Aspergillus nidulans and Chronic Granulomatous Disease: A Unique Host–Pathogen Interaction

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Invasive fungal infections are a major threat for patients suffering from chronic granulomatous disease (CGD), a primary immunodeficiency caused by a defect in the nicotinamide adenine dinucleotide phosphate (NADPH)–oxidase. Interestingly, Aspergillus (Emericella) nidulans is the second most encountered mold in CGD patients, causing almost exclusively invasive infections in this specific host, and is characterized by its aggressive behavior. A proper diagnosis is complicated by the often mild clinical presentation, the low sensitivity of the currently used diagnostic tools, and the difficulties in accurate identification of the Emericella species. According to the hitherto accepted view on the role of the NADPH-oxidase in the innate host defense pathway, the pathogenesis of A. nidulans in CGD cannot be explained. This synopsis covers the current understanding of invasive infections caused by A. nidulans in the CGD patient and is intended to direct further research by indicating gaps in our knowledge and to guide optimal management strategies.

Aspergillus nidulans (Teleomorph Emericella nidulans) has been an important research organism for studying eukaryotic cell biology for over half a century. It has contributed to our understanding of cell cycle control, DNA repair, mutation, recombination, cytoskeletal function, mitochondrial DNA structure, and human genetic disease [1]. Much less attention had been given to A. nidulans as an opportunistic pathogen in humans until recently, when it was recognized as a major cause of invasive aspergillosis (IA) in patients with chronic granulomatous disease (CGD). CGD is a rare (birth prevalence, 1:200 000) inherited immunodeficiency disorder of the nicotinamide adenine dinucleotide phosphate (NADPH)–oxidase in which phagocytes fail to generate superoxide anion and downstream reactive oxygen species (ROS) [2, 3]. CGD is a genetically heterogeneous disease caused by mutations in any of the 5 structural components of NADPH-oxidase, including the membrane-bound glycoproteins gp91phox (phagocyte oxidase), p22phox, and the cytoplasmatic components p47phox, p67phox, and p40phox [4]. As a result of the defect in the key innate host defense pathway, CGD patients suffer from life-threatening bacterial and fungal infections and inflammatory sequelae. Invasive fungal infections are often the first manifestation, revealing the underlying disease.

Aspergillus spp. are the most important encountered fungal pathogens [4]. In more detailed information extracted from the published data of CGD registries, the percentage of patients who had at least 1 infectious episode caused by Aspergillus spp. ranges from 26% in Europe [5] up to 46% in Japan [6]. Furthermore, Aspergillus spp. are the most common isolated causative pathogens in cases of pulmonary infections, brain abscesses, and osteomyelitis [2, 3, 5, 7] (Table 1). As a cause of death, fungal infections stand at the top with Aspergillus spp. being responsible for one-third to half of all deaths. Suggestion was made that CGD patients were at greater risk of A. nidulans infection than other immune-compromised patient populations and that A. nidulans was more virulent than A. fumigatus.
important areas for future research aiming at optimizing patient care.

### ASPERGILLUS nidulans

**Species Identification and Molecular Characterizations**

Identification of *A. nidulans* is based predominantly upon the morphology of the conidia and conidiophores. *Aspergillus nidulans* is a homothallic species capable of producing the teleomorph (sexual stage) without mating studies. The dual nomenclature of members of the *Aspergillus* section *Nidulanti* may be confusing for the clinician, as the ability of the fungus to produce a sexual state depends on the culture conditions.

The application of molecular tools has had major impact on the taxonomy of fungi. Multilocus sequence-based phylogenetic analyses have emerged as the primary tool for inferring phylogenetic species boundaries and relationships within subgenera and sections. Sequence analyses of the internal transcribed spacer region appears to be appropriate for identification of *Aspergillus* isolates to the subgenus/section level [17]. Partial β-tubulin or calmodulin are the most promising loci for *Aspergillus* identification to the species level.

**In Vitro Susceptibility Testing**

The efficacy of antifungal agents is different for the various *Aspergillus* spp., and *A. nidulans* reveals to be more resistant to amphotericin B compared to *A. fumigatus* [18]. Minimum inhibitory concentrations (MICs) for the mold-active azoles show a good susceptibility profile of *A. nidulans*, particularly for posaconazole [19, 20]. Although a pediatric dosage has not been defined, posaconazole as salvage therapy in CGD shows a good susceptibility profile [18]. Partial β-tubulin or calmodulin are the most promising loci for *Aspergillus* identification to the species level.

