Aspergillus terreus: How Inoculum Size and Host Characteristics Affect Its Virulence

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(See the article by Slesiona et al, on pages 1268–77.)

Invasive aspergillosis is the most common filamentous fungal infection observed in immunocompromised patients [1]. Although the majority of these cases are caused by Aspergillus fumigatus, approximately 15% are due to Aspergillus terreus [1, 2]. Aspergillus terreus appears to be an emerging cause of infection at some institutions (eg, the Medical University Hospital of Innsbruck in Austria and the M. D. Anderson Cancer Center in Houston, Texas), and the endemic appearance of A. terreus has been demonstrated [3].

Overall, our clinical understanding of this historically difficult-to-treat pathogen is meager. Aspergillus terreus causes infections ranging from superficial infections to allergic bronchopulmonary aspergillosis, aspergilloma, and invasive disease in severely immunocompromised hosts [4]. Previous studies demonstrated that A. terreus infections were associated with dissemination, resulting in higher patient mortality, compared with other Aspergillus species [2, 4].

Aspergillus terreus is a common soil saprophyte and the only member of the genus Aspergillus that produces globose, heavy-walled hyaline cells laterally on the hyphae, called accessory conidia or aleuriocnidia [5–7]. Accessory conidia can be produced singly or in clusters in vitro and in vivo. Identification of accessory conidia in tissues of infected patients is a strong indication that the infecting organism is A. terreus. Deak et al [6, 7] suggested that accessory conidia may play a role in the dissemination of disease, and recent data indicated that accessory conidia can induce elevated inflammatory responses in a model of pulmonary aspergillosis.

A significant number of A. terreus isolates are resistant to amphotericin B, a polyene that had been the standard of care for the treatment of invasive fungal infections prior to the introduction of newer azoles [4, 8]. The exact mechanism of amphotericin B resistance has not yet been defined, but the level of catalase production in A. terreus may contribute to amphotericin B resistance [9]. The newer agents, such as voriconazole, posaconazole, and caspofungin, are associated with more-successful outcomes, compared with amphotericin B [1, 4]. There have been few animal models of A. terreus infections, but murine and rabbit pulmonary aspergillosis models showed the ability of A. terreus to produce disease [6, 10].

In this issue of the Journal, Slesiona et al report surprising results using infection models for A. terreus, reminding us that fundamental differences between A. fumigatus and A. terreus exist and that host pulmonary responses to A. terreus differ according to the immunosuppressive regimen used. To study A. terreus–mediated disease, this group developed an embryonated egg model and models using leukopenic and corticosteroid-treated mice and compared the patterns of infection and inflammation. In the murine A. terreus models of pulmonary aspergillosis, the key findings were as follows: (1) significantly (ie, 100 times) higher infectious doses were required for lethal infections, compared with A. fumigatus; and, under steroid treatment, both (2) in vivo germination was delayed and (3) an immune evasion by different cytokine secretions was observed. In addition, (4) liver toxicity was detected in A. terreus models, in contrast to A. fumigatus infections (Table 1). These results are important for understanding the immune response to A. terreus, although the reasons for such differences from A. fumigatus might only be partially explained by them.

Among the most important findings of this work, the first was that the rate of A. terreus infection increased with the size of the fungal inoculum, but this inoculum effect was unaffected by host characteristics when compared with data.
obtained with *A. fumigatus* [11, 12]. Lethal infections are regularly induced with 1–5 × 10^6 *A. fumigatus* conidia [12]. However, the reasons for a fitness reduction in *A. terreus*, compared with *A. fumigatus*, are not known. The question is, what controls *A. terreus* virulence? From the data obtained in the work of Slesiona et al, we can deduce that the host microenvironment clearly favors infections due to *A. fumigatus*, supported by both fungus-derived and host-related factors. Knowledge of the factor controlling the virulence of *A. terreus* is central for understanding the emergence of fungal diseases and for predicting the effect of fungi on individual hosts and host populations. If these data can be translated into clinical practice, the implications might give a simple answer to the question, why is *A. fumigatus* the most prevalent species among *Aspergillus*-mediated infections? The likely answer is that because *A. fumigatus* is most prevalent in the environment [13], relatively low inocula are necessary for a successful infection.

The second most important finding was that *A. terreus* morphological forms (germings and hyphae) varied between animal hosts. On day 5 of in vivo studies, germings were mainly obtained from leukopenic mice but hyphae were predominantly obtained from corticosteroid-treated mice. The question to be raised here is why such reduced fungal growth occurs in the early stage of infection in leukopenic mice. An aggressive host response, an inadequate environment, and a lack of nutrition should all be considered. This observation is contrary to what might be expected, as the absence of neutrophils predisposes fungi to maximal growth rates [14]. These germings lead to aggressive disease within 14 days, and mortality was 100%. The authors themselves argue that germination may be specifically delayed for intracellular conidia. This explanation might be only partially true or may be unrealistic because infiltration was sparse in cells of leukopenic mice.

