Received: 31 March 2021 | Revised: 6 August 2021 | Accepted: 7 August 2021

DOI: 10.1111/myc.13361

ORIGINAL ARTICLE

mycoses Diagnosis, Therapy and Prophylaxis of Fungal Diseases WILEY

Immune reconstitution inflammatory syndrome in non-HIV cryptococcal meningitis: Cross-talk between pathogen and host

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Funding information
National Natural Science Foundation of China, Grant/Award Number: 81571968 and 81971911

Abstract

Background: Cryptococcal meningitis (CM)-associated immune reconstitution inflammatory syndrome (IRIS) is associated with high mortality, the epidemiology and pathophysiology of which is poorly understood, especially in non-HIV populations.

Objectives: We aim to explore the incidence, clinical risk factors, immunological profiles and potential influence of leukotriene A4 hydroxylase (*LTA4H*) on non-HIV CM IRIS populations.

Methods: In this observational cohort study, 101 previously untreated non-HIV CM patients were included. We obtained data for clinical variables, 27 cerebrospinal fluid (CSF) cytokines levels and *LTA4H* genotype frequencies. Changes of CSF cytokines levels before and at IRIS occurrence were compared.

Results: Immune reconstitution inflammatory syndrome was identified in 11 immunocompetent males, generating an incidence of 10.9% in non-HIV CM patients. Patients with higher CrAg titres (> 1:160) were more likely to develop IRIS, and titre of 1:1280 is the optimum level to predict IRIS occurrence. Baseline CSF cytokines were significantly higher in IRIS group, which indicated a severe host immune inflammation response. Four *LTA4H* SNPs (rs17525488, rs6538697, rs17525495 and rs1978331) exhibited significant genetic susceptibility to IRIS in overall non-HIV CM, while five cytokines were found to be associated with rs1978331, and baseline monocyte chemotactic protein 1 (MCP-1) became the only cytokine correlated with both IRIS and *LTA4H* SNPs.

Conclusions: Our study suggested that non-HIV CM patients with high fungal burden and severe immune inflammation response were more likely to developed IRIS. *LTA4H* polymorphisms may affect the pathogenesis of IRIS by regulating the level of baseline CSF MCP-1.

Ling-Hong Zhou and Hua-Zhen Zhao contributed equally to this article.

KEYWORDS
chemokines, cryptococcal meningitis, immune reconstitution inflammatory syndrome, *LTA4H*, non-HIV patients

1 | INTRODUCTION

Cryptococcal meningitis (CM) is a leading cause of mortality in HIV-infected patients.[1] Though commonly seen as an opportunistic infection in HIV/AIDS, an increasing number of non-HIV CM patients have been reported with fatality approaching 30% in some areas.[2,3] Mortality associated with this condition is affected by cryptococcosis-associated immune reconstitution inflammatory syndrome (IRIS), with up to 36% HIV CM patients dead from this syndrome.[4] In fact, IRIS or IRIS-like entity, described as an paradoxical clinical worsening after giving antifungal therapy and achieving negative CSF cultures, were also recognised in non-HIV immunocompromised patients and even immunocompetent CM patients. The pathophysiology of IRIS remains unclear, but it hypothesised to be due to an exaggerated and dysregulated inflammatory response as the immune system recovers following initiation of antiretroviral therapy (ART).[5] Risk factors associated with IRIS in HIV CM patients include high fungal burden in the cerebrospinal fluid (CSF),[6] poor CD4+ T-cell count recovery,[7] increased plasma interleukin-5 (IL-5) and IL-7 levels,[8] and elevated CSF interferon gamma (IFN-γ), IL-4, IL-10, IL-17, chemokine (C-X-C motif) ligand 10 (CXCL10), C-C Motif Chemokine Ligand 3 (CCL3) and monocyte chemotactic protein 1 (MCP-1) levels.[9–13] Nevertheless, precise predictive or diagnostic biomarkers for IRIS are still limited, and no systemic clinical data or biomarkers for non–HIV-associated IRIS in CM patients have been reported. A better understanding of IRIS pathogenesis in non-HIV CM patients is crucial to recognise high-risk patients, develop rational diagnostic and make immunomodulatory strategies.

Leukotriene A4 hydroxylase (*LTA4H*) is a key enzyme involved in inflammatory cascades associated with arachidonic acid pathways that catalyses hydrolysis of leukotriene A4 (LTA4) into leukotriene B4 (LTB4).[14] Previous studies in zebra fish have demonstrated that the mutations of *LTA4H* genes may affect the inflammatory phenotype via changing LTB4 level.[14,15] In large population-based studies, polymorphisms of *LTA4H* genes including rs1978331, rs2660898 and rs2540474 have been identified associated with the susceptibility of tuberculosis, including pulmonary tuberculosis and tuberculous meningitis (TBM).[16] Tobin et al also founded that rs17525495, a single nucleotide polymorphisms (SNPs) located close to the promoter site of *LTA4H*, regulating the gene activity and was associated with inflammatory phenotype and clinical response to dexamethasone in TBM: TT genotype has a hyperinflammatory presentation and respond well to dexamethasone, whereas CT and CC genotypes have a moderate and hypo inflammatory presentations, respectively, and would not benefit from dexamethasone.[14,16,17] Though *LTA4H* genotype has been found no impact on response to dexamethasone therapy in CM,[18] these preliminary findings suggested that *LTA4H* genotype might be a critical determinant of non–HIV-associated IRIS in CM patients.