**Table 1. Epidemiology of Fungal Infections in CGD: Comparison of Published Registry Data**

<table>
<thead>
<tr>
<th>Geographical Region (Ref)</th>
<th>No. Patients</th>
<th>Aspergillus Infections (%)</th>
<th>Lungs</th>
<th>Skin</th>
<th>Liver</th>
<th>Brain</th>
<th>Bone</th>
<th>Septicemia</th>
<th>Prophylaxis (%)</th>
<th>Death (%)</th>
</tr>
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<tbody>
<tr>
<td>Europe [5]</td>
<td>409</td>
<td>26</td>
<td>61</td>
<td>5</td>
<td>3</td>
<td>7</td>
<td>16</td>
<td>2</td>
<td>53</td>
<td>Main cause</td>
</tr>
<tr>
<td>USA [3]</td>
<td>368</td>
<td>33</td>
<td>78</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>12</td>
<td>0</td>
<td>NA</td>
<td>35</td>
</tr>
<tr>
<td>UK [2]</td>
<td>94</td>
<td>27</td>
<td>85</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>10</td>
<td>0</td>
<td>93</td>
<td>50</td>
</tr>
<tr>
<td>Italy [7]</td>
<td>60</td>
<td>34</td>
<td>53</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>24</td>
<td>0</td>
<td>NA</td>
<td>50</td>
</tr>
<tr>
<td>Spain [46]</td>
<td>13</td>
<td>20</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>77</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Sweden [8]</td>
<td>21</td>
<td>24</td>
<td>86</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0</td>
</tr>
</tbody>
</table>

