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The top three areas of basic research on *Aspergillus fumigatus* in 2011

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Over 450 peer-reviewed papers containing the keyword *Aspergillus fumigatus* were published in 2011. Although an impossible task, I have selected three clusters of papers describing exciting recent advances in research on *A. fumigatus*. The first is the novel approach of *in vivo* imagining of experimental aspergillosis by the use of ^{68}Ga-labeled siderophores, internalized by the fungus, and detected via positron emission tomography, to image the site infection. This work may lead to improved diagnosis of aspergillosis. The second important finding is that NK lymphocytes, not thought to be involved in host resistance to aspergillosis, can kill aspergilli through direct contact, either through perforin or interferon-γ or both. The third area pertains to a novel first-in-class antifungal drug, E1210 (Eisai), which inhibits GPI anchoring of fungal-associated cell wall proteins. Thus far, it shows promising *in vitro* activity against a broad range of fungi including Aspergilli, as well as those that are difficult to treat with currently available therapies. Overall, these three areas demonstrate the exciting promise, progress, and utility of basic research against *A. fumigatus*.

Keywords: fumigatus; antifungal; NK cell; imaging

Aspergillus fumigatus is the most common opportunistic mold pathogen of humans, causing invasive diseases in immunocompromised patients.[1] The explosive fourfold increase in the incidence of invasive pulmonary aspergillosis (IPA) that has occurred over the last 30 years has triggered a parallel 400% increase in the number of scientific research papers devoted to studying *A. fumigatus*. Highlighting this increased interest, three of these papers have been published in the prestigious publications Nature and Science over the last five years alone.[2–4] Interestingly, approximately two-thirds of the 2011 publications on *A. fumigatus* focused on basic scientific research of this mold, while only a third dealt with clinical aspects, suggesting we are still trying to gain a better molecular understanding of this pathogen, before we can manipulate its weaknesses to improve treatment.

In this review, I highlight three outstanding basic science research papers published in 2011, which mark the way for future advances in basic and clinical research of invasive aspergillosis.

Imaging IPA by hijacking the iron uptake system of the fungus

Developing imaging modalities that are able to detect IPA with high sensitivity and specificity should allow the early initiation of appropriate antifungal treatment in high-risk patients. Here, Decristoforo *et al.* from the Innsbruck Medical University in Austria have utilized a novel approach to this challenging problem. During lung infection, *A. fumigatus* encounters an essentially iron-free environment. All available iron is tightly bound by host chelators, and in particular the protein transferrin. However, in the lungs, *A. fumigatus* can acquire iron by activating two independent high-affinity iron-uptake systems.[5] These originally evolved to enable *A. fumigatus* to overcome iron shortages in its natural soil environment. The most important of the iron uptake systems uses siderophores, small secreted molecules with very high binding affinity to ferric (Fe^{+3}) iron. During infection, *A. fumigatus* upregulates gene clusters involved in the biosynthesis of the

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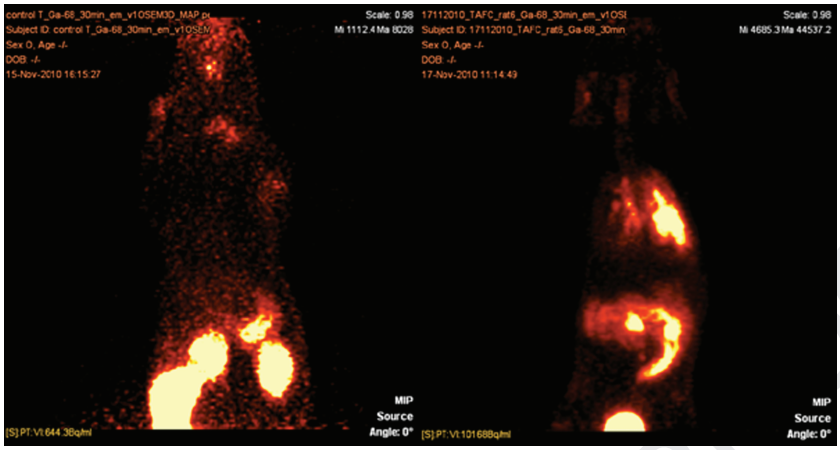

