Clinical and Experimental Immunology, 2025, **219**, uxaf053
https://doi.org/10.1093/cei/uxaf053
Advance access publication 23 August 2025
Research Article

Research Article

Immunophenotyping of apparently immunocompetent hosts with cryptococcosis reveals IL-17 deficiency as a unifying susceptibility factor

Katie Townsend[1,2], Shichina Kannambath[3], Grant Hayman[4], Rainer Doffinger[5], Lourdes Ceron-Gutierrez[5], Soraya Ebrahimi[5], Vlada Pavlova[5], Philip Gothard[6], Michael Brown[6], Fariba Tahami[7], Dakshika Jayaratnam[8,9], Anna L Goodman[9,10,11], Derek Macallan[12,13], Thomas S Harrison[12,13,14], Magda Dziadzio[15], Jonathan Lambourne[16], Tanaraj Perinpanathan[17], Laurence John[17], Neil Stone[6], Tihana Bicanic[12,13,‡] and David M. Lowe[1,2,*,‡]

[1]Institute of Immunity and Transplantation, University College London (UCL), London, UK
[2]Department of Clinical Immunology, Royal Free London National Health Service (NHS) Foundation Trust, London, UK
[3]Genomics Facility, Institute of Cancer Research, London, UK
[4]Department of Immunology, Epsom and St Helier University Hospitals NHS Trust, Carshalton, UK
[5]Department of Clinical Biochemistry and Immunology, Addenbrooke's Hospital, Cambridge, UK
[6]Hospital for Tropical Diseases, Division of Infection, University College London Hospitals NHS Foundation Trust, London, UK
[7]Department of Immunology, Great Ormond Street Hospital NHS Trust, London, UK
[8]HCAI, Fungal, AMR, AMU & Sepsis Division, UK Health Security Agency, London, UK
[9]Department of Infectious Diseases, Guy's and St Thomas' NHS Foundation Trust, London, UK
[10]Centre for Clinical Infection and Diagnostics Research, Department of Infectious Diseases, School of Immunology and Microbial Sciences, King's College London, London, UK
[11]MRC Clinical Trials Unit, UCL, London, UK
[12]Institute for Infection and Immunity, St George's, University of London, London, UK
[13]Infection Clinical Academic Group, St George's University Hospitals NHS Foundation Trust, London, UK
[14]MRC Centre for Medical Mycology, University of Exeter, Exeter, UK
[15]Department of Specialist Allergy and Clinical Immunology, University College London Hospitals, London, UK
[16]Department of Infection, Barts Health NHS Trust, London, UK
[17]Department of Infectious Diseases, Northwick Park Hospital, London North West University Healthcare Trust, London, UK

[‡]Contributed equally.
[*]Correspondence: David M. Lowe, UCL Pears Building, University College London, Rowland Hill Street, London NW3 2QG, UK. Email: d.lowe@ucl.ac.uk

Abstract

Introduction: We describe the immunophenotyping and genetic analysis of human immunodeficiency virus (HIV)-uninfected apparently immunocompetent adults presenting with disseminated cryptococcosis. Cryptococci are environmentally ubiquitous fungi that may cause disseminated infection, including meningitis. Cryptococcosis occurs predominantly in immunocompromised hosts and most commonly in the context of HIV infection. In apparently immunocompetent patients, cryptococcal disease is rare, often diagnosed later and associated with higher mortality. The immunologic work-up and management of this patient group are challenging and poorly studied.

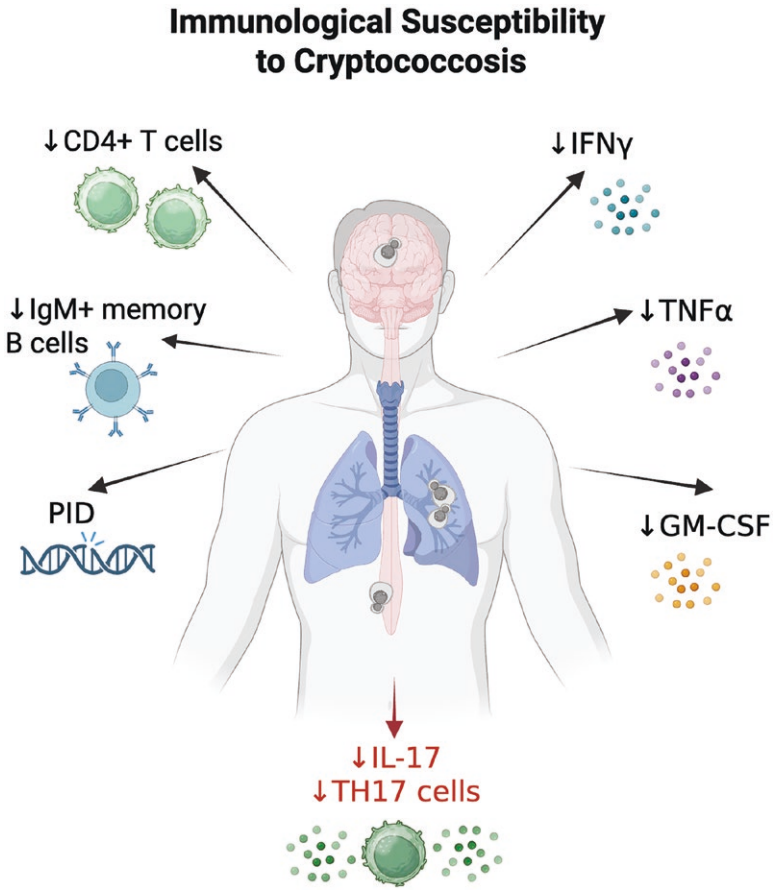
Methods: Between 2015 and 2021, eight apparently immunocompetent adults at the time of diagnosis with cryptococcosis underwent extensive diagnostic immunological work-up, including T-/B-cell subsets, immunoglobulins, T-cell proliferation and phenotyping, serum-specific antibody responses, mannose binding lectin, measurement of selected cytokines, anti-cytokine autoantibodies and targeted genetic next-generation sequencing.

Results: The production of interleukin (IL)-17 following phytohaemagglutinin (PHA) stimulation was significantly reduced in all eight patients with cryptococcosis compared to healthy controls (median IL-17 concentration in whole blood stimulation assay 88.1pg/mL in patients; 452.1pg/mL in controls, $P = 0.0047$). In 5/5 patients tested, the percentage of $CD4^+$ T-cells positive for IL-17, including memory $CD4^+CD45RO^+$ $IL\text{-}17^+$ T cells, after stimulation with staphylococcal enterotoxin B (SEB) was significantly reduced (≤0.4% cells). Reduced IgM^+ memory B cells were noted in 4/5 tested. 4/8 patients were found to have CD4 lymphopaenia. One patient with *Cryptococcus gattii* infection had autoantibodies against granulocyte-macrophage colony-stimulating factor (GM-CSF). No underlying genetic causes were identified.

Conclusions: Patients had several immunological risk factors, but reduced IL-17 production was a striking feature across the cohort—a phenotype that may facilitate tailored immunotherapeutic approaches.