*Abbreviations: CGD, chronic granulomatous disease; NA, not available.*
<table>
<thead>
<tr>
<th>Case (Ref)</th>
<th>Sex</th>
<th>Genetic Type</th>
<th>Age, y</th>
<th>Previous Fungal Infection</th>
<th>Site of Disease</th>
<th>Mechanism of Spread</th>
<th>Prophylaxis</th>
<th>Treatment</th>
<th>Surgery</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 [25]</td>
<td>M</td>
<td>NA</td>
<td>16</td>
<td>No</td>
<td>Osteomyelitis, long bone</td>
<td>No spread</td>
<td>IFN-γ</td>
<td>ABLC, AMBL, ITC</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>2 [47]</td>
<td>M</td>
<td>NA</td>
<td>6</td>
<td>No</td>
<td>Lung, chest wall, vertebrae</td>
<td>Direct</td>
<td>TMP-SMX</td>
<td>AMB, ABLC, ITC</td>
<td>Yes</td>
<td>Died</td>
</tr>
<tr>
<td>3 [30]</td>
<td>M</td>
<td>X-CGD</td>
<td>20</td>
<td>No</td>
<td>Lung, 3rd rib, femur, skull</td>
<td>Direct</td>
<td>NA</td>
<td>AMB, ITC, 5-FC</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>4 [48]</td>
<td>M</td>
<td>X-CGD</td>
<td>4</td>
<td>No</td>
<td>Lung, 8th–9th ribs, T6-L1 vertebrae</td>
<td>Direct</td>
<td>NA</td>
<td>AMB, AMBL, gran Tx</td>
<td>Yes</td>
<td>Died</td>
</tr>
<tr>
<td>5 [48]</td>
<td>M</td>
<td>X-CGD</td>
<td>9</td>
<td>No</td>
<td>Lung, 4th rib</td>
<td>Direct</td>
<td>NA</td>
<td>AMB</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>6 [48]</td>
<td>M</td>
<td>X-CGD</td>
<td>13</td>
<td>Yes, A. nidulans pneumonia and osteomyelitis 4th rib at the age of 9</td>
<td>Progression of 4th rib lesion, T3-T4 with paraparesis</td>
<td>Direct</td>
<td>NA</td>
<td>AMB</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>7 [10]</td>
<td>M</td>
<td>X-CGD</td>
<td>6</td>
<td>Yes, Aspergillus spp.</td>
<td>Lung, pleura</td>
<td>No spread</td>
<td>No IFN-γ</td>
<td>AMB, ITC, gran Tx</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>8 [10]</td>
<td>M</td>
<td>X-CGD</td>
<td>19</td>
<td>No</td>
<td>Lung, pleura, chest wall, vertebrae, skin, skull, brain</td>
<td>Direct</td>
<td>IFN-γ after first event but stopped—relapse &lt;1 y</td>
<td>AMB, ABLC, ITC, 5-FC, gran Tx</td>
<td>Yes</td>
<td>Died</td>
</tr>
<tr>
<td>9 [10]</td>
<td>M</td>
<td>X-CGD</td>
<td>16</td>
<td>Yes, Aspergillus spp.</td>
<td>Lung, pleura, vertebrae, chest wall</td>
<td>Direct</td>
<td>KTC</td>
<td>AMB, ABLC, ITC, gran Tx</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>10 [10]</td>
<td>M</td>
<td>X-CGD</td>
<td>7</td>
<td>No</td>
<td>Lung, pleura, vertebrae, chest wall, sinuses, brain</td>
<td>Direct</td>
<td>NA</td>
<td>AMB, ABLC, gran Tx</td>
<td>Yes</td>
<td>Died</td>
</tr>
<tr>
<td>11 [10]</td>
<td>M</td>
<td>X-CGD</td>
<td>4</td>
<td>No</td>
<td>Lung</td>
<td>No spread</td>
<td>IFN-γ until 1 mo before A. nidulans infection</td>
<td>AMB, ITC</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>12 [31]</td>
<td>M</td>
<td>NA</td>
<td>6</td>
<td>No</td>
<td>Lung, ribs, vertebrae T1–T8, spinal cord</td>
<td>Direct</td>
<td>No</td>
<td>AMB</td>
<td>Yes</td>
<td>Died</td>
</tr>
<tr>
<td>13 [34]</td>
<td>M</td>
<td>X-CGD</td>
<td>8</td>
<td>No</td>
<td>Lung, rib</td>
<td>Direct</td>
<td>Clindamycin</td>
<td>AMB, gran Tx</td>
<td>No</td>
<td>Survived</td>
</tr>
<tr>
<td>14 [49]</td>
<td>M</td>
<td>NA</td>
<td>10</td>
<td>No, but pneumonia not responding to antimicrobial therapy, including tuberculostatic drugs: subtotal right upper lobectomy at age 6</td>
<td>Lung, pleura, axillary abscess, 2nd–3rd ribs, vertebrae</td>
<td>Direct</td>
<td>No</td>
<td>AMB</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>15 M X-CGD</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Lung, brain</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Survived</td>
</tr>
<tr>
<td>16 F NA</td>
<td>4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Lung</td>
<td>No spread</td>
<td>NA</td>
<td>AMB</td>
<td>No</td>
<td>Survived</td>
</tr>
<tr>
<td>17 M X-CGD</td>
<td>19</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Lung, chest wall, brain</td>
<td>NA</td>
<td>NA</td>
<td>AMB, ABLC, ITC, 5-FC, FLC, gran Tx</td>
<td>No</td>
<td>Died</td>
</tr>
</tbody>
</table>
Table 2 continued.

