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Viviana GIUSTINI, Alessandra SOTTINI, Stefano BELLERI, Diego BERTOLI, Aldo Maria ROCCARO, Alessandro MONTANELLI, Matteo FILIPPINI, Marco FONTANELLA, Luisa IMBERTI

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Cryptococcal-related meningoencephalitis in a patient with sarcoidosis and CD4 lymphocytopenia: thorough immunological characterization of lymphocyte homeostasis

Viviana Giustini,[1] Alessandra Sottini,[1] Stefano Belleri,[1] Diego Bertoli,[1;2] Aldo M. Roccaro,[1;3] Alessandro Montanelli,[4] Matteo Filippini,[5] Marco Fontanella,[6] Luisa Imberti[1]*

[1]Centro di Ricerca Emato-oncologica AIL (CREA), Clinical Chemistry Laboratory, Diagnostic Department, ASST Spedali Civili di Brescia, Brescia, Italy; [2]Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy; [3]Clinical Research Development and Phase I Unit; ASST Spedali Civili di Brescia, Brescia, Italy; [4]Clinical Chemistry Laboratory, Diagnostic Department, ASST Spedali Civili di Brescia, Brescia, Italy; [5]Department of Anesthesia, Critical Care and Emergency, ASST Spedali Civili di Brescia, Brescia, Italy; [6]Division of Neurosurgery, Spedali Civili and University of Brescia, Brescia, Italy.

*Corresponding author at Centro di Ricerca Emato-oncologica AIL (CREA), ASST Spedali Civili, P.le Spedali Civili, 1, 25123 Brescia, Italy limberti@yahoo.it

ABSTRACT

Cryptococcal meningoencephalitis is the most common infective complication observed in patients with CD4 lymphocytopenia, including sarcoidosis. T-cell immunity is well characterized in HIV-related infections and data regarding immunity in cryptococcosis animal models is now available; on the contrary, little is known about the immune status in non-HIV-related infections.

We report on reduced production of new T cells observed in a patient with sarcoidosis, CD4 lymphocytopenia, and cryptococcal-related meningoencephalitis. Although T cells presented with an intact proliferative capacity, they were oligoclonally expanded showing an effector memory phenotype. However, the deleterious activity of effector memory cells could have been controlled by the expansion of the regulatory T cell subset with the highest suppressive capability. This information provide a better understanding of the immune response to cryptococcus occurring in non-HIV-associated cases, the predisposition to infection, and the role of different cell subtypes in controlling the disease in humans.

Keywords: Cryptococcal meningoencephalitis - Idiopathic CD4 lymphocytopenia - T-cell receptor repertoire - Thymic output.

INTRODUCTION

Cryptococcosis is one of the major cause of meningoencephalitis in both HIV-infected and -uninfected individuals with 20% and 30% mortality, respectively, in developed Countries[1]. Cases of HIV-unrelated cryptococcosis has been observed in a variety of circumstances, including prolonged corticosteroid immunosuppressive treatment, solid organ transplantation, hematological disease, diabetes mellitus, cirrhosis, sarcoidosis, and idiopathic CD4 lymphocytopenia (ICL)[2]. Susceptibility to cryptococcosis can be broadly categorized as a defect in adaptive immune responses, especially in T-cell immunity. For instance, in patients with sarcoidosis, cryptococcosis has been ascribed to an impaired T-cell-mediated immunity, which can be explained by a combination of $CD4^+$ T cell sequestering in granulomas and suppression of T-cell proliferation by regulatory $CD4^+$ T cells (Treg), leading to effector T-cell anergy[3-5]. In ICL patient, (a disease defined as the persistence of low CD4+ T lymphocytes number, below 300/μl, without any secondary known causes of lymphopenia[6]), cryptococcosis is the most common infection (26.6%)[7], and it is attributed to cellular immunity defects, including expansion of memory T-cell subsets as well as reduced T-cell receptor (TCR) diversity and signaling[8].