Received 12 December 2024; Revised 4 July 2025; Accepted for publication XX XXXX XXXX

Graphical Abstract



Keywords: cryptococcosis, cryptococcal meningitis, immunodeficiency, IL-17/ interleukin-17, CD4 lymphopaenia, IgM⁺ memory B cells

Abbreviations: AIDP: acute inflammatory demyelinating polyneuropathy; APECED: autoimmune polyendocrinopathy and ectodermal dysplasia; CNS: central nervous system; CM: cryptococcal meningoencephalitis; CT CAP: computed tomography of chest abdomen and Pelvis; GM-CSF: granulocyte-macrophage colony-stimulating factor; GRID: genomics of rare immune disorders; HIV: human immunodeficiency virus; ICL: idiopathic CD4⁺ lymphopaenia; IFN: interferon; IgA, IgG, IgM: immunoglobulins A, immunoglobulin G, immunoglobulin M; IL-(17): Interleukin-(17); IUIS: International Union of Immunological Societies; LPS: lipopolysaccharide; MIC: minimum inhibitory concentration; NGS: next-generation sequencing; PAP: pulmonary alveolar proteinosis; PET CT: positron emission tomography-computed tomography; PHA: phytohaemagglutinin; PID: primary immunodeficiency; PMA: phorbol 12-myristate 13-acetate; SEB: staphylococcal enterotoxin B; TNF: tumour necrosis factor

Introduction

Cryptococcus is an opportunistic human fungal pathogen that can cause pulmonary disease, mass-like lesions (cryptococcomas) and a disabling and frequently fatal meningoencephalitis [1]. As an environmental saprophyte, exposure to *Cryptococcus* via inhalation is universal, and infection, as evidenced by anti-capsular antibody to cryptococcus [2] appears to be common, yet disseminated disease is rare. Globally, cryptococcal meningoencephalitis (CM) poses a major threat in patients with human immunodeficiency virus (HIV) infection and CD4⁺ T-cell counts < 100 cells/μL, who have a 6% prevalence of disseminated infection (cryptococcal antigenemia) and in whom CM accounts for an estimated 19% of all deaths [1, 3]. Patients undergoing solid organ transplantation are also at increased risk, with an estimated incidence of cryptococcosis of ~2% [4]. However, as access to early antiretroviral therapy and antifungal prophylaxis following transplantation has improved, the proportion of non-HIV, non-transplant patients with cryptococcal infection has increased, particularly in resource-rich settings. In large United States cohorts, such cases now form up to 40% of all patients with cryptococcosis, many of whom have no immediately apparent immunodeficiency [5]. In this group, diagnosis may be delayed and response to therapy is poor, with acute mortality rates of 40–50% [6, 7], exceeding those for patients with HIV-associated CM in developed countries [8]. This may reflect an inferior understanding of the immunopathogenesis and thus treatment of cryptococcosis in the apparently immunocompetent host [9–11], compared to in the context of HIV.

In HIV-infected patients, a defect in CD4⁺ T-cell mediated immunity is the major determinant of cryptococcal infection susceptibility and severity, with reduced CD4⁺ T-cell counts and dysregulation of or defective pro-inflammatory cytokine (interferon-gamma, IFN-γ and tumour necrosis factor-alpha, TNF-α) responses of particular importance [9, 12, 13]. Immunotherapy with adjunctive IFN-γ has been shown to improve cryptococcal clearance [9, 12]. CD8⁺ T-cells are also likely to contribute to antifungal immunity [14]. Immunological abnormalities in the B-cell compartment have also been described as potential risk factors, including reduced IgM⁺ memory B cells [15].

In non-HIV-infected, non-transplanted hosts, idiopathic CD4⁺ lymphopaenia (ICL) is a recognized risk factor for

cryptococcosis [16, 17]. Production of the pro-inflammatory cytokine IL-17, particularly by IL-17$^+$ CD4$^+$ helper T cells, is of known importance in antifungal immunity, with *in vitro* and animal studies suggesting a direct role in cryptococcal immunity [18–23]. CM has been described in individuals receiving therapeutic anti-IL-17 monoclonal antibodies [24], appearing in the Food and Drug Administration label for brodalumab [25]. A role for other cytokines in susceptibility to cryptococcosis is demonstrated by its development in the presence of anti-cytokine autoantibodies, for example, against IFN-γ [26]; or against granulocyte-macrophage colony-stimulating factor (GM-CSF) in cases of *C. gattii* [27]. Reduced IgM memory B-cells have also been described, mirroring findings in HIV-infected individuals [28]. Human genetic polymorphisms in immune response genes (Fc-γ receptor, mannose binding lectin, cytokines) have been linked to cryptococcal susceptibility in both HIV-infected and -uninfected hosts [29–32].

Echoing these pathomechanisms, rare primary immunodeficiency (PID) syndromes known to predispose to fungal (including cryptococcal) infection include those with combined defects including impaired T-cell function or number (e.g. CD40-Ligand deficiency); impaired phagocyte function (e.g. chronic granulomatous disease); or impaired IL-17 signalling (e.g. Hyper-IgE syndrome, autoimmune polyendocrinopathy and ectodermal dysplasia, APECED, STAT1 gain of function) [33, 34]. Indeed, invasive cryptococcal disease has been described several times in patients with Hyper-IgE syndromes [35–37] and STAT1 gain-of-function mutations [38–40], where the major immunological abnormality is impaired IL-17 signalling [41]. However, cryptococcosis is relatively rare even in these conditions, possibly influenced by the use of prophylactic azole antifungals and a paucity of data from regions with the highest rates of cryptococcal infection.

Here we describe detailed immunological analyses of eight apparently immunocompetent HIV-uninfected adults presenting to the hospital with disseminated cryptococcosis.

Methods

Study population

Case histories of patients presenting with disseminated cryptococcal infection were retrospectively collated across 6 tertiary referral hospitals in London, UK. Detailed immunophenotyping and immunogenetic assessments were performed prospectively as part of routine patient clinical management and care following referral for specialist Immunologist assessment. Laboratory healthy control samples were collected contemporaneously for required immunological assays, according to diagnostic laboratory standard operating procedures, from healthy adults with informed consent (Research Ethics Committee [REC] approval reference numbers 04/Q0501/119 and 06/Q0508/16). Patients were additionally counselled and written consent obtained before undergoing genetic testing (ethical approval from East of England Cambridge South National REC reference numbers 13/EE/0325). Case histories were summarized and laboratory data collected by the patients' clinicians/ study authors from clinical and laboratory records at individual centres. Individuals with no identifiable defined primary or secondary immune deficiency syndromes were included for analysis in this study. Other individuals with invasive cryptococcosis, subsequently found to have underlying definitive primary or secondary causes of immune deficiency, were also considered for comparison (e.g. STAT3 hyper IgE syndrome, lymphoma). We make a distinction between these two cohorts of patients, as those without an immunodeficiency diagnosis have less clearly defined management, which may be associated with a worse prognosis [6–8]. A fully anonymised case series was established to collate data on clinical outcomes for this rare cohort; thus, additional ethical approval was not required as per current UK Health Research Authority guidance.

Diagnostic immunological and genetic assays

The following assays were performed on patient samples: T- and B-cell lymphocyte subsets; immunoglobulins (IgA, IgG, and IgM); and mannose-binding lectin levels. T-cell proliferation was compared to contemporaneously processed healthy controls following stimulation with several broad-acting T-cell stimuli [42]. Peripheral blood mononuclear cells isolated from patient or control blood samples were stained with carboxyfluorescein diacetate succinimidyl ester (CFSE), aliquots were left unstimulated or stimulated with anti-CD3, costimulator CD28, or phytohaemagglutinin (PHA), incubated for 96 hours at 37°C, then washed and stained with fluorochrome-conjugated T-cell markers, and proliferation index for T cells was calculated by gating CD3 + lymphocytes and measuring CFSE by flow cytometry.

Anti-cytokine antibody testing was performed using multiplexed particle-based flow cytometry as described [43]. In brief, recombinant human cytokines were covalently coupled to activated carboxylated beads through incubation at 20 µg/ml for 3 h at room temperature on a rotator. Cytokine-coupled beads were washed, stored in blocking buffer (10 mM PBS, 1% BSA, 0.05% NaN_3), then incubated with plasma or serum from patients or controls for 1 h in 96-well filter plates at room temperature in the dark on a shaker. Fluids were aspirated and beads were washed, incubated for 30 min with PE-labelled anti–human IgG-Fc antibody, washed and resuspended in 100 µl PBS/Tween for analysis. Successful coupling of the cytokines to their respective bead sets was verified with specific monoclonal antibodies.

Cytokine release assays were performed following *in vitro* whole blood incubation of patients or contemporaneous healthy controls with stimuli including PHA, as described [44]. Blood samples were diluted 1:5 (Roswell Park Memorial Institute medium) into 96-well F plates and stimulated with PHA; supernatants were analysed after 24 hours for cytokine production compared to unstimulated wells. Cytokine levels were determined by multiplexed particle-based flow cytometry on a Luminex analyser, according to the manufacturer's recommendations (Fluorokine MAP; R&D Systems). All cytokine measurements were standardized on lymphocyte or monocyte counts obtained from full blood count as follows: IFNγ and IL-17A levels were reported as pg/10^6 lymphocytes, and IL-6, TNFα, and IL-1β were reported as pg/10^5 monocytes.