A third interesting finding was the observation of pulmonary fungal persisters in convalescent animals and the absence of interleukin 10 (IL-10) release in corticosteroid-treated animals. Resistance to aspergillosis is often associated with the immune status of the host [15]. The lack of IL-10 production in *A. terreus* infections is contrary to observations in *A. fumigatus* infections, where IL-10 production peaked during infection [16]. IL-10 induces a T-helper cell 2 response, activates macrophages, and silences the T-helper cell 1 immune response [17]. So the evidence of viable conidial persisters in this group might be related to the inability to kill *A. terreus*. Furthermore, this work highlights the absence of interleukin 17/interleukin 22, a finding that supports the hypothesis of ineffective fungal elimination due to an imbalance of the immune response.

Whether these observations are specific for *A. terreus* infections needs to be investigated in more detail. So far, several studies have linked changes in the fungal cell wall to differences in immune response. Deak et al [6, 7] demonstrated that early germings of *A. fumigatus* display concentrated β-glucan at the tips, whereas this was not observed at the tips of *A. terreus* germings. Also, it was demonstrated that the 2 different *A. terreus* conidia have unique properties, suggesting that they may behave differently during the infection process. Overall, such modifications may partially explain the differences obtained; however, this view is likely to be overly simplistic. We have to take into account that once fungi are located in phagosomes, various fungal genes are differentially regulated (as revealed by microarray analysis), indicating the possible presence of numerous adaptive responses [15]. Similarly, the concentration and subcellular location of many fungal cell-wall proteins and carbohydrates may fluctuate during infection [15]. Whether these immunologic differences translate into differences in survival of mice challenged with *A. terreus* and/or *A. fumigatus* remains to be determined. From their data on carrier status, Slesiona et al conclude that *A. terreus* infections may reflect endogenous infection rather than

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**Table 1. Aspergillus terreus Infections and Differences in Host Response According to Underlying Immunosuppressive Regimens**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cyclophosphamide-Treated (Leukopenic) Mice</th>
<th>Corticosteroid-Treated Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doses required for successful infection (lethal infection)</td>
<td>1 × 10^6, 1 × 10^7</td>
<td>1 × 10^7</td>
</tr>
<tr>
<td>Monitoring of infection</td>
<td>Via bioluminescence imaging</td>
<td>Signals 4–6 d after infection; infection in all animals, yet 50% improved</td>
</tr>
<tr>
<td>Mortality, %</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Histology</td>
<td>Lungs: Germings on day 5</td>
<td>Hyphae on day 5</td>
</tr>
<tr>
<td></td>
<td>Liver and hepatocytes: No fungi, fatty degeneration</td>
<td>No fungi, fatty degeneration</td>
</tr>
<tr>
<td>Innate immunity and cytokines</td>
<td>Sparse immune cells infiltration; inconspicuous cytokine levels</td>
<td>Influx of neutrophils and monocytes; lack of interleukin 10 and interleukin 17 release; survival of intracellular <em>A. terreus</em> conidia</td>
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Aspergillus terreus; this is in accordance with our own results, in which we showed by RAPD strain-typing methods that A. terreus has diverse genotypes [3]. Consider that the agent-host-environment model of A. terreus airborne transmission is a framework for Aspergillus infection. Hence, the onset of an A. terreus infection might be exogenous (uptake of high inoculum) or endogenous (long-term survival of inhaled conidia). The latter mode of action suggests reactivation of conidia present in a dormant focus, as possibly shown in corticosteroid-treated mice. Prolonged and profound immunosuppression might be a prerequisite for such activation; however, the design of the study does not permit a more detailed analysis of these modifying factors.

The fourth observation by Slesiona et al, the fatty degeneration of liver cells in the absence of fungal tissue infestation, also deserves attention. Aspergillus terreus produces a variety of secondary metabolites that are economically important, such as lovastatin, an antihypercholesterolemic drug, and cyclosporine A [18]. On the other hand, most of the metabolites reported to be produced are considered mycotoxins, including citreoviridin, patulin, citrinin, emodin, terretonin, geodin, territrem, gliotoxin, and cytochalasin E [18]. It is imaginable that production of such a broad array of toxins could generate fatty degeneration of hepatocytes, as described in A. terreus infections. However, proof of this hypothesis and identification of potential toxins is necessary. Recently, it was demonstrated that gliotoxin reduced the viability of astrocytes, neurons, and primary microglia due to the induction of apoptosis [19]. A similar mode of action on hepatocytes might be valid for the toxins of A. terreus.

Finally, Slesiona et al have shown that embryonated hen eggs provide a suitable screening model for virulence studies of A. terreus. Overall, this work nicely shows how thoughtful experiments can give insights into unforeseen mechanisms and courses of infection; thus, inoculum, host ecology, and host genetics affect the virulence of A. terreus. This species has extraordinary value among the various Aspergillus species.

Notes

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References