. Moyes
Centre for Host-Microbiome Interactions, Faculty of Dentistry, Oral and Craniofacial Sciences, King's College London; Centre for Host-Microbiome Interactions, King's College London, Floor 17, Tower Wing, Guy's Hospital. SE1 9RT, London, United Kingdom. E-mail Aize Pellon: aize.pellon@kcl.ac.uk; *David L. Moyes:* david.moyes@kcl.ac.uk

Background. Candida albicans is a fungal pathobiont present in 70% of healthy individuals that can cause mild superficial mucosal infections in otherwise healthy patients. Incidence is high, with approximately 20% of women suffering from an occurrence of vulvovaginal candidiasis each year. However, these infections can lead to life-threatening systemic diseases in immunocompromised individuals with an associated mortality of 45–75%. Epithelial cells (ECs) in mucosae do not simply act as physical barriers, but drive complex immune responses to the fungus facilitating its clearance and recruiting immune cells to the infection foci. Remarkably, in recent years metabolic reprogramming in response to infections has been widely described in immune cells, where it modulates their immune responses depending on the detected stimulus. Understanding whether and how metabolism regulates immune responses in ECs will identify key novel therapeutic intervention points that will enable the improvement of current therapies.
Goal. To determine the metabolic changes occurring in ECs upon *C. albicans*, and their impact on the regulation of antifungal responses.
Methods. We infected oral epithelial cell lines with *C. albicans* strains and analysed changes in carbohydrate metabolism, measuring gene expression changes, glucose uptake, lactate production, and respiration rates. We determined the role of specific metabolic pathways in immune modulation in ECs using specific inhibitors (oligomycin for oxidative phosphorylation, OxPhos; 2-DG for glycolysis) and determined the impact on *C. albicans* infection responses, including EC survival, cytokine expression, and activation of signalling pathways involved in anti-*Candida* immunity.
Results. Infection with *C. albicans* induced a metabolic shift in ECs, as suggested by increased expression of glucose transporter and glycolytic genes. We also observed differential respiration rates, proving that physiological changes were occurring in ECs in response to the infection. Therefore, we applied a panel of chemical inhibitors of the main metabolic pathways, including OxPhos, glycolysis, glutaminolysis, pentose phosphate pathway and fatty acid oxidation. Importantly, inhibition of OxPhos led to a dramatic reduction of *C. albicans*-induced cell damage and cytokine production (G-CSF and GM-CSF) in ECs. This was matched by a dampening of *C. albicans* growth, indicating that OxPhos prevents induction of fungistatic mechanisms yet to be defined. Although strong c-Fos activation was observed in OxPhos-inhibited cells, G-CSF and GM-CSF release was reduced. Remarkably, inhibition of glycolysis and glucose transporters did not affect ECs survival, but regulated activation of immune response-related pathways and cytokine expression. In fact, glucose presence and dosage in the medium was essential not only for ECs, but for fungal growth.
Conclusions. Oral ECs are responsible for orchestrating complex immune responses to *C. albicans* that are essential for mucosal homeostasis and infection clearance. Our data suggest that these responses are tightly regulated by central metabolism pathways, such as OxPhos and glucose processing. Further analysis both *in vitro* and *in vivo* will allow us to determine which of these pathways can be used for potentiating anti-*Candida* immunity at the mucosae.

Candida auris and its pathogenic siblings

Auke de Jong[1,2]
[1]*Department of Medical Mycology. Westerdijk Fungal Biodiversity Institute. Utrecht;* [2]*Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands. E-mail:* a.jong@wi.knaw.nl

Annually, an estimated 1.5 million people die from invasive fungal infections. The advance of life expectancy, the rise of immunosuppressive treatments, higher survival of patients living with cancer or chronic disease and the use of catheters are all factors attributed to the emergence of opportunistic fungal pathogens over the last decades.
Candida species are considered the most frequent fungi encountered in hospital settings, accounting for more than 400,000 cases of bloodstream infections each year, making them the third to fourth most common cause of invasive fungal infections worldwide. *Candida albicans* is recognized as the main causative pathogen of candidiasis. However, new species are on the rise, with the globally emerging multidrug-resistant *Candida auris* being the most infamous example. Treatment options of *C. auris* are limited due

to antifungal resistance, misidentification and its ability to persistently colonize hospital environments. Since its first description in 2009, *C. auris* has been reported in over 30 countries on five continents, causing candidemia outbreaks with crude mortality rates ranging from 32 to 66%.

The *Candida* genus is composed of a highly heterogeneous group of 500 species. So far, around 30 *Candida* species have been described as opportunistic pathogens, but new species are added to this list at a steady pace. To explain the emergence of new fungal pathogens such as *C. auris* it is important to have an understanding of which virulence traits set *C. auris* and its pathogenic siblings apart from their harmless family members.

Extensive research on *C. albicans* has revealed several traits being important for its pathogenicity and virulence, including the production of lytic enzymes, morphogenesis, thermotolerance and biofilm production. *C. auris* was shown to express most of these traits to a similar extent, but also to possess seemingly unique traits such as the ability to persistently colonize the host skin and withstand high osmotic stress. These virulence-related properties have been fragmentally studied among the major pathogenic *Candida* species. A robust approach to assess the genomic and phenotypic characteristics of the pathogenic Candida species will shed a light on the virulent traits of *C. auris* and how this correlates with other pathogenic species.