2 | MATERIALS ANS METHODS

2.1 | Study design and participations

A total of 101 non-HIV patients who did not receive any antifungal treatment before with proven infected CM diagnosis were recruited between January 2014 and December 2017 at Huashan Hospital (Shanghai, China). No restriction in terms of age or sex was applied. Of these patients, 11 (10.9%) males subsequently developed IRIS during follow-up. To explore the immunological profile of IRIS, baseline CSF cytokines expressions were compared between patients with and without IRIS, as well as CSF cytokines change before and at IRIS occurrence. To further explore the role of *LTA4H* in the pathogenesis of non-HIV CM-related IRIS, a case-control genetic association study was then conducted, and correlations among *LTA4H* SNPs, CSF cytokines and IRIS occurrence were examined. Detailed clinical data were collected from the previously untreated cohort, including demographic characteristics, predisposing factors, manifestations of cryptococcal disease, laboratory examinations, image results, pathological findings, managements of antifungal therapy, prognosis and outcomes. Because all IRIS cases were reported in immunocompetent cases, further subgroup analysis in immunocompetent patients was performed to test whether there were associations between the IRIS patients and immunocompetent CM patients. The study was approved by Human Research Ethics Committee of Huashan Hospital. Oral consent was obtained from all CM patients for the surplus samples and clinical data.

2.2 | Definitions

An IRIS event for non-HIV CM was defined based on proposed definition in HIV-infected individuals and criteria for transplant settings[19]: (1) initial clinical response to antifungal therapy with partial or complete resolution of signs or symptoms, fever, or other lesions, or reduction in CSF cryptococcal antigen concentration or quantitative culture; (2) reappearance or worsening of previous manifestations after an initial response, or appearance of new manifestations consistent with the infection, and/or inflammatory process of cryptococcosis, despite receipt of appropriate therapy; (3) symptoms or signs could not be explained by alternative infection or malignant disease in the affected site, by the expected clinical course of a previously recognised agent, or by the adverse effects of therapy.

2.3 | CSF cytokines detections

Cerebrospinal fluid samples were collected from each patient at CM diagnosis, at and after IRIS event occurrence. CSF was centrifuged at 800 *g* for 10 min, and frozen at −80°C for subsequent use. We measured 27 CSF cytokines concentrations in duplicate (Human 27-Plex Panel; Bio-Rad) according to manufacturer protocol via a Luminex 100 system in all CSF samples.

2.4 | SNPs selection

Genomic DNA was extracted from peripheral blood samples according to the standard protocols of the QIAamp DNA Blood Mini kit (Qiagen). Genotyping of the SNPs was performed by multiplex SNaPshot technology using an ABI fluorescence-based assay allelic discrimination method (Applied Biosystems). We searched the NCBI and Hapmap databases with the criteria of a minor allele frequency (MAF) > 0.1 and R2 > 0.8 in the Chinese Han population to select SNPs in *LTA4H*. As supplements, SNPs that have been reported to be associated with infection disease were also selected into the candidate genes. As a result, a total of 19 SNPs were finally selected and genotyped SNPs and primers are detailed in the Supplementary Material.

2.5 | Statistical analysis

Continuous variables were expressed with mean and standard error or median and interquartile range (IQR) as appropriate and analysed using either a t test or Mann-Whitney test. Proportions were compared with the χ^2 test or Fisher's exact test. Matched nonparametric data were compared using Wilcoxon signed-rank test. We evaluated the frequency of genotypes and alleles using the χ^2 test between patients with and without IRIS. Allele frequency and genotype distribution differences were analysed with the use of SNPstats, an online software. Prediction of IRIS occurrence was analysed using multivariate logistic regression model, where all baseline factors from univariate analysis with p value < .05 were assumed relevant to the final multivariate model. The results of the multivariate analysis were expressed as odds ratio (OR) and the corresponding 95% confidence intervals (CIs).

Statistical analysis was performed with the SPSS statistical package version 17.0 and GraphPad Prism 6.0. All tests were two-sided and a value of $p < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Study cohort and demographic risk factors for IRIS

In this cohort study, we included 101 non-HIV CM cases between January 2014 and December 2017. The median age was 45 years (IQR, 35–58 years) and male to female ratio was 2.26:1. Of these patients, 11 males developed IRIS and the mean time from antifungal treatment initiation to IRIS was 23 days (IQR, 12.85–49 days), which represents an IRIS incidence rate of 10.9%. Table 1 summarised the demographic, clinical and laboratory characteristics at baseline between the two groups. Patients with an IRIS, compared with those without IRIS were more observed in male patients (p = .046), had less underlying conditions (p = .016), lower baseline CSF glucose levels (p = .006) and presented with more cranial nerve injury manifestation (p = .048). When subgroup analyses made only in immunocompetent cases, results are similar to the overall patient group, but cranial nerve injury manifestation showed no significance and CSF direct microscopy was more likely positive in IRIS group (p = .045).

