Invasive fungal infection in an elderly patient with defective inflammatory macrophage function

L. Y. A. Chai[1,2], R. Naesens[3], A. L. Khoo[1], M. V. Abeele[4], K. van Renterghem[5], R. Cartuyvels[3], M. G. Netea[1] and I. C. Gyssens[1,6,7,8]

1) Department of Medicine, Radboud University Nijmegen Medical Centre, and Nijmegen Institute for Infection, Inflammation and Immunity (N4i), Nijmegen, The Netherlands, 2) Division of Infectious Diseases, University Medicine Cluster, National University Health System, Singapore, Singapore, 3) Laboratory of Medical Microbiology, Jessa Hospital, Hasselt, Belgium, 4) Department of Geriatric Medicine, Jessa Hospital, Hasselt, Belgium, 5) Department of Urology, Jessa Hospital, Hasselt, Belgium, 6) Department of Infectious Diseases, Jessa Hospital, Hasselt, 7) Hasselt University, Diepenbeek, Belgium and 8) Department of Medical Microbiology, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands

Abstract

Macrophages are known to be involved in pathogen recognition and mediate host immune responses, but, in the clinical setting, their purported central role in opportunistic fungal infections has not been demonstrated to date. Herein, we describe a patient with invasive testicular aspergillosis in whom we found, for the first time, a defect in macrophage function.

Corresponding author: I. C. Gyssens, Jessa Ziekenhuis, Campus Virga Jesse, Stadsomvaart 11, B-3500 Hasselt, Belgium
E-mail: i.gyssens@aig.umcn.nl

Opportunistic fungal infections are encountered in the setting of immunocompromised hosts. This has been well described in patients who have received induction chemotherapy for leukaemia or who are undergoing haematopoietic stem cell transplantation, as well as in those receiving immunosuppressive drugs such as corticosteroids [1]. Immune recognition of *Aspergillus fumigatus* is mediated by an array of pattern recognition receptors (PRRs) present on macrophages, neutrophils and other host immune cells. Toll-like receptor (TLR)2, TLR4 and the C-type lectin receptor Dectin-1 are the major PRRs that recognize *A. fumigatus* [2] and consequently activate the host defence mechanisms. The indispensable role assumed by neutrophils in defence against the fungi is readily highlighted by the increased risk of aspergillosis in patients with prolonged neutropenia [1,3]. However, to date, the purported key role played by macrophages has not been demonstrated in the clinical setting. This because, in contrast to the relative ease with which neutrophil counts can be obtained, quantification of macrophage numbers and function in the patient is challenging.

On a similar note, postoperative aspergillosis has been described [4], but the underlying pathophysiology remains unknown. We recently managed an 80-year-old man who had testicular aspergillosis following urethrotomy [5]. The patient had a history of post-injection gluteal abscess caused by *Acremonium* species, but was otherwise not known to be immunocompromised. Nonetheless, given the atypical disease presentation in this setting, functional assays were performed to assess the response of the patient's immune cells to the fungal pathogen.

Peripheral blood mononuclear cells (PBMCs) were isolated from the patient (during voriconazole treatment) and from healthy controls by density centrifugation, as previously described [6]. Differentiation of monocyte-derived macrophages (MDMs) was performed with either 10% human pooled serum, macrophage colony-stimulating factor (M-CSF) or granulocyte–macrophage colony-stimulating factor (GM-CSF) [7]. The cells were stimulated with the respective fungal components (live conidia and inactivated *A. fumigatus* hyphal strain V05-27, 1×10^6/mL, and *Acremonium kiliense*, 1×10^6/mL) or specific PRR ligands (TLR2 ligand Pam3Cys (10 μg/mL), TLR4 ligand *Escherichia coli* lipopolysaccharide (LPS) (2 ng/mL) and Dectin-1 ligand β-glucan (10 μg/mL)) for 24 or 48 h at 37°C. Interleukin (IL)-6 and tumour necrosis factor (TNF)-α concentrations (at 24 h) and interferon (IFN)-γ concentrations (at 48 h) were measured with commercial ELISA kits. Cell membrane expression of TLR2, TLR4 and Dectin-1 were analysed with a FACSCalibur flow cytometer.