Intracellular cytokine staining for percentage of IL-17$^+$ CD4$^+$ helper T cells, including the CD45RO$^+$ memory population, was measured compared to healthy controls after stimulation with superantigen staphylococcal enterotoxin B (SEB) (directly crosslinking T-cell receptors to major histocompatibility complex II for polyclonal T-cell proliferation and differentiation). Anti-cytokine antibodies were compared

to contemporaneously processed healthy control samples (including IFN-γ, IFN-α, IFN-ω, IFN-β, IL-12, IL-23, GM-CSF, IL-6, IL-17A, IL-17F, IL-22, G-CSF, and IL-8).

Assays were performed in immunology diagnostic UK National Health Service laboratories.

All patients also underwent targeted genetic screening for known or suspected immunodeficiency genes via the Genomics of Rare Immune Disorders (GRID) panel, as described [45]. This panel includes 279 genes described to cause PID, including all those in the 2015 International Union of Immunological Societies classification of PID [46], with notable relevant genes to the interleukin-17 (IL-17) pathway and fungal immunity: *AICDA, AIRE, DOCK8, CD40LG, CD40, CYBA, CYBB, GATA2, IL17A, IL17F, IL17RA, IL17RC, NCF1, NCF2, NCF4, STAT3,* and *UNG*.

Statistical analysis

Data were collated and analysed using GraphPad Prism version 10 (GraphPad Software, Boston, MA, USA). For comparison between patient and control cytokine production, a two-tailed Mann–Whitney test was used.

Results

Case histories

Clinical presentation

Median age at presentation was 43 (range 30-51), with 5 male and 3 female cases. All patients had disseminated cryptococcosis, with symptomatic CNS involvement in 6 of 8 (manifesting as subacute headache with or without fever, vomiting, confusion, visual and hearing loss; MRI brain imaging showed multiple hyperintense white matter lesions with or without evidence of hydrocephalus), two of whom had concomitant lung lesions on imaging. One patient had spinal osteomyelitis with an epidural abscess; another had disseminated abscesses in the lung, liver, bone and muscle. The causative organism *was C. gattii* in one patient presenting with CNS and lung focal lesions- the seven other patients cultured *C. neoformans* from CSF, bone or muscle biopsies. In terms of past medical history, two patients had a history of recurrent childhood bacterial infection (otitis media+/– sinusitis, tonsillitis); one patient had a recent diagnosis of eczema; one patient had a history of mild mixed connective tissue disease not requiring immunosuppressive therapy. The other four patients reported no past medical history.

Treatment and outcome

All eight patients received inpatient treatment with intravenous amphotericin B and flucytosine induction therapy (duration 4–6 weeks), followed by consolidation (8 weeks) then maintenance therapy with fluconazole, according to guidelines [47]. One patient received steroids following induction therapy to manage raised intracranial pressure; steroids had been stopped in this patient prior to immunological evaluation. No other patients received immunosuppressive therapy as part of clinical management.

In total, seven of the eight patients survived, one patient died at 10 months from initial diagnosis due to recurrent, progressive CNS disease despite antifungal therapy. Survivors were followed up in specialist infectious diseases outpatient clinics for a median (range) of 3 years (18 months–10 years), as well as evaluated in specialist Immunology clinics. At last follow-up, three had persistent neurological impairment (leg weakness requiring wheelchair in the spinal osteomyelitis case; cognitive deficit and visual deficit in two with meningitis); four had made a full recovery.

Anonymised clinical case histories are summarized in Table 1.

Basic immunological testing revealed variable evidence of CD4 lymphopaenia in some patients.

Table 2 summarizes the immune investigations for each patient. All patients had negative HIV serologies and none had significant hypogammaglobulinemia.

Four patients were found to have CD4 lymphopaenia with counts ranging from 0.034-0.207 × 10^9/L (normal range 0.3-1.4 × 10^9/L), which persisted following successful treatment and resolution of CM in all three surviving patients. Supplementary figure S1 shows the time from diagnosis of cryptococcosis to immunophenotyping blood tests. One of these patients had a low $CD4^+$ T-cell percentage of total lymphocytes despite lymphocytosis at presentation, with progressive severe panlymphopaenia and CD4 lymphopaenia associated with a protracted, complex clinical course resulting in death. Notably, this patient had persistently absent CD2 expression despite the presence of $CD3^+$ cells, potentially suggestive of a T-cell malignancy or primary T-cell defect. However, investigations for haematological malignancy, including positron emission tomography-computed tomography (PET CT), bone marrow histology and immunophenotyping, did not identify malignancy, and no genetic disorder was identified via the GRID panel (see below). Two patients had significant impairment of T-cell proliferation in the context of CD4 lymphopaenia. Five patients had evidence of at least borderline impairment of T-cell proliferation on one occasion, but not a profound or sustained abnormality.

Four patients had reduced B-cell counts, albeit none less than 70% of the lower limit of normal (Table 2). Where tested, several patients also had abnormalities of B-cell phenotype; the most consistent abnormality being a reduced percentage of IgM^+ memory B cells in four patients (<7.4% total B cells).

Mannose binding lectin levels were within normal limits (>1 mg/L) in 5 patients, borderline (0.98 mg/L) in one and absent in two.

Whole blood cytokine release assay demonstrates impairment of IL-17 release with polyclonal T-cell stimulation.

We proceeded to measure cytokine release in a whole blood stimulation assay [44]. Notably, patients demonstrated reduced IL-17 release in response to broad T-cell stimulation with PHA when compared to contemporaneous healthy control blood, with significantly lower release in the patients overall (median [IQR] IL-17 concentration 88.1 [158.5] pg/mL in patients vs 452.1 [528.0] pg/mL in controls, Mann–Whitney U test p = 0.0047, Figure 1a), albeit one patient's result was in the normal range. Two patients underwent repeat testing during convalescence and the same pattern was observed. Notably, there was no correlation between IL17 production and either CD4 + T-cell count or T-cell proliferation results.

Of note, other patients with invasive cryptococcosis seen over the same period, who were subsequently shown to have

Table 1. Clinical case histories of 8 HIV-uninfected individuals presenting with invasive cryptococcosis.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Presenting symptoms	Severe headache, visual loss	Back pain, leg weakness Received 1y presumptive anti-TB treatment initially	Severe headache, nausea/ vomiting, visual deterioration, hearing loss, leg weakness Received intravenous immunoglobulin initially for presumed AIDP pre-CM diagnosis	Severe headache, nausea/ vomiting, visual deterioration	Severe headache, nausea/ vomiting, fever	Progressive headache, agitation, confusion 4 weeks after cataract operation, cervical + axillary lymphadenopathy Prior treatment for presumed TB lymphadenitis	Haemoptysis, night sweats, weight loss, flu-like symptoms, headache. Received concomitant treatment for presumed TB.	Painful swelling of right thigh
Focus of cryptococcal infection	CNS, lung	Bone (spine) and epidural	CNS, lung, bone	CNS, lung	CNS	CNS, lymph node	CNS, possibly disseminated	Buttock abscess, disseminated to bone, liver, lung
Imaging findings	MRI brain: Ring-enhancing extra-axial lesion. CT Chest: 4cm cavitary lung lesion in the left lower lobe, presumed cryptococcoma	Spinal MRI: multifocal osteomyelitis and epidural abscesses with cord compression and lymphadenitis	MRI brain: multiple small hyperintense foci in white matter of both cerebral hemispheres + focal hyperintensity in the subcortical white matter of left posterior frontal lobe. Chest: lung nodule, presumed cryptococcoma PET CT: right femoral lesion. CNS 7 months post initial treatment: 3 new large masses, mass effect, surrounding oedema (frontal, occipital lobes)	CT Chest: right upper lobe lesion, presumed cryptococcoma MRI brain: 2 months post initial treatment: obstructive hydrocephalus	MRI brain: small patches of white matter abnormality, focal lesions in right internal capsule and left caudate nucleus which enhances. Widened cerebral aqueduct suggesting mild communicating hydrocephalus. Chest: diffuse bronchial wall thickening, patchy limited bibasal peribronchial ground glass change. Subpleural opacity in left upper lobe apex.	MRI brain: obstructive Hydrocephalus	MRI brain: no abnormality CT Chest: apical inflammatory changes CT Abdomen: multiple small intra-abdominal abscesses, abnormal peritoneal thickening + fat-stranding	CT CAP: multiple lesions scattered through lungs and liver.