As baseline CSF CrAg titre has previously been shown to be associated with IRIS occurrence in HIV, we further determined if there is an optimum titre level to predict IRIS occurrence in non-HIV population. Either compared with overall control group or immunocompetent control group, our results showed that patients with titres of more than 1:160 were more likely to develop IRIS (p = .031 and 0.024, respectively), and titre of 1:1280 seemed to be the optimum level to predict IRIS occurrence (p = .000 and .001) (Table 2). Moreover, when compared with baseline CSF CrAg titre, titres at IRIS occurrence are significant lower (CSF titres>1280:10/11 vs. 4/11, p = .024), indicating an obvious fungal clearance.

3.2 | Associations of CSF cytokines with IRIS

Baseline CSF cytokine levels were compared between the IRIS group and non-IRIS group (Figure 1A) We observed higher levels for all cytokine parameters except for IL-13 and IL-17, and 11 cytokines were significantly higher in IRIS group: IL-1ra (p = .000), IL-4 (p = .017), IL-9 (p = .002), IL-10 (p = .002), IL-15 (p = .004), FGF-basic (p = .005), MCP-1 (p = .014), MIP-1α (p = .001), MIP-1β (p = .001), TNF-α (p = .040) and VEGF (p = .003). IL-12/IL-10 ratio which represented Th-1/Th-2 balance was significantly lower in the IRIS group (p = .025). When compared with immunocompetent subgroup, results were similar but IL-7 showed a higher level in IRIS group (p = .045).

To determine the changes of inflammatory pattern in central nervous system, comparison of CSF cytokine concentrations at baseline and at IRIS occurrence was also performed. The expressions of 11 cytokines were decreased significantly at the onset of IRIS, including IL-4 (p = .047), IL-7 (p = .008), IL-9 (p = .047), IL-10 (p = .009). IL-15 (p = .037), FGF-basic (p = .005), GM-CSF (p = .013), MIP-1α (p = .005), PDGF-bb (p = .028), MIP-1β (p = .005) and VEGF (p = .005). Notably, the remaining 15 except for IL-13 also exhibited a downward trend with no statistically significant difference (Figure 1B). IL-12/IL-10 ratio was significantly increased at the time of IRIS event (p = .028).

3.3 | Associations of LTA4H genotype with IRIS

The distribution of genotypes and carriage rate of allele for the 19 SNPs were tested. Four samples failed in genotyping of rs2540491 and

TABLE 1 Baseline characteristics of non-HIV CM patients with and without IRIS

Characteristics	IRIS patients ($n = 11$)	IRIS patients vs. non-IRIS patients: Non-IRIS patients ($n = 90$)	*p* Value	IRIS Patients vs. Immunocompetent non-IRIS patients: Immunocompetent non-IRIS patients ($n = 38$)	*p* Value
Sex (male)	11/11 (100%)	59/90 (65.6%)	.046	28/38 (73.7%)	.090
Age	51.0 (31.0, 63.0)	45.0 (36.0, 57.0)	.810	43.5 (37.0, 51.25)	.662
Time to diagnosis	27.0 (19.5, 58.25)	47 (37, 60)	.410	37 (16.75, 69.75)	.256
Underlying conditions[a]	0/11 (0%)	52/90 (57.8%)	.016	/	/
Fever	9/11 (81.8%)	64/90 (71.1%)	.695	28/38 (73.7%)	.723
Headache	11/11 (100.0%)	86/90 (95.6%)	1.000	37/38 (97.4%)	1.000
Vomiting	5/11 (45.5%)	45/90 (50%)	.776	14/38 (36.8%)	.729
Epilepsy	5/11 (45.5%)	17/90 (18.9%)	.104	7/38 (18.4%)	.108
Craniopathy	8/11 (72.7%)	33/90 (36.7%)	.048	17/38 (44.7%)	.196
Acute death	0/11 (0)	7/90 (7.8%)	1.000	2/38 (5.3%)	1.000
Severe cases	6/11 (54.5%)	26/90 (28.9%)	.167	8/38 (21.1%)	.055
ESR	26 (16.75, 35)	35 (17.75, 49.25)	.907	25 (16, 49)	.775
CRP	15.75 (7.22, 23.3)	8 (3.16, 18.3)	.158	10.91 (3.33, 18.93)	.481
PCT	0.12 (0.78, 0.46)	0.08 (0.05, 0.155)	.095	0.08 (0.05, 0.16)	.217
Serum calcium	2.24 (2.15, 2.30)	2.14 (2.05, 2.22)	.160	2.19 (2.09, 2.22)	.09
LP pressure (>=200 mmH_2O)	9/11 (81.8%)	55/87 (63.2%)	.311	26/37 (70.3%)	.702
CSF WBC count (10^6/L)	69 (22, 155)	65 (25, 181.5)	.785	120.5 (35.25, 225.75)	.465
CSF Lymphocyte (10^6/L)	57 (16, 93.75)	47 (17.5, 125)	.913	70.0 (23.0, 131.5)	.388
CSF Glucose ≤1.1 mmol/L	8/11 (72.7%)	24/90 (26.7%)	.006	10/38 (26.3%)	.010
CSF Protein (mg/L)	1170 (691.5, 2073.75)	1122 (636, 1484)	.651	1162 (639, 1569)	.632
CSF Chloride (mmol/L)	109 (106.5, 111)	116 (110, 119)	.006	115.5 (111, 118)	.009
CSF Culture	10/11 (90.9%)	70/90 (77.8%)	.536	30/38 (78.9%)	.662
CSF direct microscopy	11/11 (100.0%)	60/90 (66.7%)	.053	26/38 (92.9%)	.045
Cryptococcemia	6/11 (64.5%)	17/87 (19.5%)	.028	7/37 (18.9%)	.047
Meningeal enhancement	7/7 (100%)	24/36 (66.7%)	.333	16/22 (72.7%)	.304
Ventricular enlargement	3/7 (42.9%)	8/36 (22.9%)	.502	4/21 (19.0)	.318
Cranial granuloma	2/7 (28.6%)	4/36 (11.1%)	.248	2/21 (9.5%)	.253
AmB-based initial therapy	11/11 (100%)	74/90 (82.2%)	.277	34/38 (89.5%)	.562
AmB ± 5-FC	11/11 (100%)	65/90 (72.2%)		30/38 (78.9%)	
AmB + fluconazole ± 5-FC	0/11 (0%)	6/90 (6.7%)		1/38 (2.6%)	
Fluconazole ±5-FC	0/11 (0%)	15/90 (16.7%)		4/38 (10.5%)	
Others[b]	0/11 (0%)	4/90 (4.4%)		3/38 (7.9%)	