Production of TNF-α and IL-6 by the patient's PBMCs in response to both live and heat-inactivated *Aspergillus* was normal (Fig. 1a). In contrast, serum-differentiated MDMs from the patient had markedly diminished TNF-α and IL-6 levels as compared with controls (Fig. 1b). There was no
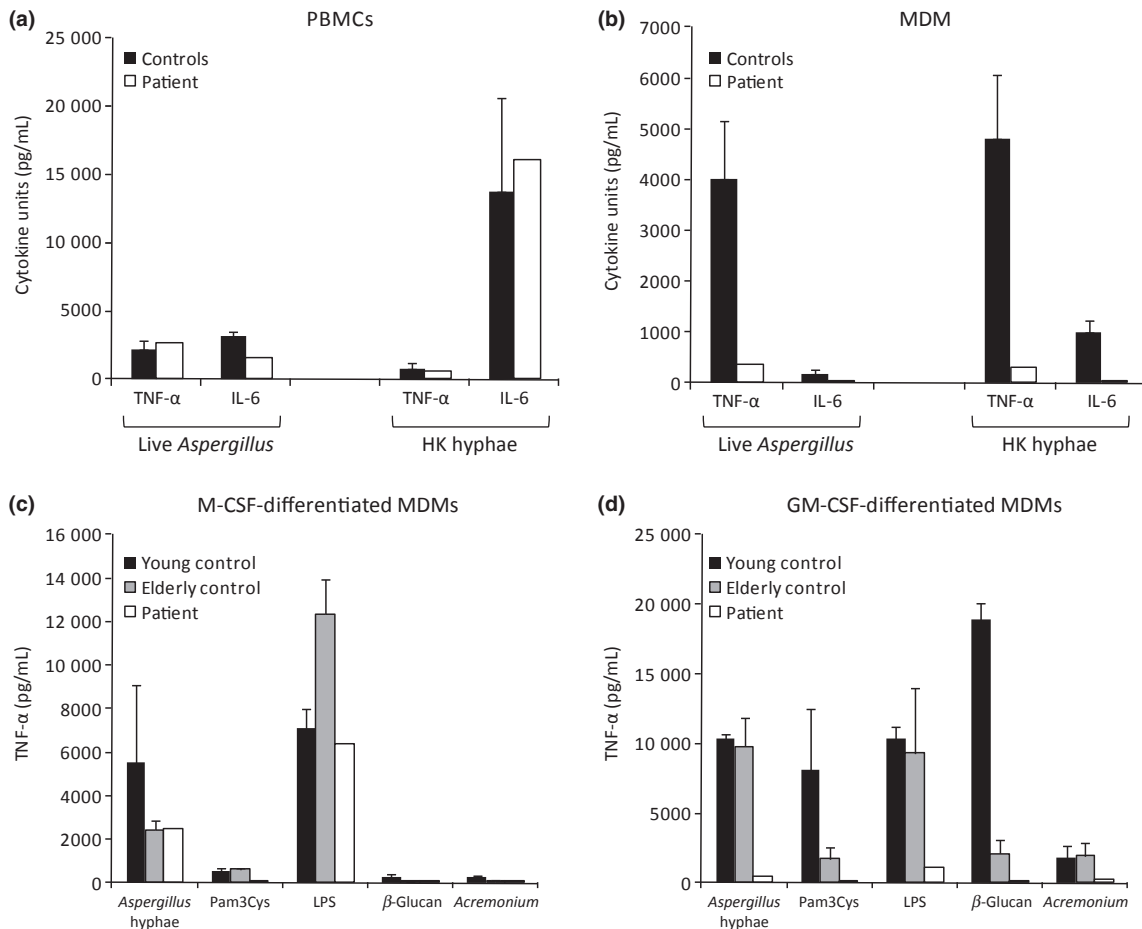
FIG. 1. (a, b) Cytokine response upon stimulation of (a) peripheral blood mononuclear cells (PBMCs) and (b) pooled serum-differentiated monocyte-derived macrophages (MDMs) of the patient and three healthy controls with live *Aspergillus* conidia and heat-inactivated hyphae (HK hyphae). (c, d) Tumour necrosis factor-α (TNF-α) response upon stimulation of (c) macrophage colony-stimulating factor (M-CSF)-differentiated and (d) granulocyte–macrophage colony-stimulating factor (GM-CSF)-differentiated MDMs from patient, three young healthy controls and three elderly healthy controls with the respective ligands as indicated. The data are expressed as means ± standard errors of the mean. IL, interleukin; LPS, lipopolysaccharide.