Table 1. Continued

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Cryptococcal Antigen (CrAg)	Not done	Serum Positive	Not done	CSF positive	CSF positive	Serum + CSF positive	CSF positive	Serum positive
Cryptococcal isolate	*C. gattii*	*C. neoformans*	*C. neoformans*	*C. neoformans*	*C. neoformans*	*C. neoformans*	*C. neoformans*	*C neoformans*
Past medical history including of infection/ autoimmunity/ syndromic association	None	Idiopathic thrombocytopenic purpura (ITP); protracted ear infection and severe varicella in childhood	Eczema	None	Connective tissue disease not requiring immune suppression, childhood otitis media (grommets), tonsillectomy; recurrent sinusitis; recurrent vulvovaginal candidiasis	Intravenous drug use	None	None
Outcome at last visit	At 10 years: Full recovery, relapse free, no features of PAP	At 2.5 years: Ongoing leg weakness and back pain; wheelchair bound.	At 10 months: Deceased	At 1.5 years: returned to work, remained visually impaired	At 6 years: Full recovery, relapse free	At 3 years: on-going neurocognitive deficits	At 2 years: full recovery	At 3.5 years: full recovery

AIDP, acute inflammatory demyelinating polyneuropathy; CM, cryptococcal meningitis; CNS, central nervous system; CrAg, cryprococcal antigen; CSF, cerebrospinal fluid; CT CAP, computed tomography chest abdomen and pelvis; MRI, magnetic resonance imaging; PAP, pulmonary alveolar proteinosis; PET CT, positron emission tomography / computed tomography; TB, tuberculosis.

Table 2. Basic immunological investigations performed for HIV-uninfected patients presenting with disseminated cryptococcosis.

Immune test	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
HIV serology	negative	negative	Negative	negative	Negative	negative	negative	negative
Serum IgA (0.7-4 g/L)	1.1	2.3	1.3	1.5	4.6	1.5	2.2	0.7
Serum IgG (7-16)	**18.4**	**21.7**	**6.1**	8.1	12.8	8.3	**16.2**	**23.4**
Serum IgM (0.4-2.3)	1.3	0.9	1.3	1	0.6	0.8	0.5	**3.4**
Serum MBL (1-4mg/L)	2.25	**>4**	**>4**	3.88	<0.05	**>4**	**0.98**	**<0.05**
Total blood Lymphocyte count (1-2.8 × 10^9/L)	1.635	1.553	**0.7**	1.011	**0.742**	1.0	1.002	1.76
Absolute (%) $CD3^+$ count (0.7-2.1 × 10^9/L) (58-88%)	1.343 (73)	1.125 (73)	**0.100 (19)** CD2 expression 0-0.08 (1)	**0.672** (67)	**0.409 (56)**	0.911 (59)	0.723 (76)	1.36 (77)
Absolute (%) $CD4^+$ count (0.3-1.4 × 10^9/L) (30-62%)	0.741 (36)	0.447 (**29**)	**0.040 (7)**	**0.207 (23)**	**0.034 (5)**	**0.115 (7)**	0.373 (37)	0.750 (42)
Absolute (%) $CD8^+$ count (0.2-0.9 × 10^9/L) (13-42%)	0.579 (34)	0.605 (39)	**0.060 (11)**	0.373 (39)	0.367 (49)	0.840 (**53**)	0.353 (35)	0.61 (34)
Absolute (%) $CD19^+$ count (0.1-0.5 × 10^9/L) (8-20%)	0.118 (8)	0.214 (14)	**0.090** (19)	0.130 (12)	**0.088** (12)	0.526 (35)	**0.088** (10)	**0.070 (3.6)**
Absolute (%) $CD16^+CD56^+$ count (0.09-0.6 × 10^9/L) (5-20%)	0.118 (15)	0.153 (10)	0.290 (61)	0.207 (19)	0.221 (30)	**0.080** (5)	**0.077** (8)	0.330 (19)
CD4/CD8 ratio (1-3.6)	1.28	**0.74**	**0.60**	**0.55**	**0.09**	**0.10**	1.06	1.23
B-cell phenotyping	Reduced IgM memory cells. Reduced plasmablasts. High % CD21-CD38- B-cells	Reduced IgM memory B-cells. Reduced plasmablasts. Increased transitional B-cells	N/A	N/A	Reduced IgM memory B-cells. Reduced switched memory B-cells.	N/A	Reduced IgM memory B-cells. Increased transitional B-cells.	No significant abnormalities
TH17 phenotyping (≥0.4% $CD4^+$ $CD45RO^+$ cells)	0.4% $CD4^+$ CD45RO + $IL\text{-}17^+$ cells (average of 2 separate assays)	0.25% $CD4^+$ $CD45RO^+$ $IL\text{-}17^+$ cells (average of 2 separate assays)	N/A	0.2% $CD4^+$ $CD45RO^+$ $IL\text{-}17^+$ cells	0.06% $CD4^+$ $CD45RO^+$ $IL\text{-}17^+$ cells	N/A	N/A	0.1% $CD4^+$ $CD45RO^+$ $IL\text{-}17^+$ cells

Table 2. Continued

Immune test	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
T-cell stimulation assays	Borderline low response to anti-CD3 stimulation but appropriate boosting with anti-CD28. Good PHA response.	Initial impaired T-cell proliferation to anti-CD3 stimulation but normal on repeat. Good PHA response.	Severely impaired T-cell activation with PHA.	Initial borderline low response to PHA stimulation (improved on repeat) but good response to anti-CD3.	Significantly impaired T-cell proliferation with anti-CD3 or PHA stimulation	Normal T-cell proliferation to PHA and anti-CD3 stimulation.	Normal T-cell proliferation to PHA stimulation, slightly impaired to anti-CD3 stimulation	Impaired proliferation following anti-CD3 and anti-CD3/CD28 stimulation; normal PHA stimulation
Cytokine release assays (except IL-17)	Reduced IFN-γ production with polyclonal T-cell stimulation	No other abnormalities	Severely Impaired response to polyclonal T-cell stimulation with reduced IFN-γ production	Globally low response to β-D glucan but good IFN-γ production with polyclonal T-cell stimulation	Slightly reduced responses (including IFN-γ) to polyclonal T-cell stimulation	Reduced IFN-γ production with polyclonal T-cell stimulation on one of two occasions.	No other abnormalities	Reduced IL-2 and IL-10 and increased IFN-γ, TNF-α and IL-6 with polyclonal T-cell stimulation.
Auto-antibodies	Positive anti-GM-CSF antibodies	No anti-cytokine antibodies	No anti-cytokine antibodies	No anti-cytokine antibodies	No anti-cytokine antibodies Positive anti-nuclear and anti- ribonucleoprotein antibodies.	No anti-cytokine antibodies Weak positive anti-smooth muscle antibody.	No anti-cytokine antibodies	No anti-cytokine antibodies
Targeted genetic sequencing	No causative mutations identified	No causative mutations identified	No causative mutations identified	No causative mutations identified	No causative mutations identified	No causative mutations identified	No causative mutations identified	No causative mutations identified
Final immunodeficiency diagnosis	Anti GM-CSF antibody Impaired IL-17 response	Impaired IL-17 response	ICL Impaired IL-17 response	ICL Impaired IL-17 response	ICL MBL deficiency Impaired IL-17 response	ICL Impaired IL-17 response	No clear immune deficiency	Impaired IL-17 response MBL deficiency Low B-cells

GM-CSF, granulocyte-macrophage colony-stimulating factor; HIV, Human immunodeficiency virus; ICL, idiopathic CD4 lymphopaenia; IgA/IgG/IgM, Immunoglobulin A/G/M; IFN, interferon; IL, interleukin; MBL, Mannose binding lectin; PHA, phytohaemagglutinin; TNF, tumour necrosis factor. **Bold** = abnormal value.