Shifting epidemiology of *Candida*

Irene Jurado

Grupo de Micología Médica, Departamento de Inmunología, Microbiología y Parasitología, UPV/EHU, Bilbao, Spain. E-mail: irenejurado06@gmail.com

Candida is one of the main causes of bloodstream infections in Europe and USA. Candidaemia is the principal clinical presentation of invasive candidiasis, which is the most frequent invasive mycosis. Invasive candidiasis is of great medical concern due to its continuous changes in the aetiology and epidemiology. Although *Candida albicans* remains the predominant worldwide cause of candidaemia and other invasive candidiasis, there are other emerging species of *Candida* (NCA – Non-*Candida albicans*) of clinical interest, such as *Candida parapsilosis, Candida glabrata, Candida tropicalis, Candida krusei* and, more recently, *Candida auris*. These species, along with *C. albicans*, are responsible of more than 90% of candidaemias.

C. parapsilosis forms tenacious biofilms on medical devices, shows less susceptibility to echinocandins and it is often the second or third most prevalent aetiology of invasive candidiasis, depending on the patient clinical group as well as on the geographical region: this species is responsible for 20–25% of all *Candida* bloodstream infections. *C. glabrata*, which has reduced susceptibility to fluconazole, follows behind, being usually the third most common isolated species (10–15%) from blood. Moreover, in some geographical regions *C. glabrata* displaces *C. parapsilosis* from the second aetiological position. *C. tropicalis* is emerging as one of the most relevant *Candida* species in terms of epidemiology and virulence and there is an increasing number of isolates resistant to fluconazole with cross-resistance to voriconazole and posaconazole. *C. tropicalis* usually ranks the fourth position (2–10%), except in some tropical and subtropical countries in Asia and Latin America, where it is the second or third most common species isolated from invasive candidiasis. Finally, *C. krusei* commonly causes 0.4–3.9% of invasive candidiasis and also shows resistance to fluconazole. Apart from those five NAC species, there are some other relevant species. One of those is the multi-drug resistant *Candida auris*, which was first described in 2009 and, since then, it has caused more than 20 outbreaks in different hospitals over the five continents. Another species is *Candida guilliermondii*, which is intrinsically less susceptible to azoles and echinocandins, and some isolates have been classified as pan-azole resistant. Some consequences of these changing features of invasive candidiasis are longer hospital stays, medical complications and disease sequelae, antifungal resistance inducing the use of uncommon therapies, and a worst patient outcome, which lead to higher morbidity and mortality associated to NAC infections. This situation begins to be reported in the aetiology of some superficial candidiasis, such as vulvovaginal and oral candidiasis, in which NAC, especially *C. glabrata*, are increasing as a cause of these fungal diseases.

Epidemiology of *Candida auris* during the persistence presence of *C. auris*

Alba Ruiz-Gaitán

Grupo de Investigación en Infección Grave, Instituto de Investigación Sanitaria La Fe; Servicio de Microbiología, Hospital Universitario y Politecnico La Fe. Valencia, Spain. E-mail: alba_ruiz@iislafe.es

Background. Candida auris is an emerging, multidrug-resistant yeast causing hospital outbreaks. The outbreaks by *C. auris* described in Spain as well as in other countries with large outbreaks, are characterized by an exponential increase in the number of cases in a short period, suggesting a high transmission rate.

Goal. We report the first 24 months of the ongoing *C. auris* outbreak in a tertiary hospital in Spain.

Methods. The epidemiological, clinical and microbiological characteristics of candidemia episodes and environmental samples by *C. auris* were also analyzed.

Results. 228 patients were involved in the case–control study (114 colonized/candidemia and 114 controls). All candidemia episodes were observed in adult patients (21–81 years old) and 87.8% of them were admitted to SICU. The most common underlying condition observed in both colonized and candidemia patients was polytrauma ($n = 13$, 32%) followed by cardiovascular disease ($n = 10$, 25%) and cancer ($n = 7$, 17%). Indwelling CVC (odds ratio {OR}, 13.48), parenteral nutrition (OR, 3.49), and mechanical ventilation (OR, 2.43) were the more frequently invasive procedures observed in these two groups and remained significant predictors of *C. auris* colonization/candidemia.

All *C. auris* isolates were resistant to fluconazole (MICs >64 mg/L) and had significantly reduced susceptibility to voriconazole (GM, 1.8 mg/L). All isolates were susceptible to itraconazole, posaconazole, isavuconazole, and echinocandins.

Environmental sampling showed presence of the *C. auris* on sphygmomanometer cuffs (25%) patient tables (10.2%), keyboards (10.2%), and infusion pumps (8.2%).

Conclusions.

- Predictor conditions to *C. auris* colonization/candidemia are similar to other Candida species. *C. auris* colonizes multiple patient's environment surfaces. All isolates are resistant to fluconazole and had significant reduced susceptibility to voriconazole.

- Due to its high transmissibility and survival in the hospital environment, *C. auris* can cause long duration outbreaks that are difficult to detect in early stages, and it makes it difficult to control and eradicate.
- The implementation of early and strict surveillance and control measures is essential to preventing the spread of the outbreak representing a significant risk to critical patients.