Granulocyte Macrophage Colony-Stimulating Factor-Specific Autoantibodies and Cerebral Nocardia With Pulmonary Alveolar Proteinosis

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In this study, we report the history of a 40-year-old man with a primary cerebral abscess caused by *Nocardia abscessus* that led to the discovery of autoimmune pulmonary alveolar lipoproteinosis (anti-granulocyte-macrophage colony-stimulating factor [GM-CSF] autoantibodies). Anti-GM-CSF autoantibodies promote immunodeficiency and should be monitored to prevent opportunistic and disseminated infections and to diagnose asymptomatic pulmonary alveolar lipoproteinosis.

Keywords. Nocardia; Pulmonary alveolar proteinosis' Anti-GM-CSF antibody

In April 2018, a 40-year-old man, who was an active smoker (10 pack-years), consulted the hospital for subacute left brachiofacial deficit and headaches. He had no medical history. He previously worked as an order picker and reported a former professional exposure to dust. On admission, he presented with moderate left facial paralysis and left brachial deficit (4/5). Pulmonary auscultation was normal. A voluminous right parietal lesion compatible with a cerebral abscess was identified on cerebral imaging and quickly drained by neurosurgeons (Figure 1). The patient underwent a full body computed tomodensitometry (CT) scan, which did not show any secondary infectious focus but did identify an unexpected diffuse interstitial lung disease with "crazy paving" aspect (Figure 1). Further pulmonary examinations showed a restrictive ventilatory disorder with a decrease in vital capacity and 60% decrease in total pulmonary capacity, associated with a severe alteration of alveolocapillary diffusion (DLCO at 31%).

Per-operative samples of surgical drainage showed partially necrotic polynuclear neutrophils in histopathology, with negative direct examination. Cultures returned positive after 72 hours for *Nocardia* spp. The matrix-assisted laser desorption ionization time-of-flight technique was used to identify *N abscessus*. Molecular biology performed on abscess samples to eliminate other pathogens such as aspergillus, mycobacterias, candidas, cryptococcus, histoplasma, and cysticercus was negative. Bronchoalveolar lavage (BAL) fluid was opalescent, microbiological culture and molecular biology searching for pneumocystis, aspergillus, mycobacteria, *Streptococcus pneumoniae*, mycoplasma, *Bordetella pertussis*, as well as for cytomegalovirus, herpes simplex virus, enterovirus, rhinovirus, respiratory syncytial virus, metapneumovirus, and influenzavirus were negative as well. Histopathologic BAL analysis revealed extracellular periodic acid-Schiff staining-positive material evocative of pulmonary alveolar lipoproteinosis. Less than 1% of lymphocytes were detected in the BAL fluid, with mainly T cells and an inverse CD4/CD8 ratio. Phenotypic and functional analyses of circulating lymphocytes did not reveal any obvious immunodeficiency: $CD4^+$ T, $CD8^+$ T, B, and natural killer cell counts were normal, as were mitogen-induced T-cell proliferation and Th1/Th2/Th17 cytokine production. Similarly, B cell function indicated by immunoglobulin production evaluation was normal. Anti-granulocyte-macrophage colony-stimulating factor (GM-CSF) autoantibodies, evaluated by the functional method of TF1 cell line proliferation inhibition, were highly positive in the serum (titer of 155), confirming the neutralizing power of antibodies and therefore the autoimmune origin of pulmonary alveolar proteinosis (PAP) [1, 2].

The patient was treated with a combination of meropenem administered intravenously for 6 weeks and a high dose of trimethoprim-sulfamethoxazole relayed per os (800/160 mg 3 times a day) from the 7th day; treatment was continued for 1 year and then replaced with a secondary prophylactic regimen with sulfamethoxazole-trimethoprim at 800/160 mg once a day (ongoing treatment). The patient clinically improved, with total neurological recuperation and total regression of the cerebral abscess on cerebral CT scan control imaging performed in October 2019. Primary PAP was initially treated with dose escalation of recombinant GM-CSF (sargramostim [LEUKINE]) subcutaneous injection, at 500 µg per day. Despite excellent hematopoietic tolerance, recombinant GM-CSF was not effective enough, because the patient presented 3 respiratory distress syndromes during the year, requiring hospitalization in intensive care units and whole lung lavages. Inefficient LEUKINE treatment was