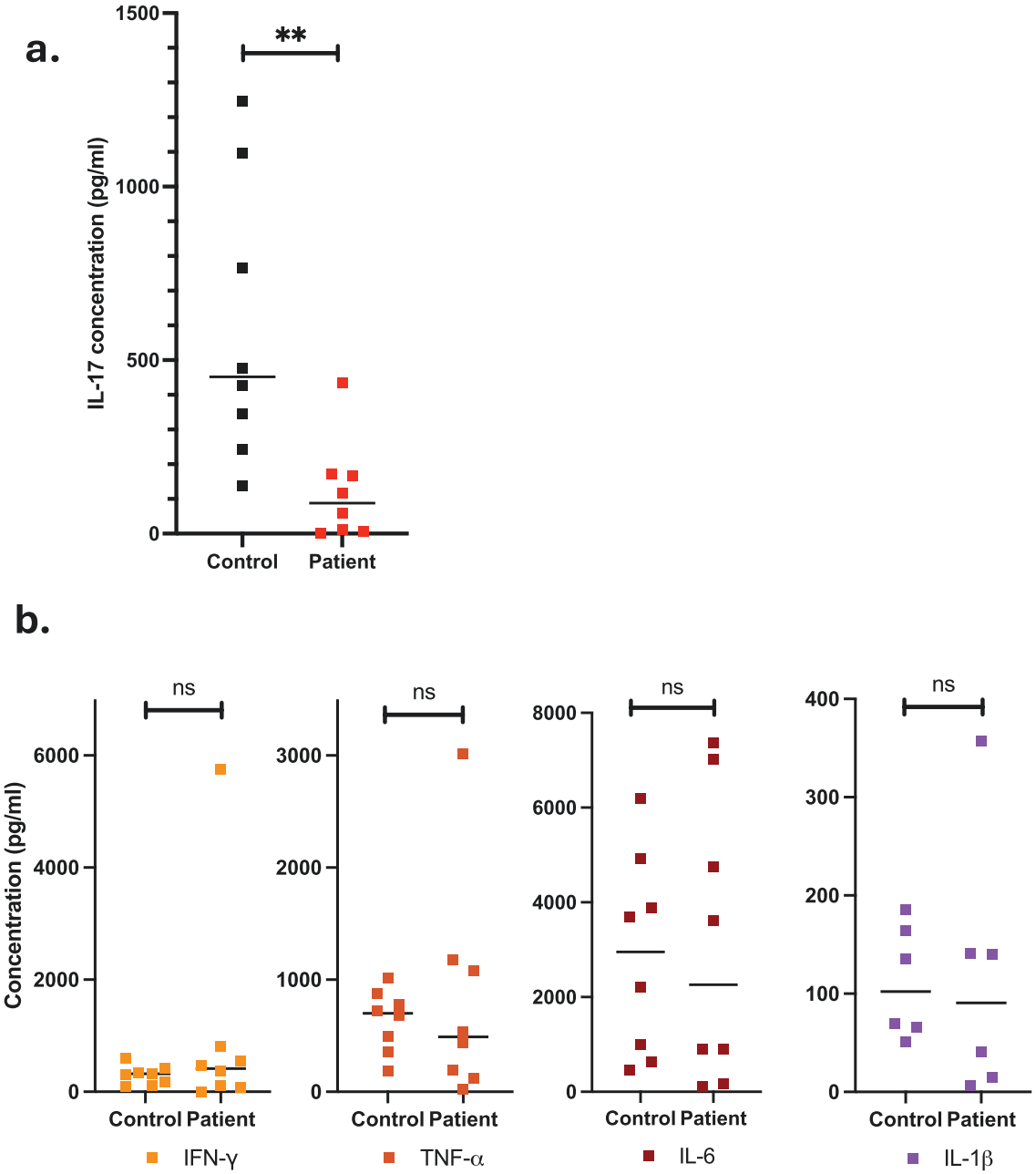


Figure 1: A. Concentration of IL-17 (pg/ml) in supernatants of whole blood from eight patients with cryptococcosis and contemporaneously processed controls following stimulation with PHA. B. Concentration of IFN-γ, TNF-α, IL-6, and IL-1β in supernatants of whole blood from eight patients with cryptococcosis and contemporaneously processed controls following stimulation with PHA. *Key*: **: $P < 0.005$ Mann–Whitney *U* test; ns = not significant Mann–Whitney *U* test.

clearly defined primary or secondary immune deficiency syndromes that would cause susceptibility to cryptococcosis and therefore not reported in detail in this series, also had markedly reduced IL-17 production in the same range as the patients presented here, without other marked abnormalities of cytokine levels or other immunological tests (Supplementary Figure S2).

In addition, individual patients in our cohort demonstrated further patterns of abnormality, the most common being reduced IFN-γ production (patients 1, 3, 5, and 6), including two specifically in response to the fungal cell wall antigen β-D-glucan. Nevertheless, there was significant heterogeneity across the cohort, and no overall difference between patients and controls in the measured concentrations of IFN-γ, TNF-α, IL-6 and IL-1β (the latter measured in six patients) following PHA stimulation (Figure 1b).

In view of the apparent reduction in IL-17 release in the whole blood assay, we also determined the percentage of $CD4^+$ T-cells positive for IL-17 after stimulation via flow cytometry in five of eight patients. Four patients demonstrated results below the lower end of the reference range (≥ 0.4% $CD4^+$ memory $CD45RO^+$ cells), with one patient with a borderline low result of 0.4% (Table 2). Supplementary Figure S3 shows representative FACS plots from a healthy control compared

to a patient with a low percentage of CD4+ CD45RO+ T cells expressing IL-17.

Anti-GM-CSF antibodies were only seen in association with *C. gattii* infection

Analysis of anti-cytokine antibodies revealed high titres of anti-GM-CSF autoantibody in the patient with *C. gattii* infection, with no clinical features of pulmonary alveolar proteinosis developing to date. All other patients were negative for autoantibodies to Th1/TH17 cytokines, type I/II interferons and GM-CSF.

Targeted next-generation sequencing

We performed next-generation sequencing of 279 genes known to be associated with primary immunodeficiency on the GRID panel, including notable relevant genes to the interleukin-17 (IL-17) pathway and fungal immunity: *AICDA, AIRE, DOCK8, CD40LG, CD40, CYBA, CYBB, GATA2, IL17A, IL17F, IL17RA, IL17RC, NCF1, NCF2, NCF4, STAT3,* and *UNG* [45] in all patients. No known causative mutations were identified in these eight patients.

Discussion

Cryptococcosis is a serious disease, occurring usually in the context of advanced HIV, severe primary immunodeficiency, or significant iatrogenic immunosuppression. We herein describe eight apparently healthy adults with disseminated disease in the absence of an identified syndrome associated with a risk of fungal infection. Immunologically, the most consistent finding was reduced IL-17 release following polyclonal T-cell stimulation, observed on two separate assays. This reduced IL-17 production was not simply due to reduced CD4+ T-cell numbers (seen in four of eight participants), as demonstrated by the reduced *proportion* of T-helper cells expressing IL-17 on flow cytometry.

This finding is consistent with other observations in patients with invasive cryptococcosis who have a well-recognized risk for opportunistic infection, genetic or otherwise [33–35, 38, 39, 48]], and with results from patients seen at our centres over the same time period with known risks. Impaired IL-17 release or response is a recognized feature of Hyper IgE Syndrome, APECED, STAT1 gain-of-function and mutations directly within the IL-17 gene or its receptor [33, 34, 39, 41]. These disorders are associated with increased risk of fungal infection, with predominant manifestations being chronic mucocutaneous candidiasis, (in part due to the ubiquitous colonization of human skin and mucosa with candida), as opposed to disseminated fungal infections or cryptococcosis. Still, whilst rare, cryptococcosis has been described in patients with Hyper-IgE syndromes [35–37], STAT1 gain-of-function [38–40], and in individuals receiving therapeutic anti-IL-17 monoclonal antibodies [24, 25]. This strongly suggests that impairment of the IL-17 pathway is a risk factor for cryptococcal disease. The discovery of underlying conditions in patients presenting with disseminated cryptococcal infection underlies the importance of specialist immunological work-up.

Patients presented in this cohort had no significant clinical features of primary immune deficiencies associated with chronic mucocutaneous candidiasis. Nonetheless, the reduction in IL-17 production within this cohort was as marked as those with PID associated with increased susceptibility to fungal infection (Supplementary Figure S2), and was the most consistent immunological finding. Normal levels of IL-6 suggest that signalling driving the differentiation of Th17 cells for IL-17 production is likely to be intact [49, 50]. We therefore hypothesise that a reduction in IL-17+ CD4+ T-helper cells directly increases the risk of invasive cryptococcal disease, for example by compromising IL-17-mediated activation of the innate lung mucosal immunity; the primary site of exposure to *Cryptococcus* [51].