While extensive characterization of T-cell immunity has been performed in HIV-related disease, there is little understanding of the mechanisms of *Cryptococcus* susceptibility in non-HIV related disease. Therefore, herein, we have performed a thorough characterization of lymphocyte homeostasis in a patient with previous history of sarcoidosis that also shared some typical immunological features with ICL.

CASE REPORT

A 42-year-old male, a worker in the local thermal baths, presented with a history of headache for 6 months followed by acute episodes of aphasia that led to urgent evaluation and computed tomography scan revealing multiple, bilateral, subcortical hypodense lesions. He was admitted to the local Hospital with suspicion of gliomatosis or lymphoproliferative cerebral disease. He was diagnosed with neurosarcoidosis 1 month later. He was subsequently readmitted 1 month later with acute and intense headache, vomiting, and photophobia. He quickly deteriorated and was intubated. It is worth noting that the patient had no fever until the final state, and that neither stiff neck nor Kernig's sign were observed throughout the course of the disease. Brain magnetic resonance imaging (MRI) revealed mild meningeal enhancement and hydrocephalus, which was urgently treated with an external lumbar catheter, leading to clinical improvement, followed by a further deterioration.

Cerebrospinal fluid (CSF) from lumbar puncture revealed high protein content (167 mg/dl), decreased glucose concentration (32 mg/dL, CSF/serum glucose ratio <0.3), and mild pleocytosis (166 cells/μL) consisting of monocytes (86/μL), neutrophil (20/μL), and lymphocytes (60/μL; 61% $CD3^+CD4^+$). Routine microscopic examination with India ink demonstrated the presence of the encapsulated yeast form of *Cryptococcus neoformans*, also present in bronchoalveolar lavage.

DISCUSSION

Blood tests showed low red blood cells (2900/μL) and normal white blood cells (5860/μL), but low total lymphocyte count (870/μL). Although a significant lymphopenia involving $CD3^+$, $CD4^+$, $CD8^+$, and $CD19^+$ cells is a common feature in patients with sarcoidosis and is related more to disease pathology than medical treatments[9], in our patient total $CD3^+$ lymphocytes were within normal range. Accordingly, T-cell proliferation was normal when peripheral blood mononuclear cells were stimulated with anti-CD3 monoclonal antibody, anti-CD3 plus interleukin-2, phorbol myristate acetate (PMA) plus ionomycin, and only slightly reduced when cultured with phytohemagglutinin (PHA) (data not shown). In addition, the number of $CD3^+CD8^+$ lymphocytes (33%; 287/μL) was within normal limits, with a clear predominance of effector memory cells, which are known to represent the predominant population elicited by chronic parasitic infections[10]. Similarly, lymphopenia did not involve the B-cell compartment since the value of $CD19^+$ B cells (19%; 165/μL) was within the range found in healthy controls (HC). Accordingly, K deleting recombination excision circles (KRECs), considered a marker of bone marrow output[11], were 3,967/mL and therefore within the range found in male age-matched HC (range: 3,932-52,472/mL).

In the patient, $CD3^+CD4^+$ cells were very low (25.8%; 226/μL). CD4 lymphopenia could be attributed to the impaired thymic output because T-cell receptor excision circles (TRECs)[11] were low in peripheral blood (565/mL) in comparison to values found in male age-matched HC (range: 708-26,917/mL). Thorough phenotypic characterization of T-cell subsets, performed by flow cytometry, confirmed the reduced thymic output because

$CD4^+CD45RA^+CCR7^+CD31^+$ recent thymic emigrants (RTE), an alternative marker of new T-cell production[12], were significantly lower compared to the normal values observed in HC (Table 1). Accordingly, naïve T cells were also reduced. This result is different from that previously found in ICL, demonstrating that the thymic output was above values found in HC[13]. In this case it was proposed that overproduction of TRECs was insufficient to maintain normal peripheral T-cell counts, because of accelerated maturation of RTE together with increased peripheral turnover.