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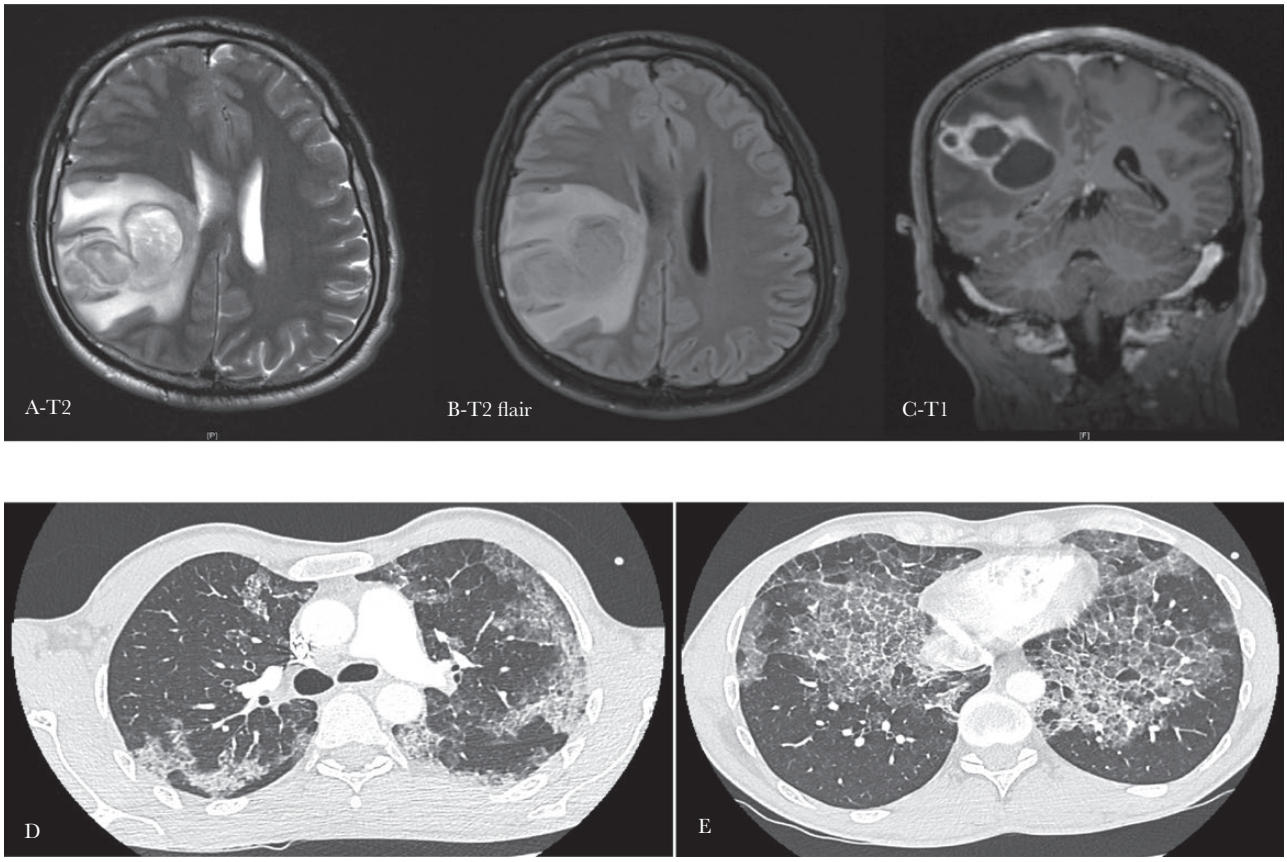

Figure 1. Cerebral and thoracic imaging of a 40-year-old man with cerebral nocardiosis and pulmonary alveolar proteinosis. (A–C). Magnetic resonance imaging showing a voluminous cerebral parietal abscess. (D–E) Thoracic computed tomodensitometry scan showing diffuse and bilateral interstitial syndrome with thickening of the interlobular septa and a "crazy paving" aspect, which is typically found in pulmonary alveolar lipoproteinosis.

interrupted and second-line rituximab was initiated with good tolerance and clinical stabilization. (The patient's written consent was obtained. The design of the work has been approved by local ethical committees under the number of registration: 2016-024.)