Note: Data are *n* (%) or median (IQR). Missing data not provided by the sites are indicated by the denominators in each variable.

Abbreviations: AmB, amphotericin; CM, cryptococcal meningitis; CrAg, cryptococcal antigen; CRP, C-reactive protein; CSF, cerebrospinal fluid; ESR, erythrocyte sedimentation rate; HIV, human immunodeficiency virus; IRIS, immune reconstitution inflammatory syndrome; LP, lumbar puncture; PCT, procalcitonin; WBC, white blood cell.

[a]Autoimmune diseases in 23 patients; diabetes mellitus and idiopathic CD4 deficiency in nine patients, respectively; liver disease, kidney disease, solid organ transplantation and solid organ malignancy in four patients, respectively; haematological malignancy in two patients and drug abuse history in one patient. One or more underlying conditions were identified in 10 patients.

[b]Others include one patient treated with voriconazole, and three patients treated with AmB liposome.

rs2540494, and one sample failed in rs35633627. Association analysis suggested that genotype distributions of four SNPs were significantly correlated with IRIS in overall CM patients (Table 3): rs17525488 C/CT (OR 0.10, 95% CI 0.01–0.90; $p = .024$), rs6538697 C/T (OR 0.13, 95% CI 0.02-1.07; $p = .046$), rs17525495 G/A (OR 0.10, 95% CI 0.01-0.90; $p = .024$) and rs1978331 G/A (OR 0.21, 95% CI 0.04–1.04; $p = .039$). We further made subgroup analyses to compare allele and genotype distributions between IRIS and immunocompetent non-IRIS patients.

TABLE 2 Baseline CSF CrAg titres of non-HIV CM patients with and without IRIS

CSF CrAg titre	IRIS patients (*n* = 11)	IRIS patients vs. non-IRIS Patients: Non-IRIS patients[a] (*n* = 89)	*p* Value	IRIS patients vs. immunocompetent non-IRIS patients: Immunocompetent non-IRIS patients (*n* = 38)	*p* Value
>2560	5 (45.5%)	6 (6.7%)	.002	3 (7.9%)	.009
>1280	10 (90.9%)	24 (27.0%)	.000	13 (34.2%)	.001
>640	11 (100%)	43 (48.3%)	.001	20 (52.6%)	.004
>320	11 (100%)	47 (52.8%)	.002	20 (52.6%)	.004
>160	11 (100%)	59 (66.3%)	.031	25 (65.8%)	.024
>80	11 (100%)	68 (76.4%)	.114	27 (71.1%)	.050
>40	11 (100%)	76 (85.4%)	.350	33 (86.8%)	.574
>20	11 (100%)	82 (92.1%)	1.000	36 (94.7%)	1.000
>10	11 (100%)	85 (95.5%)	1.000	37 (97.4%)	1.000

Note: Data are *n* (%).

Abbreviations: CrAg, cryptococcal antigen; CSF, cerebrospinal fluid; IRIS, immune reconstitution inflammatory syndrome.

[a]CSF CrAg titres were available in 89 non-IRIS patients.