Flow cytometry demonstrated that the proportion of lymphocyte memory subpopulations was differentially affected by lymphocytopenia. Residual $CD3^+CD4^+$ T cells of the patient showed a prevalence of $CD4^+CD45RA^-CCR7^-$ effector memory (T_{EM}) over $CD4^+CD45RA^-CCR7^+$ central memory (T_{CM}) cells while, in HC, T_{CM} were more abundant that T_{EM} counterpart (Table 1). T_{EM} cells have the potential to home to peripheral lymphoid tissues, where they produce a variety of microbicidal cytokines and therefore display a rapid effector function *ex vivo*[14]. Different cytokines, produced by host T helper subsets are important in the progression and outcome of cryptococcal infection with a Th1 cytokine profile associated with clearance of fungal infection and a Th2 profile associated with cryptococcal dissemination and host damage. In our patient, the percentage and total number of Th1 and Th2 subsets were similar to those found in HC (data not shown), without a clear dominance of either of the two subsets.

Recent studies consistently and specifically highlight the importance of Treg in modulating exuberant Th2 responses during cryptococcal infection, without notable effects on Th1[15,16]. Accordingly, lethal disease caused by *Cryptococcus* appeared to be a consequence of the combined failure to control replication and immunopathology caused by induced Th2 cell responses[16]. In a mouse model of experimental cryptococcosis, Treg cells accumulated in the lung parenchyma and utilize CCR5 to localize with and suppress Th2 effector cells[15,16]. A similar mechanism might have occurred in our patient since Treg central memory cells were the preferentially represented subset, despite very low levels of $CD4^+CD25^+CD127^{low/-}$ Treg (Table 1). These cells have more effective activity than their effector memory counterpart, because they express CCR7 thus favoring lymph nodes homing, where they expand upon antigen stimulation and suppress effector cell responses[17].

A further peculiarity of peripheral cell subsets in this patient was the increased percentage and number of $CD4^-CD8^-$ gamma/delta T cells (12%; 103/μL). These cells do not promote fungal clearance in the host response to *Cryptococcus neoformans*, and gamma/delta T cell deficient mice are able to control fungal infection better than controls, due to a switch towards a Th1 profile[18]. Gamma/delta T cells constitutively display a restricted TCR repertoire and recognize mostly unknown non-peptide antigens. They act as bridge between innate and adaptive immunity and play a protective role in immune-surveillance[19]. The analysis of TCR gamma chain diversity, performed by CDR3 spectratyping, revealed the clonal expansion in our patient of two different populations, one identified in peripheral blood ($V\gamma9$-$J\gamma1.3/2.3$) and the other in CSF ($V\gamma11$-$J\gamma1.3/2.3$). This indicated that the antigenic-driven expansion, probably triggered by the massive cryptococcal infection[20], acts differently in different districts.

It is known that both the extent of new T-cell production and the quantity of memory $CD4^+$ and $CD8^+$ T-cell expansions define the diversity of the TCR repertoire that, in turn, is very important for host defense against infections. In different pathological conditions, ranging from immunodeficiencies to parasitic, allergic, and autoimmune diseases, the organism develops oligoclonal expansions of T lymphocytes. The analysis of the TCR beta chain repertoire analyzed by multiplex PCR followed by CDR3 spectratyping[21] indicates generalized oligoclonal expansions in our patient (Figure 1).

CONCLUSIONS

Altogether, these data indicate that the peripheral homeostasis of conventional T cells and Treg in this patient were unusual. Although T cells were functioning, the low number of newly produced lymphocytes, the presence of T_{EM} cells, and the oligo- or clonal TCR repertoires might have influenced the progression and outcome of cryptococcal infection. However, the deleterious activity of effector T cells may be controlled by the expansion of central memory Treg.

This information allows for a better understanding about the immune responses to *Cryptococcus neoformans* in non-HIV-associated cases, predisposition to infection, and the role of different cell subtypes in controlling the disease. This information may be important since drugs specific for *Cryptococcus neoformans* cure are limited; in contrast, immunomodulatory adjunctive therapies based on advances in our understanding of host immunity are promising, but need to be carefully tailored for each patient according to individual immune status.