<table>
<thead>
<tr>
<th>Case (Ref)</th>
<th>Sex</th>
<th>Genetic Type</th>
<th>Age, y</th>
<th>Previous Fungal Infection</th>
<th>Site of Disease</th>
<th>Mechanism of Spread</th>
<th>Prophylaxis</th>
<th>Treatment</th>
<th>Surgery</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 [50]</td>
<td>F</td>
<td>p67phox</td>
<td>3</td>
<td>No, but borderline positive Mantoux test, consolidation on RX, no improvement with tuberculostatic therapy or antibiotics</td>
<td>Endocarditis, skin lesions, blood</td>
<td>Hematogenous</td>
<td>TMP</td>
<td>AMB</td>
<td>Yes</td>
<td>Died</td>
</tr>
<tr>
<td>19 [26]</td>
<td>M</td>
<td>X-CGD</td>
<td>21</td>
<td>Yes, <em>A. fumigatus</em> pneumonia at the age of 10, brain focus at the age of 13</td>
<td>Lung, popliteal abscess, soft tissues hemithorax, spinal cord, T5-T7 vertebrae</td>
<td>Direct/hematogenous</td>
<td>TMP-SMX, ITC, IFN-γ; poor compliance</td>
<td>AMB, AMBL, VOR, CAS, gran Tx</td>
<td>Yes</td>
<td>Died</td>
</tr>
<tr>
<td>20 [27]</td>
<td>M</td>
<td>X-CGD</td>
<td>4</td>
<td>No</td>
<td>Lung, T2-T5 vertebrae, spinal cord</td>
<td>Direct</td>
<td>TMP-SMX, ITC</td>
<td>AMB, AMBL, VOR, POS, CAS, gran Tx</td>
<td>Yes</td>
<td>Survived (ex vivo gene therapy)</td>
</tr>
<tr>
<td>21 [29]</td>
<td>M</td>
<td>X-CGD</td>
<td>13</td>
<td>No</td>
<td>Lung</td>
<td>No spread</td>
<td>TMP-SMX, ITC but diarrhea and serum levels (-)</td>
<td>AMBL, VOR, CAS</td>
<td>No</td>
<td>Survived</td>
</tr>
<tr>
<td>22 [28]</td>
<td>M</td>
<td>p22phox</td>
<td>5</td>
<td>No</td>
<td>Lung, chest wall cutaneous abscess</td>
<td>Direct</td>
<td>NA</td>
<td>AMB, VOR, ITC, gran Tx</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>23 [51]</td>
<td>M</td>
<td>X-CGD</td>
<td>8</td>
<td>No</td>
<td>Lung, 6th rib, chest wall fistula over the rib, psoas abscess</td>
<td>Direct</td>
<td>TMP-SMX, ITC; stopped 4 wk prior to <em>A. nidulans</em> infection</td>
<td>AMB, AMBL, gran Tx</td>
<td>Yes</td>
<td>Survived (BMT)</td>
</tr>
<tr>
<td>24 [32]</td>
<td>M</td>
<td>X-CGD</td>
<td>4</td>
<td>No</td>
<td>Lung, chest wall, TB-T11 vertebrae, 7th rib</td>
<td>Direct</td>
<td>NA</td>
<td>AMB, 5-FC, ITC gran Tx</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>25 [33]</td>
<td>M</td>
<td>NA</td>
<td>5</td>
<td>NA</td>
<td>Lung, chest wall, vertebrae, spinal cord syringomyelia</td>
<td>Direct</td>
<td>NA</td>
<td>VOR</td>
<td>Yes</td>
<td>Survived</td>
</tr>
</tbody>
</table>

Abbreviations: 5-FC, 5-flucytosine; ABLC, amphotericin B lipid complex; AMB, amphotericin B deoxycholate; AMBL, amphotericin B liposomal; BMT, bone marrow transplantation; CAS, caspofungin; FLC, fluconazole; gran Tx, granulocyte transfusion; IFN-γ, interferon-γ; ITC, itraconazole; KTC, ketoconazole; TBF, terbinafine; TMP-SMX, trimethoprim-sulfamethoxazole; NA, not available; PCZ, pozaconazole; VOR, voriconazole.
INVASIVE A. nidulans INFECTIONS

Twenty-five cases of IA due to A. nidulans in CGD patients were previously reported. The major clinical features are summarized in Table 2. Twenty-three were male, and median age was 7.5 years (range, 3–21 years). Of those whose genetic pattern was reported (n = 19), 89% were X-linked. The most common localization (72%) is lung invasion with direct spread to adjacent chest-wall structures (Figure 1). The presenting signs and symptoms were often mild, with low-grade fever, local pain or swelling, malaise, and cough, but could be completely silent with asymptomatic new lung infiltrates detected during a routine visit.