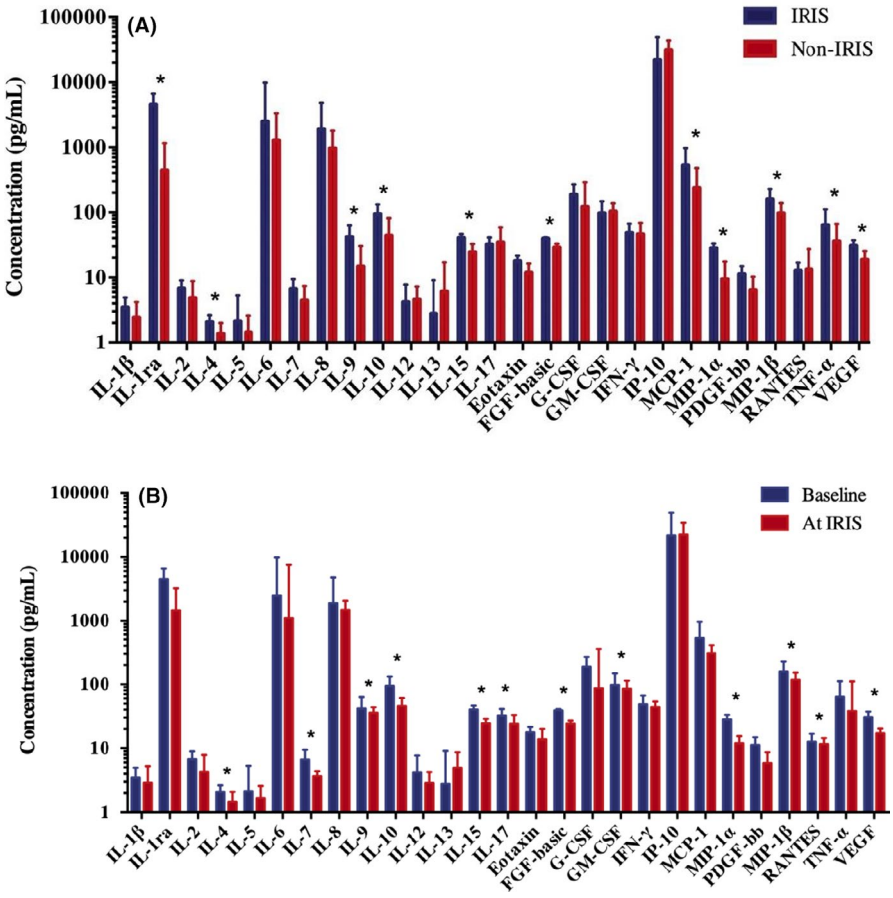

FIGURE 1 Comparisons of baseline CSF cytokines levels between non-HIV CM patients with or without IRIS, and CSF cytokines levels at baseline and after IRIS occurrence. (A) Summary cytokine levels between IRIS patients (*n* = 11) and non-IRIS patients (*n* = 90). (B) Summary cytokine levels at baseline (*n* = 11) and after IRIS occurrence (*n* = 10). Bar graphs show median extended to interquartile range (IQR) with whiskers. CSF, cerebrospinal fluid; FGF-basic, fibroblast growth factor-basic; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IP, interferon inducible protein; IRIS, immune reconstitution inflammatory syndrome; MCP, monocyte chemoattractant protein; M-CSF, macrophage colony-stimulating factor; MIP, macrophage inflammatory protein; RANTES, regulated on activation in normal T-cell expressed and secreted; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor

Similar to the results from overall group, associations were also found in rs17525488 C/CT, rs6538697 C/T, rs17525495 G/A and rs1978331 G/A, and additional one SNPs were identified as detailed in Table 3. Interestingly, rs1978331 G/A became the most significant SNP that correlated with IRIS after adjustment for gender (OR: 0.14, 95%CI: 0.03–0.77, *p* = .013).

3.4 | Associations of LTA4H SNPs with CSF cytokines

Univariate analysis found that 19 cytokines (IL1-β, IL-5, IL-6, IL-7, IL-8, IL-12, IL-13, IL-17, IFN-γ, IP-10, RANTES, GM-CSF, IL-1ra, IL-4,

TABLE 3 Allele and genotype distributions of five *LTA4H* SNPs associated with IRIS