However, we acknowledge that impaired IL-17 release may not have been the only risk, and several individuals demonstrated additional immunological abnormalities. IFN-γ release was reduced in some patients after polyclonal stimulation of whole blood. Notably, two individuals demonstrated impaired release of IFN-γ and other cytokines in the whole blood assay in response to β-D-glucan. This raises the possibility of a specific failure to respond to fungal antigens. Deficiencies of IFN-γ or its receptor, or autoantibodies against this cytokine, usually predispose to mycobacterial infection [52]; however, invasive fungal disease is also described [53].

Four patients had a reduction in circulating CD4+ T cells, and T-cell proliferation was significantly reduced in several (especially following anti-CD3/CD28 stimulation). Idiopathic CD4 lymphopaenia is a poorly understood condition with a variable phenotype, and some individuals do suffer from opportunistic infections, including cryptococcosis [16, 17, 54]. We believe that the degree of CD4+ lymphopaenia in two of these individuals, those with severely impaired proliferation on stimulation with PHA, was sufficiently low to have contributed to the risk of cryptococcal infection.

One patient with *C. gattii* infection tested positive for anti-GM-CSF antibodies, a previously described risk factor for infection with this organism [27]. Interestingly, this patient also had impaired IL-17 release, suggesting two distinct immunological susceptibilities. Whilst *C. neoformans* is typically seen in HIV-infected or otherwise immunocompromised patients and *C. gattii* in immunocompetent patients, there is considerable overlap in immunopathogenesis, including a role for Th1 and Th17 cells in both species [55, 56]. *C. gattii* infection is even rarer than *C. neoformans*; immune profiling of individuals with this remains poorly characterized [55, 56]. Patients with *C. neoformans* did not have detectable anti-cytokine antibodies.

Undetectable mannose-binding lectin was observed in two patients. This is also a known risk factor for cryptococcal infection [32], but as mannose-binding lectin deficiency is common [57] and usually clinically mild or silent, we consider this to be a minor contributor to immunological risk [58].

Reduced percentage of IgM+ memory B-cells was a common feature where assessed; this has been implicated as a risk factor for cryptococcal disease in patients with and without HIV [15, 28]. Total circulating immunoglobulin levels (including IgM) were not decreased in our patients, as also seen in HIV-infected and uninfected cohorts previously described [15, 28].

We obtained DNA from most patients and interrogated sequencing data for evidence of mutations in known genes associated with primary immunodeficiency [45], including those known to affect IL-17, but this cohort of eight patients had no known pathogenic causative mutations. One patient with persistently low CD4+ T-cell percentage had absent CD2 expression despite the presence of CD3+ T-cells, in the absence

of identifiable malignancy. This highlights the possibility of an as-yet-unknown genetic disorder causing primary T-cell immune deficiency in this patient. It remains possible that undescribed genetic disorders, polygenic causes, or epigenetic phenomena may explain the abnormalities seen in these patients; our cohort is too small to interrogate this.

There are some limitations to our findings. Our cohort is small, reflecting the rarity of cryptococcosis in the UK. In this context, the cohort was not systematically recruited but relied on referrals to a specialist London Immunology centre within a limited geographic area. Investigations were determined on clinical grounds and therefore not every test was performed on every patient; healthy controls were recruited contemporaneously and opportunistically for laboratory diagnostic tests as per standard practice; and were therefore not necessarily matched with cases in terms of demographics. Some patients were further into convalescence from the acute infection than others at the time of testing, albeit this is likely to make the consistency of findings on the IL-17 assay more robust and reduce the probability that the findings were a consequence rather than cause. Unfortunately, it was not possible in this real-world setting to obtain samples at a standardized time point following infection. In the two patients who underwent repeat testing, these results remained consistent. We cannot prove definitively that reduced IL-17 release was a cause rather than a consequence of the infection, but the universality of the finding and stability over time, including in those who had recovered from infection, suggests the former. The genetic panel used at the time the patients were seen only included 279 genes compared to nearly 500 now identified, and we cannot therefore definitively exclude monogenic disease.

Collectively, our results indicate that individual apparently immunocompetent patients who develop cryptococcosis may have several contributing and interacting immunological risk factors for cryptococcal infection, including $CD4^+$ lymphopaenia, reduced numbers of memory IgM^+ B-cells, impaired IFN-γ production and anti-GM-CSF antibodies (*C. gattii infection*). Our findings suggest that reduced IL-17 release appears to be an additional key susceptibility element. These findings align with current knowledge on anti-cryptococcal immunity but require further validation in a larger prospective cohort with standardized inclusion criteria. Monitoring of individuals on anti-IL17 monoclonal antibodies may also provide valuable insight.

Importantly, although some of these abnormalities may be demonstrated on routine laboratory tests (e.g. lymphopaenia), most would only be detected by specialized assays, making it difficult to identify in advance people who may be vulnerable to cryptococcal infection. We therefore advocate that all apparently immunocompetent patients presenting with cryptococcal infection should be referred for further evaluation and investigation by an immunologist.

Supplementary data

Supplementary data are available at *Clinical and Experimental Immunology* online.

Acknowledgments

Not applicable.

Author Contributions

Katie Townsend (Conceptualization, Data curation, Formal analysis, Methodology, Writing - original draft, Writing - review & editing), Shichina Kannambath (Conceptualization, Data curation, Writing - original draft), Grant Hayman (Data curation), Rainer Doffinger (Data curation, Formal analysis), Lourdes Ceron (Data curation), Soraya Ebrahimi (Data curation), Vlada Pavlova (Data curation), Philip Gothard (Data curation), Michael Brown (Data curation), Fariba Tahami (Data curation, Investigation), Dakshika Jayaratnam (Data curation), Anna Goodman (Data curation, Writing - review & editing), Derek Macallan (Data curation, Writing - review & editing), Thomas Harrison (Data curation), Tanaraj Perinpanathan (Data curation), Laurence John (Data curation), Neil Stone (Data curation), Tihana Bicanic (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing - original draft, Writing - review & editing) and David M Lowe (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing - original draft, Writing - review & editing.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Conflict of Interest

T.B. has received speaker fees, advisory board fees and research fellowship funding from Gilead Sciences, speaker and advisory board fees from Mundipharma and research grants from Pfizer and MSD. D.M.L has received personal fees from Gilead for an educational video and from Merck for a roundtable discussion, speaker fees from Biotest, Takeda and Astra-Zeneca and support to attend a conference from Octapharma. D.M.L. also holds research grants from GSK and Bristol Myers Squibb and has received consultancy fees from GSK paid to his institution, all outside the current work. All other contributing authors (K.T, S.K, G.H, R.D, L.C-G, S.E, P.G., M.B, F.T, D.J, A.L.G, D.M, T.S.H, M.D., J.L, T.P, L.J., and N.S.) have no conflicts of interest to disclose.

Ethical Approval

This study was performed in line with the principles of the Declaration of Helsinki. This is an observational study. Detailed immunophenotyping and immunogenetic assessments were performed prospectively as part of routine patient clinical management and care following referral for specialist Immunologist assessment. Laboratory healthy control samples were collected according to diagnostic laboratory standard operating procedures, from healthy adults with informed consent (Research Ethics Committee [REC] approval reference numbers 04/Q0501/119 and 06/Q0508/16). Patients were additionally counselled and written consent obtained before undergoing genetic testing (ethical approval from East of England Cambridge South National REC reference numbers 13/EE/0325). A fully anonymised case series was established to collate data on clinical outcomes for this rare cohort; thus, further ethical approval was not required as per current UK Health Research Authority (HRA) guidance.

Permission to Reproduce

Graphical abstract created in BioRender. Townsend, K. (2025) https://app.biorender.com/citation/675afb9bface9e805eaa34db

Data Availability

The data that support the findings of this study are available from the corresponding author D.M.L, upon reasonable request.