Diagnosis

Information on diagnostic tools was provided in 22 of the 25 patients reviewed in this study. Twenty-one of them fulfilled the criteria for proven invasive mold infection. Histopathology and direct microscopy or culture of a biopsy taken during open surgery was conclusive in 67% (n = 14) of the proven cases. Pulmonary radiologic features were present in 75%. Interestingly, beyond description of “pulmonary consolidation” and “lung infiltrates,” no specific signs as nodules, air crescent formation, halo, signs, or cavitations were described or could be detected on the published images. Computed tomography and magnetic resonance established extra-pulmonary extension to soft tissues, bones, and spinal cord injury. Local extension of disease from lung parenchyma to adjacent structures and osteomyelitis of the thoracic skeleton have been found particularly associated with underlying CGD [24].

In 4 of the reported CGD cases, information about circulating antigens in serum could be extracted [25–28]. Three of them were negative despite the extensiveness of the disease. Only van ’t Hek described positive galactomannan ratios in a X-linked CGD patient suffering from invasive A. nidulans infection and chest-wall invasion [28]. Information on diagnostic polymerase chain reaction was retrieved in 2 proven cases and 1 probable; however, results were negative or inconclusive [25, 27, 29]. Four cases mentioned strongly positive anti-Aspergillus antibodies [26, 30–32].

Treatment and Outcome

Combined antifungal treatment and extensive and early surgical debridement was used in most patients (83%). All patients except 2 received presumptive treatment with amphotericin B. Conventional amphotericin B deoxycholate (range, 0.6–1.5 mg/kg/d intravenously) was used in 91%. Usually, treatment was initiated as a monotherapy (71%); combination therapy was started only in 6 cases by the addition ofitraconazole, 5-flucytosine, or caspofungin. Use of voriconazole was first reported in 1998 and used in 5 cases, only once as a first-line treatment [33]. Twelve patients (50%) received granulocytes, and 7 patients (30%) received interferon-γ (IFN-γ) in addition to the antifungal therapy. The use of IFN-γ as adjunctive therapy of IA in CGD patients has not been investigated by controlled studies and remains controversial. In those who did not receive surgery and survived, infection was limited to the lung or with minimal involvement of adjacent structures [29, 34].

Unambiguous data on clinical outcome were lacking, and follow-up ranged from “still under treatment” to 8 years. At the time cases were published, the mortality rate was 32%. The exact mortality rate of A. nidulans invasive infection in the CGD patient is difficult to determine, and an underestimation cannot be ruled out because of the huge variability in follow-up.

PATHOGENESIS AND HOST DEFENSE

By comparing invasive A. nidulans infections in the CGD host (n = 25) with those caused by A. fumigatus (n = 44), A. nidulans infections are often asymptomatic, behave more aggressively, and are significantly more likely to result in death. Primary lung involvement was followed in 75% (vs 14% for A. fumigatus) by extensive tissue destruction and direct spread to adjacent chest-wall structures [9–11]. Only 2 studies with p47phox−/− mice provide histopathology data on A. nidulans infections, but no direct comparison was made to A. fumigatus infections [35, 36]. Besides the fact that comparable fatal A. fumigatus infections were observed in the X-linked murine CGD model and that the histopathology data do support a role for aberrant inflammation, not more can be concluded from these studies.

Most studies focusing on innate immune responses against Aspergillus spp. have used A. fumigatus due to the fact that this species is the most commonly encountered causative agent of IA. Knowledge of the host response against other Aspergillus spp. is scarce, in particular with respect to A. nidulans. The first line of host–defense is directed against conidia, the infective form of the filamentous fungi, and consists of macrophages. The macrophages will kill the germinating spores intracellularly by mainly nonoxidative processes. The second line of defense against mold infections is superoxide production by neutrophils, a powerful mechanism to kill the invasive hyphal structures of filamentous fungi such as Aspergillus spp., and is missing in CGD [37] (Figure 2).
Studies on the role of NADPH-oxidase activity in killing *A. fumigatus* conidia by alveolar macrophages (AM) have produced contrasting interpretations. Experimental studies with AM from gp91phox$^{-/-}$ mice showed phagocytosis and killing rates comparable to AM from normal mice, indicating that NADPH-oxidase–independent mechanisms in murine AM are pivotal to the inhibition of conidial germination [38–40]. In contrast, AM from p47phox$^{-/-}$ mice were unable to kill *A. fumigatus* conidia [41]. Furthermore, inhibitors of NADPH-oxidase that decreased the production of reactive oxidant intermediates inhibited the killing of *A. fumigatus* in normal murine AM [42]. By comparing the killing ratio of *A. fumigatus* and *A. nidulans* by gp91phox$^{-/-}$ AM and healthy AM, we confirmed that gp91phox$^{-/-}$ AM were at least as efficient in killing these 2 species as healthy AM are [43]. Although differences in animal strains, morphotypes, cell sources, and methods to assay the fungal damage might be responsible for this discrepancy, this heterogeneity underscores the complexity of fungal resistance and the fact that other mechanisms than killing by ROS must be involved as well.