In this study, we report the first case of *N abscessus* cerebral infection with anti-GM-CSF autoantibodies and documented PAP (Tables 1 and 2). In the literature, 3 cases of nocardial infection with anti-GM-CSF antibodies and documented PAP have been reported: 36 cases of PAP associated with nocardial infection without specifying the presence of anti-GM-CSF autoantibodies and 4 cases of nocardial infection with anti-GM-CSF autoantibodies without PAP were found (Tables 1 and 2). These observations highlight the promotive role of anti-GM-CSF autoantibodies in the occurrence of these 2 diseases, nocardial infection, and PAP.

Pulmonary alveolar proteinosis is mostly autoimmune (90% of cases), and in such cases, it is characterized by a high level of anti-GM-CSF autoantibodies, whereas hereditary PAP results in mutations in genes encoding the GM-CSF receptor [3].

Secondary infection is the most common and threatening complication of PAP, occurring in 5%–13% of cases and accounting for 10%–20% of deaths [3]. Patients with PAP are known to be more susceptible to bacterial, mycobacterial, and fungal infections such as nocardiosis, mycobacteriosis, aspergillosis, and cryptococcosis [3]. The association between PAP and opportunistic infection has been reported since the first description of the disease in 1958 by Rosen et al [4], with 2 cases of cryptococcosis and 2 cases of nocardiosis among the 27 patients described. More recently, a review of opportunistic infections occurring in 75 patients with PAP found 43% positivity for *Nocardia* spp infection, followed by mycobacterial and fungal infections representing 37% and 20% of the patients, respectively (Table 1) [5-12]. Disseminated or meningeal cryptococcal infections have led investigations to identify the presence of anti-GM-CSF autoantibodies in patients without a history of PAP (Table 2) [13-17]. Similarly, by screening the serum of 7 patients presenting with central nervous system or disseminated nocardiosis, Rosen et al [13] detected anti-GM-CSF autoantibodies in 5 of the 7 samples. None of the patients had PAP initially, and 2 developed PAP during follow-up replace by ref 13-17 (Table 2). Other cases of anti MG GM-CSF-CSF auto antibody without PAP have been described.

We decided to treat the patient with prolonged trimethoprim-sulfamethoxazole as a secondary prophylaxis because we considered the patient immunocompromised. In addition, whereas

Table 1. Nocardiosis (Cerebral and/or Disseminated) Associated With Pulmonar Alveolar Proteinosis With or Whithout Anti-GM-CSF Autoantibodies

Number of Patients/ Date/Country	Age/Sex	Infectious Focus	Species	Anti-GM-CSF Antibodies	Treatment	Evolution	Ref
32 patients 1950–2010 Worldwide	65% male 35% female Mean age 35	75% pulmonar (n = 24) 19% (n = 6) cerebral 6% other (n = 2)	*Nocardia asteroides* 19 (59%) *Nocardia brasiliensis* 1 (3%) *Nocardia farcinica* 1 (3%) *Nocardia* spp 11 (34%)	Not performed	Unspecified antibiotherapy (n = 20) Surgery (n = 6)	41% died	5
1 patient 2010 Japan	37/male	Pulmonar	NA	Presence	NA	NA	6
2 patients 1990–2010 China	NA	NA	NA	NA	NA	NA	7
1 patient 2014 Spain	50/male	Pulmonar	*N farcinica*	Not performed	Amikacin 6 weeks and TMP-SMX 6 months	Full recovery	8
1 patient 2015 Iran	42/male	Cerebral abscesses	*N asteroids*	Not performed	TMP-SMX, meropenem and amikacin 2 months, relayed TMP-SMX	No improvement	9
1 patient 2017 Spain	49/male	Cerebral abscess	*N farcinica*	Not performed	12 months of AMC and minocycline	Full recovery	10
1 patient 2002–2016 Brazil	NA	NA	NA	Not performed	Adapted antibiotherapy (not specified)	Full recovery	11
1 patient 2020 United States	62/male	Pulmonar	*N brasiliensis*	Presence	Amikacin 6 weeks and TMP-SMX 6 months	Full recovery	12
1 patient 2018 France	40/male	Cerebral abscess	*Nocardia abscessus*	Presence	Meropenem 6 weeks and TMP-SMX 12 months	Full recovery	Our case

Abbreviations: AMC, amoxicillin/clavulanate; GM-CSF, granulocyte macrophage colony-stimulating factor; NA, not available; Ref, reference; TMP-SMX, trimethoprim-sulfamethoxazole.