Figure 1. Micro-PET image (maximum intensity projection) of two rats, one hours postinjection of ^{68}Ga-Triacetylfusarinine (TAFC): left: control uninfected rat, activity is seen in kidney and bladder (urinary excretion), right: rat with severe invasive pulmonary aspergillosis (two days after intrapulmonary instillation of *A. fumigatus* conidia) showing additional high uptake in the left, infected lung (arrow). (Courtesy of C. Decristoforo, Department of Nuclear Medicine, Innsbruck Medical University, Austria and P. Laverman, Department of Nuclear Medicine, Radboud University Nijmegen Medical Center, The Netherlands).

siderophores fusarinine (FsC) and triacetylfusarinine (TAFC).[6] Once synthesized and secreted, TAFC and FsC sequester iron even when it is bound to human transferrin, enabling fungal infection to proceed. *A. fumigatus* mutant strains lacking the ability to synthesize FC and TAFC are completely avirulent in mice.[7] Siderophore uptake in *A. fumigatus* is actively mediated by Siderophore Iron Transporters (SIT), permeases of the major facilitator superfamily.[5] Importantly, mammalian cells lack SIT homologs and cannot actively take up siderophores. In two recent papers,[8,9] Petrik *et al.* took advantage of this distinction and designed various purified siderophores in which chelated iron was replaced by radioactive Gallium-68 (^{68}Ga). This element has a similar charge and size to iron and is widely used for imaging by positron emission tomography (PET). Once injected into rats infected intratracheally with *A. fumigatus*, the labeled siderophores were actively and selectively concentrated into the infecting fungus, enabling it to be clearly visualized inside the lungs by PET (Fig. 1). In essence, the researchers exploited the same siderophores used so successfully by the fungus to survive during infection, labeled with ^{68}Ga, as "Trojan horses" to allow specific imaging of the infectious process. Of the ^{68}Ga-labeled siderophores tested, *A. fumigatus* TAFC and bacterial ferrioxamine E were shown to be the best siderophore for clearly and selectively imaging *A. fumigatus* infection. Labeling was sensitive enough to differentiate between severe and mild infection as early as three days after administration of the fungus.[8] Other fungi and some bacteria are able to actively take up TAFC or ferrioxamine E. Consequently, although further research will be necessary to address the sensitivity and specificity of this method versus existing PCR and ELISA technologies, it may be possible in the near future to use this technology to accurately detect and localize invasive fungal infection at its earliest onset.

NK cells—a novel line of defense against IPA

To date, it has been widely accepted that the major known lines of innate immune defense active against IPA are those mediated by macrophages, neutrophils and dendritic cells.[10] Here, two groups, led by Juergen Loeffler from Wurzburg University and by Thomas Lehrnbecher from Goethe University, Germany, suggest an additional cellular player: The NK (natural killer) cell.[11,12] NK cells are a third class of lymphocyte, related to B and T cells. First recognized for their ability to autonomously recognize and destroy cancer cells, they are now known to participate in the defense against viruses, bacteria, and protozoans.[13] Their response to pathogens generally requires signals (both contact dependent and soluble) from accessory cells such as dendritic cells and macrophages, and involves the release of interferon γ (IFN-γ) and direct cytolytic killing.

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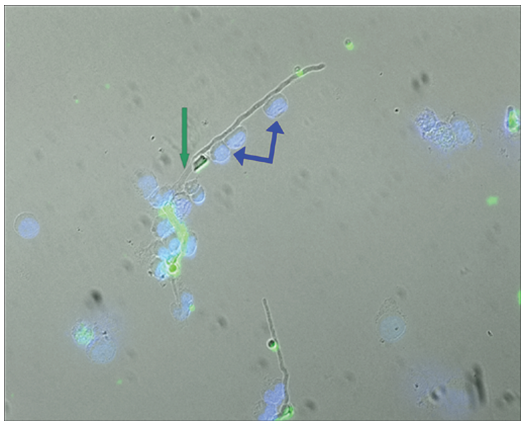