**Figure 1.** Computed tomography scan of *A. nidulans* infection in a patient with chronic granulomatous disease. Note the extensive chest wall invasion and subcutaneous infiltration (arrow).

**Figure 2.** *A. nidulans* unique interaction with the CGD host. A functional NADPH-oxidase is crucial both as antimicrobial effector complex and as regulator of inflammation: a balance that is skewed to a state of hyperinflammation upon interaction with *A. nidulans*. Various mechanisms known to play a role in the host immune response induced by *A. nidulans* are schematically illustrated. *A.* Inhaled *A. nidulans* conidia will be killed by alveolar macrophages mainly by nonoxidative processes. *B.* Early PMN recruitment is crucial in preventing germination of *A. nidulans* and is partly NADPH dependent. The microbicidal activity of CGD PMN is maintained toward *A. nidulans* but not to *A. fumigatus*. *C.* CGD patients shown to have lower expression of PRR. *A. fumigatus* is able to modulate the host TLR responses. Influence of *A. nidulans* on the CGD PRR expression and modulation of the response still needs to be elucidated. *D.* *A. nidulans* infections are able to boost the proinflammatory state of the CGD cell, which results in an increase of measurable TNF-α and a decrease of IL-10. L-tryptophan metabolism in human CGD cells is normal in response to fungal pathogens; however, IL-17A is strikingly low in response to *A. nidulans* and *A. fumigatus*. *E.* ROS likely dampen inflammasome activation and NADPH-oxidase–defective human PBMCs are a source of elevated IL-1β. Infection of human CGD leukocytes with *A. nidulans* has shown significantly more IL-1β secretion compared to *A. fumigatus*. Abbreviations: CGD, chronic granulomatosus disease; IL, interleukin; NADPH, nicotinamide adenine dinucleotide phosphate; PBMCs, peripheral blood mononuclear cells; PMN, polymorphonuclear neutrophil; PRR, pattern recognition receptor; TNF, tumor necrosis factor.
Reactive-oxygen species like H$_2$O$_2$ seem to act as chemotactants [44]. Furthermore, by studying IA in an experimental murine model, it was suggested that early polymorphonuclear neutrophil (PMN) recruitment is crucial. PMN recruitment to the lungs shows to be slower in gp91$^{phox-/-}$ mice, resulting in increased germination. More extensive hyphal proliferation and tissue invasion were observed in the lungs of gp91$^{phox-/-}$ mice, indicating that when the lungs are exposed to large numbers of conidia, early PMN recruitment and formation of oxidative-active aggregates are essential in preventing germination of A. fumigatus conidia [39]. These data were confirmed by microarray data of murine C57BL/6 and gp91$^{phox-/-}$ AM exposed to A. fumigatus conidia in vivo: the most marked early transcriptional changes did not indicate obvious NADPH-oxidase involvement but occur in genes involved in PMN recruitment [40].

We recently explored the central role of the NADPH-oxidase, and the resulting ROS, in direct antifungal host defense. In vitro infection of circulating human leucocytes revealed that resistance to A. nidulans is not directly ROS related. We showed that A. nidulans, in contrast to A. fumigatus, is not susceptible to ROS. Infection of healthy PMN and peripheral blood mononuclear cells (PBMCs) by live A. nidulans did not result in any measurable ROS release, and the microbicidal activity of CGD PMN was maintained toward A. nidulans but not to A. fumigatus [43]. These results indicate that the etiology of A. nidulans infections in CGD cannot be explained by the simple absence of the direct microbicidal effect of ROS.