difference in IFN-γ-inducing capability between patient and control cells (data not shown). We studied this deficient response in MDMs by specifically differentiating PBMCs into type 1 or type 2 macrophages with growth factors. The use of M-CSF in the process of MDM differentiation gives rise to type 2-like resident macrophages, whereas GM-CSF-differentiated MDMs have type 1-like proinflammatory macrophage characteristics [8,9]. The defect was further delineated as being a dysfunction in the GM-CSF-differentiated MDM response. As compared with both young and elderly healthy controls, GM-CSF-differentiated MDMs of the patient showed diminished TNF-α and IL-6 responses, not only to *Aspergillus*, but also to *Acremonium* species, relative to the M-CSF-differentiated MDMs. In addition, GM-CSF-derived macrophages displayed deficient cytokine production following stimulation with Pam3Cys, LPS and β-glucan, which are spe-

cific ligands for the TLR2, TLR4 and Dectin-1 receptors, respectively (Fig. 1c,d shows TNF-α results; the data for IL-6 were similar to those for TNF-α, and are not shown). On the other hand, M-CSF-differentiated macrophages of the patient showed normal production of proinflammatory cytokines.

In tandem with the functional responses elicited as above, morphologically the patient's macrophages also showed poor differentiation under the influence of GM-CSF, yielding smaller cells than controls (Fig. 2a–c). In addition, flow cytometric analysis of the GM-CSF-differentiated macrophages revealed reduced TLR2, TLR4 and Dectin-1 expression relative to the controls (Fig. 2d–f).

The above findings point to a defect in inflammatory macrophage function, and this is the first clinical case to be
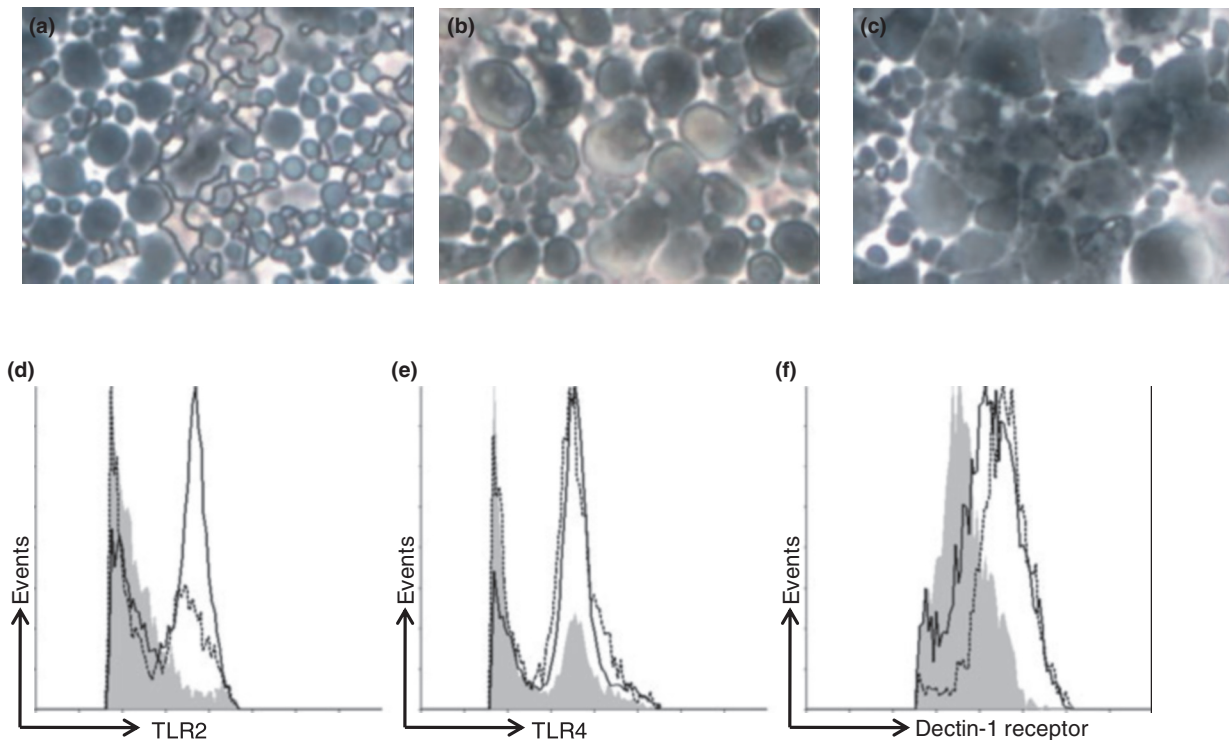
FIG. 2. (a, c) Direct microscopy of granulocyte–macrophage colony-stimulating factor (GM-CSF)-differentiated monocyte-derived macrophages (MDMs) from (a) the patient, (b) a healthy young control and (c) a healthy elderly control (Leica direct viewing microscope series, ×400 magnification). (d, f) Histogram overlay of (d) Toll-like receptor (TLR)2, (e) TLR4 and (f) Dectin-1 receptor expression on GM-CSF-differentiated MDMs of the patient (grey shade), a healthy young control (continuous line) and a healthy elderly control (dotted line).

described and linked to susceptibility to a locally invasive *Aspergillus* infection.