SNP	Position	Genotype	IRIS patients ($n = 11$)	Non-IRIS patients ($n = 90$)	Immunocompetent non-IRIS patients ($n = 38$)	IRIS patients vs. non-IRIS patients		IRIS Patients vs. Immunocompetent non-IRIS patients	
						OR (95% CI)	*p* Value	OR (95% CI)	*p* Value
rs17525488	12:96035660	CT/CT (Dominant)	8 (72.7%)	44 (48.9%)	19 (50.0%)	0.36 (0.09-1.44)	.135	0.37 (0.09-1.63)	.303
		C/CT (Over-dominant)	1 (9.1%)	41 (45.6%)	19 (50.0%)	0.12 (0.01-0.97)	.024	0.10 (0.01-0.86)	.017
		C/C (Recessive)	2 (18.2%)	5 (5.6%)	0 (0.0%)	3.78 (0.64-22.36)	.167	NA (0.00-NA)	.047
		C (Allelic)	5 (22.7%)	51 (28.3%)	19 (25.9%)	0.74 (0.21-2.12)	.579	0.88 (0.29-2.72)	.827
rs6538697	12:96009832	T/T (Dominant)	8 (72.7%)	46 (51.5%)	19 (50.0%)	0.39 (0.10-1.57)	.175	0.37 (0.09-1.63)	.303
		C/T (Over-dominant)	1 (9.1%)	39 (43.3%)	19 (50.0%)	0.13 (0.02-1.07)	.046	0.10 (0.01-0.86)	.017
		C/C (Recessive)	2 (18.2%)	5 (5.6%)	0 (0.0%)	3.78 (0.64-22.36)	.167	0.00 (0.00-NA)	.047
		T (Allelic)	17 (77.3%)	131 (72.8%)	57 (75.0%)	0.79 (0.28-2.24)	.653	0.88 (0.29-2.72)	.827
rs17525495	12:96035599	G/G (Dominant)	8 (72.7%)	44 (48.9%)	19 (50.0%)	0.36 (0.09-1.44)	.247	0.37 (0.09-1.63)	.303
		G/A (Over-dominant)	1 (9.1%)	41 (45.6%)	19 (50.0%)	0.12 (0.01-0.97)	.024	0.10 (0.01-0.86)	.017
		A/A (Recessive)	2 (18.2%)	5 (5.6%)	0 (0.0%)	3.78 (0.64-22.36)	.167	NA (0.00-NA)	.047
		G (Allelic)	17 (72.3%)	129 (71.7%)	57 (75.0%)	0.74 (0.26-2.12)	.579	0.88 (0.29-2.72)	1.000
rs1978331	12:96015423	A/A (Dominant)	7 (63.6%)	28 (31.1%)	12 (31.6%)	0.26 (0.07-0.95)	.045	0.26 (0.06-1.08)	.081
		G/A (Over-dominant)	2 (18.2%)	46 (51.1%)	23 (60.5%)	0.21 (0.04-1.04)	.039	0.14 (0.03-0.77)	.013
		G/G (Recessive)	2 (18.2%)	16 (17.8%)	3 (7.9%)	1.03 (0.20-5.22)	1.000	2.59 (0.38-17.92)	.311
		A (Allelic)	16 (72.7%)	102 (56.7%)	47 (61.8%)	0.49 (0.18-1.31)	.149	0.61 (0.21-1.73)	.348

(Continues)

TABLE 3 (Continued)

SNP	Position	Genotype	IRIS patients (n = 11)	Non-IRIS patients (n = 90)	Immunocompetent non-IRIS patients (n = 38)	IRIS patients vs. non-IRIS patients		IRIS Patients vs. Immunocompetent non-IRIS patients	
						OR (95% CI)	p Value	OR (95% CI)	p Value
rs2540491	12:96024731	T/T (Dominant)	6 (60.0%)	26 (29.9%)	12 (31.6%)	0.28 (0.07-1.09)	.077	0.31 (0.07-1.30)	.145
		C/T (Over-dominant)	2 (20.0%)	45 (51.7%)	23 (60.5%)	0.23 (0.05-1.16)	.093	0.16 (0.03-0.88)	.033
		C/C (Recessive)	2 (20.0%)	16 (18.4%)	3 (7.9%)	1.11 (0.21-5.73)	1.000	2.92 (0.42-20.44)	.276
		T (Allelic)	14 (70.0%)	97 (55.7%)	47 (61.8%)	0.54 (0.20-1.47)	.222	0.70 (0.24-2.01)	.500

Note: Data are n (%).

Abbreviations: IRIS, immune reconstitution inflammatory response syndrome; NA, not available; SNP, single nucleotide polymorphism; TLR, Toll-like receptor.

IL-9, FGF-basic, MCP-1, MIP-1β and VEGF) were significantly associated with 16 SNPs before antifungal therapies, of which 8 cytokines were over expressed in IRIS patients (Figure 2). We further investigated the associations between baseline CSF cytokines and the four IRIS-related SNPs. Five cytokines (IL-12, IL-13, IFN-γ, MCP-1 and RANTES) were found to be associated with rs1978331, while the remaining three SNPs were only related to MCP-1, indicating the core situation of this chemokine in IRIS.

To understand how SNP affecting CSF inflammations, we analysed the CSF cytokine concentrations according to rs1978331 genotype (Figure 3). Patients with rs1978331 G/A had a higher concentration of IL-12 (p = .010), IL-13 (p = .006) and IFN-γ (p = .011) compared with other groups. Moreover, we identified a low concentration of MCP-1 in patients with genotype G/G, intermediate concentration in those with genotype G/A and high concentration in those with genotype A/A. A significant difference was found between the three groups (p = .036).

3.5 | Multivariate analysis of IRIS occurrence

We further developed a logistic regression model for IRIS occurrence that includes the following factors: age, sex, predisposing factors, craniopathy, CSF glucose, CSF cryptococcal antigen titre, blood culture, 11 cytokines and rs1978331 genotype. Increased MIP-1β (OR 11.89, 95% CI 1.76–80.46; p = .011) and IL-15 (OR 15.69, 95% CI 2.30–107.00; p = .005) levels were independently predictive of occurrence.

4 | DISCUSSION

Immune reconstitution inflammatory syndrome is a common complication of ART in HIV CM patients, with an incidence rate ranging from 8% to 49% in the literature.[4,9,11,20,21] In SOT associated CM patients, IRIS occurrence rate ranged from 4.8% to 14%.[22-24] However, few studies of IRIS cases have been reported in non-HIV non-SOT CM patients. In the current study, 10.9% of CM patients experienced IRIS after antifungal treatment. Though this incidence is comparable to previous studies in HIV CM patients and SOT CM patients, considering its low awareness, high morbidity and mortality, exploring the mechanism of this disease is of great significance. In this observational cohort study, we characterised the incidence and clinical features of IRIS in patients with non-HIV CM and investigated whether fungal burden, baseline CSF cytokines and *LTA4H* genotypes could identify those patients at risk of IRIS.