In the early 1980s, it was suggested that abnormal pH regulation within the phagosome of CGD phagocytes might have a role in defective killing [45]. The basis of this assumption was that the initiation of superoxide production is normally accompanied by phagosomal alkalinization as a result of the proton-acceptor function of superoxide anions. This pH change was proposed to be essential for the activation of granule-derived enzymes within the phagosome. Later on, this scheme was adjusted by showing that it is the pH-dependent, compensatory potassium surge across the vacuolar membrane, which is responsible for the release and activation of cationic granule proteins, form the anionic sulfated proteoglycan matrix [41]. In patients with CGD, however, the NADPH-oxidase function, alkalinization, and potassium influx is absent, resulting in impairment of these killing mechanisms. Whether this plays a significant role in the pathogenesis of A. nidulans is subject to debate, as earlier virulence studies of A. nidulans mutants in p47$^{phox-/-}$ mice indicate that pathogenicity was not influenced by fungal virulence factors as catalases and pH responsiveness [35, 36]. Interestingly, the use of pH-response mutants of A. nidulans in neutropenic mice showed a dramatic attenuation of ability to cause invasive disease [52; for references S1 to 66, see supplementary materials]. This observation shows that extrapolation of data from neutropenic mouse models of IA is insufficient to understand the pathophysiology of IA in CGD patients.

Phagocytic cells possess several nonoxidative fungal mechanisms, including antimicrobial peptides (eg, defensins, histatin 5) and hydrolases, which are effective in preventing germination or at killing intra- and extracellular fungi [53]. Drosomycin-like defensin shows antifungal activity [54]. This synthetic drosomycin-like defensin was designated based on a putative human homologue of the Drosophila-derived drosomycin, known for its antifungal properties. In this in vitro study, the susceptibility of A. fumigatus and A. nidulans to drosomycin-like defensin were remarkably different; the growth of A. nidulans was inhibited by the synthetic drosomycin-like defensin, but not by drosomycin. In contrast, A. fumigatus was susceptible to both of these defensins. The role of defensins and other cationic proteins stored in the granules of phagocytes from CGD patients in the host defense against filamentous fungi is not yet known and needs to be investigated.

It is clear that the almost exclusive contribution of NADPH-oxidase to microbial killing is a justified subject of debate, and recent studies indicate a critical role of the NADPH-oxidase as regulator of the immune homeostasis at multiple levels [55–57].

We have observed that the absence of the respiratory burst is associated with a dysregulated production of pro- and anti-inflammatory cytokines and further contributes to the pathogenesis of IA in CGD patients [58]. A more proinflammatory cytokine response was shown after stimulation with A. fumigatus conidia, while the balance shifted to an anti-inflammatory response after hyphal stimulation. The opposite was seen in healthy controls. In general, the induction of a T-helper 1 (Th1)–type response, characterized by IFN-γ, tumor necrosis factor (TNF)–α, and interleukin (IL)–12 production, is protective against the development of IA. In contrast, defense against IA is impaired by IL-4 and IL-10. The respiratory burst in phagocytes is differentially regulated by Th1- and Th2-type cytokines: TNF–α enhances superoxide production by neutrophils, while IL-10 impairs the respiratory burst in macrophages [37]. In a paper by Romani et al. [55], dysregulation of the L-tryptophan metabolism in mice with defects in NADPH-oxidase, resulting in overproduction of IL-17, has been proposed to link ROS defects with hyperinflammation and susceptibility to pulmonary aspergillosis. However, in humans with CGD, tryptophan metabolism was shown to be intact, indicating that the mechanism of fungal susceptibility is different in CGD humans from CGD mice [59–60]. We evaluated in both gp91$^{phox-/-}$ and p47$^{phox-/-}$ CGD patients the L-tryptophan metabolism and cytokine profiles in response to Candida albicans, A. fumigatus, and A. nidulans. Indeed, in contrast to mice, both CGD genotypes display a normal tryptophan metabolism. PBMCs of CGD patients produced more proinflammatory cytokines after stimulation, and
IL-17A production was strikingly low in response to fungal pathogens when compared to healthy controls [61]. Although it seems that an efficient anti-Aspergillus defense relies more on a Th1 immune response [62], the absence of an adequate IL-17 response might contribute to their inability to clear fungal infections.