References

1. Chang CC, Harrison TS, Bicanic TA, Chayakulkeeree M, Sorrell TC, Warris A, et al. Global guideline for the diagnosis and management of cryptococcosis: an initiative of the ECMM and ISHAM in cooperation with the ASM. Lancet Infect Dis 2024, **24**, e495–512. doi:10.1016/S1473-3099(23)00731-4
2. Goldman DL, Khine H, Abadi J, Lindenberg DJ, Pirofski L, Niang R, et al. Serologic evidence for Cryptococcus neoformans infection in early childhood. Pediatrics 2001, **107**, E66. doi: 10.1542/peds.107.5.e66
3. Rajasingham R, Govender NP, Jordan A, Loyse A, Shroufi A, Denning DW, et al. The global burden of HIV-associated cryptococcal infection in adults in 2020: a modelling analysis. Lancet Infect Dis 2022, **22**, 1748–55. doi:10.1016/S1473-3099(22)00499-6
4. Husain S, Camargo JF. Invasive aspergillosis in solid-organ transplant recipients: guidelines from the american society of transplantation infectious diseases community of practice. Clin Transplant 2019, **33**, e13544. doi:10.1111/ctr.13544
5. George IA, Spec A, Powderly WG, Santos CAQ. Comparative Epidemiology and Outcomes of Human Immunodeficiency virus (HIV), non-HIV non-transplant, and solid organ transplant associated cryptococcosis: a population-based study. Clin Infect Diseases 2018, **66**, 608–11. doi:10.1093/cid/cix867
6. Nguyen MH, Husain S, Clancy CJ, Peacock JE, Hung CC, Kontoyiannis DP, et al. Outcomes of central nervous system cryptococcosis vary with host immune function: results from a multicenter, prospective study. J Infect 2010, **61**, 419–26. doi:10.1016/j.jinf.2010.08.004
7. Brizendine KD, Baddley JW, Pappas PG. Predictors of mortality and differences in clinical features among patients with cryptococcosis according to immune status. PLoS One 2013, **8**, e60431. doi:10.1371/journal.pone.0060431
8. Pyrgos V, Seitz AE, Steiner CA, Prevots DR, Williamson PR. Epidemiology of cryptococcal meningitis in the US: 1997-2009. PloS One 2013, **8**, e56269. doi: 10.1371/journal.pone.0056269
9. Jarvis JN, Casazza JP, Stone HH, Meintjes G, Lawn SD, Levitz SM, et al. The phenotype of the Cryptococcus-specific CD4+ memory T-cell response is associated with disease severity and outcome in HIV-associated cryptococcal meningitis. J Infect Dis 2013, **207**, 1817–28. doi:10.1093/infdis/jit099
10. Ssebambulidde K, Anjum SH, Hargarten JC, Chittiboina P, Shoham S, Seyedmousavi S, et al. Treatment recommendations for non-HIV associated cryptococcal meningoencephalitis including management of post-infectious inflammatory response syndrome. Front Neurol 2022, **13**, 994396. doi:10.3389/fneur.2022.994396
11. Anjum S, Dean O, Kosa P, Magone MT, King KA, Fitzgibbon E, et al. Outcomes in previously healthy cryptococcal meningoencephalitis patients treated with pulse taper corticosteroids for post-infectious inflammatory syndrome. Clin Infect Dis 2021, **73**, e2789–98. doi:10.1093/cid/ciaa1901
12. Jarvis JN, Meintjes G, Rebe K, Williams GN, Bicanic T, Williams A, et al. Adjunctive interferon-gamma immunotherapy for the treatment of HIV-associated cryptococcal meningitis: a randomized controlled trial. AIDS 2012, **26**, 1105–13. doi:10.1097/QAD.0b013e3283536a93
13. Jarvis JN, Meintjes G, Bicanic T, Buffa V, Hogan L, Mo S, et al. Cerebrospinal fluid cytokine profiles predict risk of early mortality and immune reconstitution inflammatory syndrome in HIV-associated cryptococcal meningitis. PLoS Pathog 2015, **11**, e1004754. doi:10.1371/journal.ppat.1004754
14. Normile TG, Rella A, Del Poeta M. Cryptococcus neoformans Δsgl1 Vaccination Requires Either CD4+ or CD8+ T-cells for complete host protection. Front Cell Infect Microbiol 2021, **11**, 739027. doi:10.3389/fcimb.2021.739027
15. Subramaniam K, Metzger B, Hanau LH, Guh A, Rucker L, Badri S, et al. IgM(+) memory B-cell expression predicts HIV-associated cryptococcosis status. J Infect Dis 2009, **200**, 244–51. doi:10.1086/599318
16. Ho DD, Cao Y, Zhu T, Farthing C, Wang N, Gu G, et al. Idiopathic CD4+ T-lymphocytopenia--immunodeficiency without evidence of HIV infection. N Engl J Med 1993, **328**, 380–5. doi:10.1056/NEJM199302113280602
17. Smith DK, Neal JJ, Holmberg SD. Unexplained opportunistic infections and CD4+ T-lymphocytopenia without HIV infection. An investigation of cases in the United States. The Centers for Disease Control Idiopathic CD4+ T-lymphocytopenia Task Force. N Engl J Med 1993, **328**, 373–9. doi:10.1056/NEJM199302113280601
18. Wozniak KL, Hardison SE, Kolls JK, Wormley FL. Role of IL-17A on resolution of pulmonary C. neoformans infection. PLoS One 2011, **6**, e17204. doi:10.1371/journal.pone.0017204
19. Valdez PA, Vithayathil PJ, Janelsins BM, Shaffer AL, Williamson PR, Datta SK. Prostaglandin E2 suppresses antifungal immunity by inhibiting interferon regulatory factor 4 function and interleukin-17 expression in T cells. Immunity 2012, **36**, 668–79. doi:10.1016/j.immuni.2012.02.013
20. Movahed E, Cheok YY, Tan GMY, Lee CYQ, Cheong HC, Velayuthan RD, et al. Lung-infiltrating T helper 17 cells as the major source of interleukin-17A production during pulmonary Cryptococcus neoformans infection. BMC Immunol 2018, **19**, 32. doi:10.1186/s12865-018-0269-5
21. LaRocque-de-Freitas IF, Rocha JDB, Nunes MP, Oliveira PAV, Nascimento DO, Freire-de-Lima L, et al. Involvement of the capsular GalXM-induced IL-17 cytokine in the control of Cryptococcus neoformans infection. Sci Rep 2018, **8**, 16378. doi:10.1038/s41598-018-34649-4
22. Murdock BJ, Huffnagle GB, Olszewski MA, Osterholzer JJ. Interleukin-17A enhances host defense against cryptococcal lung infection through effects mediated by leukocyte recruitment, activation, and gamma interferon production. Infect Immun 2014, **82**, 937–48. doi:10.1128/IAI.01477-13
23. Guo X, Mao X, Tian D, Liao Y, Su B, Ye C, et al. Cryptococcus neoformans infection induces IL-17 production by promoting STAT3 phosphorylation in CD4+ T Cells. Front Immunol 2022, **13**, 872286. doi:10.3389/fimmu.2022.872286
24. Driver J, Zaayman M, Silfvast-Kaiser A, Menter A. Cryptococcal meningitis associated with interleukin-17 inhibitor use for psoriasis. Dermatol Ther 2022, **35**, e15609. doi:10.1111/dth.15609
25. The United States Food and Drug Administration. Siliq (brodalumab) Label. https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/761032lbl.pdf
26. Browne SK, Burbelo PD, Chetchotisakd P, Suputtamongkol Y, Kiertiburanakul S, Shaw PA, et al. Adult-onset immunodeficiency in Thailand and Taiwan. N Engl J Med 2012, **367**, 725–34. doi:10.1056/NEJMoa1111160
27. Saijo T, Chen J, Chen SC, Rosen LB, Yi J, Sorrell TC, et al. Anti-granulocyte-macrophage colony-stimulating factor autoantibodies are a risk factor for central nervous system infection by Cryptococcus gattii in otherwise immunocompetent patients. mBio 2014, **5**, e00912–14. doi:10.1128/mBio.00912-14
28. Rohatgi S, Nakouzi A, Carreño LJ, Slosar-Cheah M, Kuniholm MH, Wang T, et al. Antibody and B Cell subset perturbations in human immunodeficiency virus-uninfected patients with cryptococcosis. Open Forum Infect Dis 2017, **5**, ofx255. doi:10.1093/ofid/ofx255