For HIV CM patients, immune reconstitution following the initiation of ART is a major cause of IRIS.[4] However, for non-HIV CM patients, especially those who do not have immunocompromised conditions, no immunological recover could be identified. On the one hand, pathogens may play an immunosuppressive role in immunocompetent patients. The capsular polysaccharide of *Cryptococcus* has been reported could directly affecting FcRγII expression in

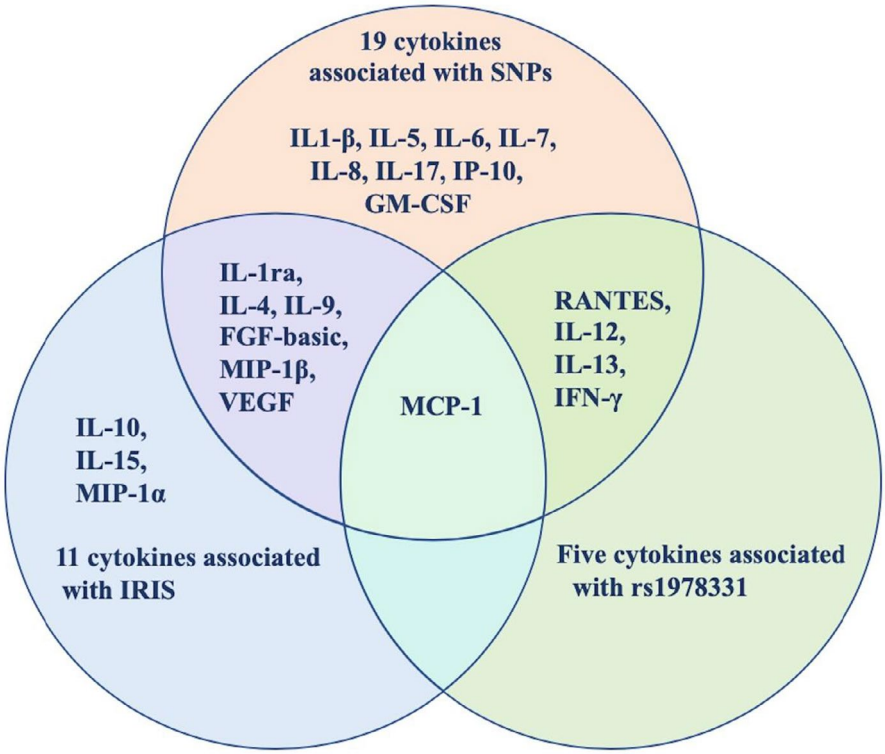

FIGURE 2 Summary of interactions among rs1987331, CSF cytokines and IRIS. Three cycles represent cytokines associated with rs1978331, IRIS and all the SNPs. Their intersection shows that MCP-1 is correlated to all. FGF-basic, fibroblast growth factor-basic; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IP, interferon inducible protein; IRIS, immune reconstitution inflammatory syndrome; MCP, monocyte chemoattractant protein; M-CSF, macrophage colony-stimulating factor; MIP, macrophage inflammatory protein; RANTES, regulated on activation in normal T-cell expressed and secreted; SNP, nucleotide polymorphism; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor

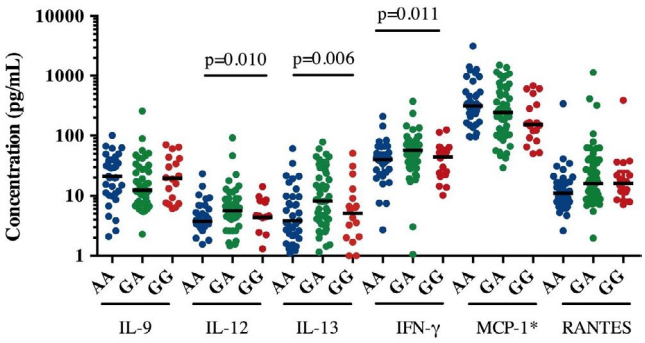

FIGURE 3 Cerebrospinal fluid (CSF) levels of cytokine expression by rs1978331 genotype in non-HIV cryptococcal meningitis patients. Comparisons were made based on overdominant models (G/A vs. G/G + A/A). Concentrations are in picogram/millilitre for all cytokines. MCP-1*, $p < .05$ in codominant model (G/G vs. G/A vs. A/A). Cytokines with $p < .05$ in univariate analysis were showed. IL, interleukin; IFN, interferon; MCP, monocyte chemoattractant protein; RANTES, regulated on activation in normal T-cell expressed and secreted

monocytes, macrophages, as well as dendritic cells,[25,26] and glucuronoxylomannan in extracellular space could prevent immune cell infiltration into brain and highly inflammatory intracranial response, which would result in an inhibitory signal that suppresses host immune response to the *C neoformans*.[27] At the same time, high baseline CSF CrAg titres also showed significant difference in univariable analysis in our study, indicating that the fungal infection could cause a immune suppression, which may lead to a subsequent partly immune reconstitution after effective antifungal treatment. On the other hand, our observation of dynamic CSF cytokine changes also provides support for this proposal that rapid decreased fungal burden could lead to strong immune reversion like what we have described in HIV population. Once the immune suppression is relieved by effective antifungal treatment, aberrant immune responses and excessive CNS inflammation would same occur, and finally results in IRIS events in non-HIV patients. Our data showed that IL-12/IL-10 ratio that represented Th-1/Th-2 balance was significantly lower at baseline in the IRIS group, and increased inversely at IRIS occurrence, which is consistent with the study from Panackal et al,[28] and indicated an immune reconstitution.