Figure 2. NK cell–*A. fumigatus* interaction is mediated by direct physical contact. NK cells were stained with a DAPI dye (blue arrows) and *A. fumigatus* hyphae were stained with a FITC dye (green arrow) displaying a direct contact after three hours of coincubation. The photo was acquired using a Zeiss fluorescence microscope, and Zeiss AxioVision LE software (version 4.7) at a magnification of 40×. (Courtesy of J. Loeffler, Medizinische Klinik und Poliklinik II, Universitatsklinikum Wurzburg, 97080, Wurzburg, Germany.)

Previous studies on the role of NK cells in IPA suggested that they are the main source of early IFN-γ in the infected lungs, and this is an important mechanism in the defense against this infection.[14,15] Now, Schmidt *et al.*[12] and Bouzani *et al.*[11] demonstrate that purified human NK cells directly recognize and destroy growing *A. fumigatus* in the absence of accessory cells (Fig. 2). Interestingly, although NK cell activation depended on direct contact with the fungus, killing was mediated by a NK-cell-secreted factor, which one group identified as perforin (a secreted pore-forming protein) while the other identified as IFN-γ.[11,12] The reason for this disparity remains unclear and will require further investigation. However, both studies point toward NK cells as a potentially important and hitherto unappreciated player in the innate defense against *A. fumigatus* and as a promising new avenue of immunotherapeutic augmentation.

E1210: A novel antifungal that targets anchoring of cell wall proteins

The cell wall is an essential component of all fungi. Since it is not found in mammalian cells, it presents an attractive drug target. Surprisingly however, of the four major existing families of antifungals (polyenes, azoles, allylamines, and echinocandins) only the latter directly targets the cell wall, by inhibiting the enzyme glucan synthase, which is responsible for synthesizing β-1,3-glucan, a major polysaccharide wall component.[16] Recently however, Asada *et al.* from the Eisai Company in Japan have published a stream of seven papers, describing the antifungal activity of a novel cell-wall-targeting drug, called E1210.[17–23] Unlike the echinocandins, which target synthesis of the cell wall polysaccharide scaffold, E1210 inhibits an early step in the glycosylphosphatidylinositol (GPI)-dependent anchoring of cell wall proteins within this scaffold. Lacking these proteins, the cell wall weakens, resulting in the fungistatic arrest of growth. The GPI anchor is synthesized and attached to target proteins within the endoplasmatic reticulum (ER) in a pathway containing approximately 11 enzymes.[24] The target of E1210 is Gwp1p, the fourth enzyme in the pathway, responsible for inositol acylation.[23,25,26] Although mammals also contain a Gwp1 homolog called PigW, it is only 28% identical to the fungal gene and is not inhibited by E1210.[23] *In vitro*, E1210 was highly effective (MIC_{90} ranges of 5–200 ng/mL range) against most fungi, including yeast (*Candida* species except *C. krusei*) and molds (*Aspergillus* species, *Fusarium* spp., black molds), as well as strains resistant to azoles and polyenes.[19–22] E1210 was moderately effective against species of zygomycetes (MIC_{90} ranges of 1–8 μg/mL). *In vivo*, E1210 was effective (> 80% two-week survival, 2.5–25 mg/kg/day) in the treatment of murine models of disseminated candidiasis (*C. albicans*), pulmonary aspergillosis (*A. fumigatus* or *A. flavus*), and disseminated fusariosis (*Fusarium solani*). E1210 was nontoxic at 100 mg/kg and was generally well tolerated at doses of up to 300 mg/kg in rats.[18] Currently pharmacodynamic and metabolic studies of E1210 are being conducted in rats, dogs, and monkeys, and these will hopefully pave the way for future clinical studies with this novel acting and promising compound leading to improved therapies especially for organisms resistant to currently available treatments.

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Conflicts of interest

The authors declare no conflicts of interest.

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