Recently, Segal et al. [56] showed that NADPH-oxidase-dependent, redox-mediated signaling is critical for termination of lung inflammation. By challenging NADPH-oxidase-deficient p47phox−/− mice and gp91phox−/− mice with intratracheal zymosan, they showed that NADPH-oxidase limits lung inflammation by attenuating the proinflammatory transcription factor NFκB and by activating Nrf2, a key redox-sensitive antiinflammatory regulatory transcription factor. Data from mononuclear cells from X-linked CGD patients were consistent with these findings.

Innate immune receptors like Toll-like receptors (TLRs) and complement receptors are important in orchestrating the host–defense and may be modulated by pathogens during the course of infection. PMN from CGD patients show lower expression levels of TLR5, TLR9, CD11b, CD18, CD35, and CXCR1 compared to those from healthy controls, whereas similar or increased receptor expressions were found in patients without CGD but with bacterial pneumonia [63]. A. fumigatus is able to modulate the host TLR responses by directly decreasing the capacity of the host cells to respond to TLR2 and TLR4 ligation, a mechanism that can be interpreted as a means to evade the host immune system or to interfere with the resultant signaling pathways [64]. Whether differences in immune-receptor expression and regulation or modulation during invasive fungal infections in CGD patients are relevant for the observed epidemiology has still to be elucidated.

Overall, it is clear that the innate response to fungal pathogens serves 2 main purposes: a direct antifungal activity and a regulatory function. Cellular mediators may serve both functions, allowing a certain degree of redundancy and compensation under specific conditions of either infection or other causes of inflammation. Inhibitors of TNF-α are commonly used to control severe inflammatory bowel disease in CGD, but these have been complicated by severe and sometimes fatal occurrence of fungal infections [65]. These observations suggest that TNF-α is playing a more prominent role in the host defense in the absence of superoxide formation. The absence of ROS and the inflammasome activation, resulting in IL-1β production, is another proposed mechanism for hyperinflammation in CGD patients. ROS likely dampen inflammasome activation and NADPH-oxidase–defective human PBMCs are a source of elevated IL-1β [66, 67]. Infection of human CGD leucocytes with A. nidulans has shown significantly more IL-1β secretion compared to A. fumigatus (unpublished data). Current studies are conducted to unravel the IL-1β processing in CGD patients infected by A. nidulans and A. fumigatus. Targeting the IL-1β secretion provides new potential therapeutic options for inflammatory conditions associated in CGD. Besides antifungal treatment, targeted dampening of inflammation during an A. nidulans infection in the CGD patient definitely needs further investigations.

CONCLUSIONS

This synopsis summarizes our current understanding of the unique interaction between A. nidulans and its preferred host, the CGD patient. The clinical epidemiology points out to a specific disease pathology being the result of the complex interaction between the pathogen and the host.

Fungal pathogenesis is a continuum between infection and inflammation. In the CGD host, the absence of a functional NADPH-oxidase complex has both an impact on displaying an efficient antimicrobial effect as well as a controlled inflammatory response. The defective NADPH-oxidase results in an impaired direct antimicrobial function but cannot explain the etiology of A. nidulans infections in CGD patients. Dysregulated cytokine production, the L-tryptophan metabolism, inactivation of Th17 cells, differential expression of innate immune receptors, impaired Nrf2 activity and inflammasome activation are discussed as immunological mechanisms underlying the dysregulated inflammatory response as observed in CGD upon interaction with A. nidulans. More extensive and in-depth analyses of these mechanisms in the unique interaction of A. nidulans in the CGD patient will definitely improve the current understanding of the role of the NADPH-oxidase in the host–immune response. More insight and detailed understanding of this challenging frontline is urgently needed to optimize diagnostic and therapeutic strategies for these often devastating invasive infections in the CGD patient. In summary, invasive A. nidulans infections are a major threat for patients suffering from CGD, while the molecular interaction between A. nidulans and the immune cells of the CGD host are hardly explored.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank our colleague Dimitri Diavatopoulos, PhD, for drawing the figure.

Financial support. This work was supported by the European Society of Paediatric Infectious Diseases/Wyeth fellowship to S. S. V. H. (grant 2008–2010).

Potential conflicts of interest. All authors: No reported conflicts.
All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Additional references can be seen online as Supplementary material.