Conventionally, in the setting of invasive aspergillosis, tissue macrophages are known to form the first line of host defence, whereas the role of neutrophils predominates following *Aspergillus* conidial germination [10]. However, until now, the role of macrophages in defence against *Aspergillus* infection has not be demonstrated *in vivo* [11]. On the other hand, the clinical association between neutropenia and invasive aspergillosis is well established in haematological patients [3]. Hohl *et al.* have described how the time to recruitment of neutrophils may be an important determinant of infection outcome in a mouse model of pulmonary invasive aspergillosis. Notably, alveolar macrophage-depleted mice did not seem to have increased susceptibility to inhaled *Aspergillus* conidia challenge [12]. However, the central role of macrophages in coordinating the host inflammatory response and its antigen-presenting capabilities cannot be disregarded.

Our patient exhibited a defect in the differentiation of type 1 (GM-CSF-induced) proinflammatory macrophages, which are believed to be recruited during the inflammation process [8,9]. This is in contrast to the type 2 (M-CSF-induced) 'resident' macrophages, which act as

immune sentinels in the tissues. The inability to mount an adequate proinflammatory TNF-α and IL-6 response here was attributable to deficient expression of the major PRRs that are primarily responsible for immune recognition of *A. fumigatus*: TLR2, TLR4 and Dectin-1. This may partly be attributable to a failure of proper differentiation into type 1 macrophages, as supported by the small size of the patient's cells following GM-CSF induction (Fig. 2a). Incidentally, the patient's GM-CSF-differentiated MDMs had also exhibited diminished cytokine responses with hindsight of his past history of *Acremonium* abscess. Furthermore, the attenuated immunity elicited from the patient while on antifungal treatment takes on added significance, as voriconazole, conversely, is known to heighten TNF-α and nuclear factor-κB production [13]. Immune senescence was a consideration that had led us to include healthy elderly controls in our *ex vivo* studies. However, it was apparent that age was not a significant factor in determining susceptibility in this case, as the macrophages of the patient also had lower production of cytokines when compared with cells isolated from elderly control volunteers (Fig. 1c,d).

Putting the above findings into perspective, it is believed that, whereas a system of redundancy in the host innate and

acquired immune system might have compensated for the inflammatory macrophage dysfunction in the patient [14], an invasive procedure had facilitated the inoculation of an opportunistic pathogen deep beyond the epithelium and the sentinel capabilities of resident macrophages. Nonetheless, the infection was subsequently dealt with adequately by medical intervention, as well as by the other intact arms of the innate and acquired host immune response. This case adds to our understanding of the relative clinical importance of the role that macrophages play in invasive fungal infections.

Acknowledgements

Transparency Declaration

The authors declare no conflicts of interest.

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Value of (1-3)-β-D-glucan, *Candida* mannan and *Candida* DNA detection in the diagnosis of candidaemia

E. Mokaddas[1], Z. U. Khan[1], S. Ahmad[1], M. R. N. Nampoory[2] and M. Burhamah[3]

1)Department of Microbiology, Faculty of Medicine, Kuwait University, 2)Hamed Al-Essa Organ Transplant Center and 3)NBK Cancer Ward, Al-Sabah Hospital, Shuwaikh, Kuwait

Abstract

This study determined the value of (1,3)-β-D-glucan (BDG), *Candida* mannan (MN) and *Candida* species-specific DNA as surrogates for diagnosis of candidaemia. Thirty-nine patients yielding *Candida* species in blood cultures were investigated for presence of BDG, MN and *Candida* species-specific DNA in serum samples. The *Candida* spp. bloodstream isolates included *C. albicans* (n = 16), *C. tropicalis* (n = 10), *C. parapsilosis* (n = 7), *C. glabrata* (n = 3) and *C. dubliniensis* (n = 3). Positivity of the three markers was as follows: *Candida* DNA for corresponding *Candida* species, 100%; BDG, 87%; MN, 59%. Despite varying sensitivities of these biomarkers, they provided a useful adjunct to the diagnosis of candidaemia.

Keywords: (1-3)-β-D-glucan, *Candida* DNA, *Candida* mannan, candidaemia, diagnosis