AUTHORS' CONTRIBUTION

LI conceived the manuscript; AS, VG, SB and DB performed immunological characterization; MF and MF contributed to patient clinical follow-up; AS, VG, SB, DB, AM, and AMR participated in revising the manuscript for important intellectual content; all authors approved the final manuscript.

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ETHICAL STATEMENT

Data were generated based on routinary assays. As a retrospective study, formal consent was not required.

CONFLICTS OF INTEREST

Luisa Imberti received a research grant, consultancy, and speaker fees from Genzyme; all other authors declare that they have no conflict of interest.

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Table 1. Flow cytometer analysis of T-cell subsets

	Patient	HC (range)	Patient	HC (range)
T-cell subsets	*percentage*		*number (μL)*	
$CD3^+$	**67.8**	63.5 – 82.7	**597**	821 - 1843
$CD3^+CD4^+$	**25.8**	30.0 – 50.3	**227**	439 - 1160
$CD4^+CD45RA^+CCR7^+CD31^+$ (recent thymic emigrants)	**6.2**	10.4 – 35.7	**14**	74 - 467
$CD4^+CD45RA^+CCR7^+$ (naïve)	**10.2**	17.7 – 45.2	**23**	150 - 636
$CD4^+CD45RA^-CCR7^+$ (central memory)	**24.1**	35.4 – 67.4	**55**	204 - 385
$CD4^+CD45RA^-CCR7^-$ (effector memory)	**56.1**	12.2 – 28.9	**127**	55 - 147
$CD4^+CD25^+CD127^{low/-}$ (Treg)	**1.9**	2.5 – 5.6	**17**	44 - 123
$CD4^+CD25^+CD127^{low/-}CD45RA^+CCR7^+$ (naïve)	**7.6**	10.8 – 38.9	**1**	9 - 48
$CD4^+CD25^+CD127^{low/-}CD45RA^-CCR7^+$ (central memory)	**62.4**	40.8 – 62.3	**10**	20 - 71
$CD4^+CD25^+CD127^{low/-}CD45RA^-$CCR7- (effector memory)	**29.0**	13.9 – 35.9	**5**	9 - 37
$CD3^+CD8^+$	**37.3**	11.3 – 30.7	**328**	264 - 1022
$CD8^+CD45RA^+CCR7^+$ (naïve)	**1.2**	6.5 – 50.5	**4**	81 - 448
$CD8^+CD45RA^-CCR7^+$ (central memory)	**2.7**	4.0 – 46.1	**9**	10 - 64
$CD8^+CD45RA^-CCR7^-$ (effector memory)	**43.7**	25.0 – 61.5	**143**	49 - 798
$CD8^+CD45RA^+CCR7^-$ (highly differentiated effector)	**52.4**	7.2 – 51.4	**172**	39 - 234
$CD3^+$CD4-CD8-	**12**	nd	**103**	nd

Table 1. T-cell subpopulations were assessed by eight-colour flow cytometry analysis on $1x10^6$ PBMC stained using various combinations of allophycocyanin-H7 anti-CD4, fluorescein isothiocyanate anti-CD45RA, phycoerythrin anti-CD3, peridinin-chlorophyll protein-Cy5.5 anti-CCR7, phycoerythrin-Cy7 anti-CD8, allophycocyanin anti-CD31 monoclonal antibodies, phycoerythrin-Cy7 anti-CD127 and brilliant violet 421 anti-CD25. One million events were collected for each tube. Data acquisition was performed with a FACSCanto II cytometer and data were analysed with FACSDiva software. nd: not done

Figure 1. T-cell receptor repertoire.

The analysis of TCR beta variable (TCRBV) gene families by CDR3 spectratyping was performed in the patient with criptococcosis (A) and in an age-matched healthy control. (B). cDNA was uesd for TCRBV family-specific multiplex PCRs allowing the detection of 23 functional TCRBV gene families. The fragment analysis of 6 FAM labelled PCR products was performed on an ABI 3500 Series Genetic Analyzer.

Figure 1. T-cell receptor repertoire.