**E-Supplement:**

**Laboratory findings:**

Immunophenotyping

The immune status of the patient showed a mild lymphocytopenia (CD4+ T-cells: 414/µl; CD8+ T-cells: 335/µl; CD19+ B-cells: 141/µl; CD3-CD56+NK-cells: 91/µl). No gross shift in the proportion of naive, memory, effector or activated T-cells was evident. Remarkably, 80% of the circulating B-cells were plasmablasts (CD19+, CD20+/-, IgD-, CD24+++, CD38+++). Naive (4/µl) and transitional (not detectable) B-cells were almost absent. The low or missing expression of CD20 on plasmablasts mocks a B-cell deficiency, if this marker is used to characterize B-cells. CD56high NK-cells were absent and a high proportion (44%) of the CD56dim population was CD16-. The patient showed a massive monocytopenia (4/µl), as has been reported during the entire period of observation. In addition, no myeloid (lin-, HLA-DR+, CD11c+, CD123-/+) or plasmacytoid (lin-, HLA-DR+, CD11c-, CD123++) dendritic cells could be detected in the peripheral blood. The frequency of Tregs (as defined as CD4+, CD25+, CD127-, CD45R0+ and CCR4+) in the peripheral blood was reduced: 14/µl compared to 45±15/µl in healthy controls. Therefore, the phenotype was compatible with the previously described MonoMAC syndrome caused by GATA2 mutations.

Diagnostic sequencing of the GATA2 gene (PCR)

A heterozygote mutation “c.1143+1G>A; p.?” was proven in the GATA2 area. The mutation affects the splice donor area in Intron 4 and will most likely lead to loss of the splice donor area and so presumably leads to aberrant splicing to a GATA2 haploinsufficiency. This mutation has not been described in the literature.

Gene expression

Compatible with the reduced numbers of myeloid cells in the periphery, the expression of inflammatory cytokines was severely impaired after stimulation of whole blood by 1ng/ml LPS (e-Figure 1a). Likewise the myeloid cell-dependent induction of the perforin and granzyme B genes in NK cells after LPS stimulation was missing (data not shown). Although quantitatively present, T-cell function was also impaired, as indicated by a reduced induction of several T-cell specific cytokine genes after PMA/Ionomycin stimulation. This was observed for both Th1 and Th2 cytokines, whereas the expression of IL17 and IL22 genes was only slightly reduced (e-Figure 1b).

Microbiology

The patient’s isolates were studied phenotypically and by molecular methods. Maximum growth of the fungus was at 37°C. It tolerated 0.1% cycloheximide, had urease activity, and no tolerance of NaCl 10%. In tissue as well as *in vitro* on Sabouraud agar septate hyhae were produced, 1.5 – 4.0 µm diameter, branched at 45° - 90°C; thick-walled chlamydospore-like structures present; conidia spherical to ovoidal, 5.0-7.5 µm in length. Sequencing of parts of the 18S ribosomal RNA gene, the complete internal transcribed spacer 1, 5.8 S and ITS 2 genes and parts of the 28S rRNA genes resulted in a > 3000 basepair long sequence. Cluster analysis in GenBank revealed a 99% identity with CBS 885.95 *Knufia perforans*, with a max. score of 3083/3754 and a coverage of 69%. Comparison with sequences deposited in the database of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands ([www.cbs.knaw.nl](http://www.cbs.knaw.nl)) revealed 99.1% large ribosomal subunit (LSU) and 96.5% internal transcribed spacer region (ITS) similarity with CBS 457.67, the type strain of *Arthrocladium caudatum*. In an unpublished research database of black fungi maintained at CBS for research purposes the strains formed a monophyletic group with three unnamed *Exophiala* isolates from Brazilian coconut shells at levels of 98-99% ITS similarity. Descriptions of these fungi as novel species are submitted.

**Methods**

Susceptibility testing

Antifungal susceptibility testing according to the CLSI M38-A2 guidelines was possible after repeated subculturing of the isolate to stimulate formation of conidia. Minimal inhibitory concentrations (µg/ml) after an incubation of 72 h at 35°C were: amphotericin B 0.5, itraconazole 0.125, posaconazole 0.125 and voriconazole 1.

Flow cytometry

Comprehensive analysis of leukocyte subpopulations was performed using the following 4 ENTIRE-HIPC panels by 8-color flow cytometry: T-cell-panel: CD45; CCR7; CD4; CD45RA; CD38; CD8; CD3; HLA-DR; Treg-panel: CD45; CD25; CD4; CCR4; CD127; CD45R0; CD3; HLA-DR ; B-cell-panel: CD45; CD24; CD19; IgD; CD38; CD20; CD27; CD3; Innate-panel: CD45; CD56; CD123; CD11c; CD16; CD3+CD19+CD20 (dump); CD14; HLA-DR(19). Briefly 100 µl of whole blood was added to the panel tubes with pre-mixed antibodies and incubated for 30 minutes at room temperature in the dark with light agitation. After red cell lysis, cells were fixed and analyzed on a LSR-II instrument. A detailed description of the methods can be downloaded at http://entire-net.eu/sops-and-cats.

Real-Time RT-PCR quantification

1 ml of heparinized whole blood was incubated with 1 ml RPMI1640 or RPMI1640 containing either 100 ng/ml PMA and 5 µg/ml ionomycin (Sigma) or 1 ng/ml LPS (Sigma) for 3 hrs at 37°. After red cell lysis, mRNA was isolated with the MagNA-Pure-LC device using the standard protocol for cells. mRNA was reverse-transcribed using avian myeloblastosis virus reverse-transcriptase and oligo-(dT) as a primer (First Strand cDNA synthesis kit; Roche Diagnostics) according to the manufacturer’s protocol in a thermocycler. After termination of the cDNA synthesis, the reaction mix was diluted to a final volume of 500 µL and stored at -20°C until analysis. Gene expression was quantified using real-time PCR with the LightCycler. Target sequences were amplified using commercially available LightCycler Primer Sets (Search-LC, Heidelberg, Germany) with the LightCycler FastStart DNA Sybr Green I Kit (Roche Diagnostics) according to the manufacturer’s protocol. The transcript concentration for the measured genes was calculated from a virtual standard curve, obtained by plotting a known input concentration of a plasmid to the PCR cycle number at which the detected fluorescence intensity reaches a fixed value. mRNA input was normalized to the expression of the housekeeping gene PPIB (transcripts/1000 transcripts PPIB).

**Figure Legend:**

E-Supplement-Figure 1a: Immunophenotyping - Expression of inflammatory cytokines was severely impaired.

E-Supplement-Figure 2b: Immunophenotyping - Expression of IL17 and IL22 genes was only slightly reduced.

E-Supplement-Figure 1a



E-Supplement-Figure 1b