Associations between high chemokine expressions and IRIS development were identified in HIV and non-HIV populations as previously reported.[9,12,29] Elevated CSF IFN-γ, IL-4, IL-10, IL-17, CXCL10, CCL3 and MCP-1 levels were recognised as biomarkers for IRIS in HIV population, whereas our data showed th1 cytokines (TNF-α), th2 cytokines (IL-4, IL-10) and chemokines (MCP-1, MIP-1α, MIP-1β) all increased at baseline CSF, indicating that IRIS is a complicated immune response in both HIV and non-HIV populations. *LTA4H* as a key enzyme for LTB4 which would result in excessive proinflammatory response has been reported associated with severe IRIS in HIV-TB co-infected individuals.[30] We then investigated whether polymorphisms of these SNPs could affect the phenotype of inflammation at baseline in CM patients. Four SNPs were identified significantly correlated with IRIS, and when compared with baseline CSF cytokines, MCP-1 was the only cytokine associated with all the SNPs, and we found that patients with the rs1978331 A/A genotype had higher CSF MCP-1 concentrations, whereas G/A and G/G genotypes have intermediate and lower CSF MCP-1 concentrations, respectively.

Since patients with rs1978331 G/A genotype have less opportunity to develop IRIS, a moderate level of MCP-1 is considered as a crucial factor for host to control the infection appropriately, with high level leading to immune reconstitution and low level, on the other hand, will lead to infection spread.

MCP-1 as one of the chemokines is produced by microglial cells and could promote a cellular infiltrate into the CNS following the exposure to *Cryptococcus*.[31] Pro-inflammatory cytokines are then excreted and macrophages are activated in response to cytokines, which could help host to defence the pathogens, accelerate the elimination and recovery from the infection.[9] However, in patients with immunosuppression at baseline, especially HIV patients, the counts of CD4 cell and CSF lymphocyte are very low and a compensatory upregulated MCP-1 is expressed, which, following the immune restoration with ART, may lead to an excessive aggregation of inflammatory cells and IRIS. Similarly, the immune suppression exists in non-HIV patients. According to our hypothesis, the suppression is a local immune response caused by abundant *Cryptococcus*, which would lead to excessive MCP-1 expression in the same way and lead to IRIS occurrence.

Despite this is the first study of IRIS in non-HIV CM patients, with comprehensive clinical and immunological data, it has some limitations. First, as an observational study, the analyses do not provide definitive evidence that associations are caused by immune suppression of high fungal burden. Second, the relative small sample size may skew the results. Given the relative low incidence rate of CM in non-HIV patients, the number of IRIS patients was small, which may restrict the statistical power. Third, the association of *LTA4H* genotype and concentrations of LTB4 was not verified. We measured the LTB4 in CSF samples from patients with IRIS, using enzyme-linked immunosorbent assay. However, LTB4 levels were undetectable in around 50% of samples (data not shown), and no conclusion could be drawn in this study.

In summary, our findings indicated that IRIS in non-HIV CM patients is an immune reconstitution caused by *Cryptococcus* which had a local immune suppression effect. The local immune responses reflected by CSF cytokines are under *LTA4H* genetic influence and correlated with IRIS development. Overall, our data suggested that IRIS in non-HIV CM is an interaction between fungi and host genetic factors. Further cytological research or animal model investigation is needed to validate our results and explore the causative mechanisms.

ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation of China (Grant No. 81571968 and Grant No. 81971911 to L-PZ). The funders of the study had no role in the study design, data collection, data analysis, data interpretation or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication. We thank Dr David Denning for his encourage and revisions for this article. We thank Shanghai Genesky Bio-Tech Genetic Core Lab for their assistance in genotyping techniques.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Ling-Hong Zhou : Methodology (equal); Writing-original draft (equal); Writing-review & editing (equal). **Hua-Zhen Zhao:** Writing-original draft (equal); Writing-review & editing (equal). **Xuan Wang:** Formal analysis (equal). **Rui-Ying Wang:** Project administration (equal). **Ying-Kui Jiang:** Methodology (equal). **Li-Ping Huang:** Data curation (equal). **Ching-Wan Yip:** Data curation (equal). **Jia-Hui Cheng:** Software (equal). **Chun-Xing Que:** Software (equal). **Li-Ping Zhu:** Conceptualization (lead).

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Zhou L-H, Zhao H-Z, Wang X, et al. Immune reconstitution inflammatory syndrome in non-HIV cryptococcal meningitis: Cross-talk between pathogen and host. *Mycoses*. 2021;64:1402–1411. https://doi.org/10.1111/myc.13361