29. Meletiadis J, Walsh TJ, Choi EH, Pappas PG, Ennis D, Douglas J, et al. Study of common functional genetic polymorphisms of FCGR2A, 3A and 3B genes and the risk for cryptococcosis in HIV-uninfected patients. Med Mycol 2007, **45**, 513–8. doi:10.1080/13693780701390140
30. Hu XP, Wu JQ, Zhu LP, Wang X, Xu B, Wang RY, et al. Association of Fcgamma receptor IIB polymorphism with cryptococcal meningitis in HIV-uninfected Chinese patients. PLoS One 2012, **7**, e42439. doi:10.1371/journal.pone.0042439
31. Rohatgi S, Gohil S, Kuniholm MH, Schultz H, Dufaud C, Armour KL, et al. Fc gamma receptor 3A polymorphism and risk for HIV-associated cryptococcal disease. mBio 2013, **4**, e00573–13. doi:10.1128/mBio.00573-13
32. Ou XT, Wu JQ, Zhu LP, Guan M, Xu B, Hu XP, et al. Genotypes coding for mannose-binding lectin deficiency correlated with cryptococcal meningitis in HIV-uninfected Chinese patients. J Infect Dis 2011, **203**, 1686–91. doi:10.1093/infdis/jir152
33. Olbrich P, Vinh DC. Inborn errors of immunity causing pediatric susceptibility to fungal diseases. J Fungi 2023, **9**, 149. doi:10.3390/jof9020149
34. Haapaniemi EM, Kaustio M, Rajala HL, van Adrichem AJ, Kainulainen L, Glumoff V, et al. Autoimmunity, hypogammaglobulinemia, lymphoproliferation, and mycobacterial disease in patients with activating mutations in STAT3. Blood 2015, **125**, 639–48. doi:10.1182/blood-2014-04-570101
35. Odio CD, Milligan KL, McGowan K, Rudman Spergel AK, Bioshop R, et al. Endemic mycoses in patients with STAT3-mutated hyper-IgE (Job) syndrome. J Allergy Clin Immunol 2015, **136**, 1411–3. doi:10.1016/j.jaci.2015.07.003
36. Nierenberg RJ, Devasagayaraj R, Nguyen H, Craciun L, Chalabi D, Nguyen H, et al. Hyper-Immunoglobulin E syndrome and cryptococcal meningitis leading to a fatal outcome in a young woman: a rare and lethal combination. Ann Clin Case Rep 2020, **5**, 1903.
37. Garty BZ, Wolach B, Ashkenazi S, Weismart Y, Rachmel A, Nitzan M. Cryptococcal meningitis in a child with hyperimmunoglobulin E syndrome. Pediatr Allergy Immunol 1995, **6**, 175–7. doi:10.1111/j.1399-3038.1995.tb00279.x
38. Marinelli L, Ristagno E, Fischer P, Abraham R, Joshi A. Cryptococcal pneumonia in an adolescent with a gain-of-function variant in signal transduction and activator of transcription 1 (STAT1). BMJ Case Rep 2020, **13**, e234120. doi:10.1136/bcr-2019-234120
39. Toubiana J, Okada S, Hiller J, Oleastro M, Lagos Gomez M, Aldave Becerra JC, et al.; International STAT1 Gain-of-Function Study Group. Heterozygous STAT1 gain-of-function mutations underlie an unexpectedly broad clinical phenotype. Blood 2016, **127**, 3154–64. doi:10.1182/blood-2015-11-679902
40. Zhang W, Chen X, Gao G, Xing S, Zhou L, Tang X, et al. Clinical relevance of gain- and loss-of-function germline mutations in STAT1: a systematic review. Front Immunol 2021, **12**, 654406. doi:10.3389/fimmu.2021.654406
41. Lionakis MS, Drummond RA, Hohl TM. Immune responses to human fungal pathogens and therapeutic prospects. Nat Rev Immunol 2023, **23**, 433–52. doi:10.1038/s41577-022-00826-w
42. Jiao J, Zhao X, Hou R, Wang Y, Chang W, Liang N, et al. Comparison of two commonly used methods for stimulating T cells. Biotechnol Lett 2019, **41**, 1361–71. doi:10.1007/s10529-019-02743-w
43. Puel A, Döffinger R, Natividad A, Chrabieh M, Barcenas-Morales G, Picard C, et al. Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. J Exp Med 2010, **207**, 291–7. doi:10.1084/jem.20091983
44. Colombo SAP, Hashad R, Denning DW, Kumararatne DS, Ceron-Gutierrez L, Barcenas-Morales G, et al. Defective interferon-gamma production is common in chronic pulmonary aspergillosis. J Infect Dis 2022, **225**, 1822–31. doi:10.1093/infdis/jiab583
45. Simeoni I, Shamardina O, Deevi SV, Thomas M, Megy K, Staples E, et al. GRID – Genomics of Rare Immune Disorders: a highly sensitive and specific diagnostic gene panel for patients with primary immunodeficiencies. bioRxiv 2019, **431544**. doi: 10.1101/431544
46. Bousfiha A, Jeddane L, Al-Herz W, Ailal F, Casanova JL, Chatila T, et al. The 2015 IUIS phenotypic classification for primary immunodeficiencies. J Clin Immunol 2015, **35**, 727–38. doi:10.1007/s10875-015-0198-5
47. Perfect JR, Dismukes WE, Dromer F, Goldman DL, Graybill JR, Hamill RJ, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of America. Clin Infect Dis 2010, **50**, 291–322. doi:10.1086/649858
48. Eyerich K, Foerster S, Rombold S, Seidl HP, Behrendt H, Hofmann H, et al. Patients with chronic mucocutaneous candidiasis exhibit reduced production of Th17-associated cytokines IL-17 and IL-22. J Invest Dermatol 2008, **128**, 2640–5. doi:10.1038/jid.2008.139
49. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 2006, **441**, 235–8. doi:10.1038/nature04753
50. Harbour SN, DiToro DF, Witte SJ, Zindl CL, Gao M, Schoeb TR, et al. T_H17 cells require ongoing classic IL-6 receptor signaling to retain transcriptional and functional identity. Sci Immunol 2020, **5**, eaaw2262. doi:10.1126/sciimmunol.aaw2262
51. Gurczynski SJ, Moore BB. IL-17 in the lung: the good, the bad, and the ugly. Am J Physiol Lung Cell Mol Physiol 2018, **314**, L6–L16. doi:10.1152/ajplung.00344.2017
52. Lake MA, Ambrose LR, Lipman MC, Lowe DM. '"Why me, why now?" Using clinical immunology and epidemiology to explain who gets nontuberculous mycobacterial infection. BMC Med 2016, **14**, 54. doi:10.1186/s12916-016-0606-6
53. Smith NL, Denning DW. Clinical implications of interferon-gamma genetic and epigenetic variants. Immunology 2014, **143**, 499–511. doi:10.1111/imm.12362
54. Yarmohammadi H, Cunningham-Rundles C. Idiopathic CD4 lymphocytopenia: pathogenesis, etiologies, clinical presentations and treatment strategies. Ann Allergy Asthma Immunol 2017, **119**, 374–8. doi:10.1016/j.anai.2017.07.021
55. Elsegeiny W, Marr KA, Williamson PR. Immunology of cryptococcal infections: developing a rational approach to patient therapy. Front Immunol 2018, **9**, 651. doi:10.3389/fimmu.2018.00651
56. Yang DH, England MR, Salvator H, Anjum S, Park YD, Marr KA, et al. Cryptococcus gattii species complex as an opportunistic pathogen: underlying medical conditions associated with the infection. mBio 2021, **12**, e0270821. doi:10.1128/mBio.02708-21
57. Thiel S, Frederiksen PD, Jensenius JC. Clinical manifestations of mannan-binding lectin deficiency. Mol Immunol 2006, **43**, 86–96. doi:10.1016/j.molimm.2005.06.018. PMID: 16023210CIDPMC7132399.
58. Eisen DP, Dean MM, O'Sullivan MV, Heatley S, Minchinton RM. Mannose-binding lectin deficiency does not appear to predispose to cryptococcosis in non-immunocompromised patients. Med Mycol 2008, **46**, 371–5. doi:10.1080/13693780701874515