Table 2. Reported Cases of Opportunistic Infections Associated With Anti-GM-CSF Autoantibodies Without Pulmonar Alveolar Proteinosis

Infectious Agent	Number of cases	Age/Sex	Infection Focus	Species	Anti-GM-CSF Antibodies	Presence of PAP	Treatment	Outcome	Ref
Nocardiosis	1	44/male	Cerebral	*Nocardia paucivorans*	Presence	Scanographic infiltrates but normal respiratory function tests, PAP diagnosis not retained	Amikacin and TMP-SMX 8 weeks, TMP-SMX and linezolide 8 weeks, then TMP-SMX alone	Full recovery	13
	2	73/male	-cutaneous, pulmonar, and subsequent cerebral nocadiosis-pulmonar aspergillosis	*Nocardia* spp *Aspergillus fumigates*	Presence	No evidence of PAP	Imipenem amikacin voriconazole, then TMP-SMX, AMC, voriconazole per os + subcutaneous GM-CSF	Neurologic relapse	
	3	61/male	cerebral nocardiosis	*Nocardia farcinica*	Presence	No evidence of PAP	Imipenem amikacin IV 8 weeks and TMP-SMX and moxifloxacin	Neurologic relapse	
	4	50/male	cerebral nocardiosis	*N paucivorans*	Presence	No evidence of PAP	12 months of TMP-SMX, imipenem, and moxifloxacin	Full recovery	
	5	52/female	cerebral and pulmonary nocardiosis and disseminated cryptococcosis	*Nocardia asteroids*	Presence	No evidence of PAP	NA	NA	
Cryptoccocosis	1	49/female	Meningitidis	Cryptococcus *gattii*	Presence	NA	NA	NA	14
	2	NA/female	Meningitidis	*C gattii*	Presence	NA	NA	NA	
	3	NA/female	Meningitidis	*C gattii*	Presence	NA	NA	NA	
	4	NA/male	Meningitidis	*C gattii*	Presence	NA	NA	NA	
	5	NA/female	Meningitidis	*C gattii*	Presence	NA	NA	NA	
	6	NA/female	Meningitidis	*C gattii*	Presence	NA	NA	NA	
	7	NA/male	Meningitidis	*C gattii*	Presence	NA	NA	NA	
	8	20/female	Meningitidis	*Cryptococcus neoformans*	Presence	Develop PAP 1 year later	AmphoB + 5-FC, relayed by FLC	Full recovery	15
	9	31/female	Meningitides	*C gattii*	Presence	NA	AmphoB + 5-FC, relayed by FLC + 5-FC	Full recovery	
	10	48/male	Cryptococcal meningitidis Pulmonar tuberculosis	*C neoformans*	Presence	NA	AmphoB, relayed by FLC Antituberculous therapy	Full recovery	
	11	47/male	Meningitidis	*C neoformans*	Presence	Develop asymptomatic PAP 4 years later	AmphoB + FLC, relayed by FLC	Full recovery	
	12	26/male	Meningitides	*C gattii*	Presence	NA	AmphoB + 5-FC	Full recovery	
	13	34/male	Meningitides	*C gattii*	Presence	NA	AmphoB + 5-FC + therapeutic LP	Sequelae	
	14	32/male	Meningitides	*C gattii*	Presence	NA	AmphoB + 5-FC + therapeutic LP	Sequelae	
	15	48/male	Pulmonary cryptococcoma, and subsequent cerebral cryptococcosis	*C gattii*	Presence	Scanographic infiltrates but normal respiratory function tests, PAP diagnosis not retained	AmphoB + 5-FC, relayed by FLC	Full recovery	16
	16	43/male	Solitary cerebral abscess	*C gattii*	Presence	NA	Surgically treated, AmphoB + 5-FC, relayed by several triazoles	Full recovery	
	17	37/male	Disseminated	NA	Presence	No evidence of PAP	AmphoB + 5-FC + therapeutic LP	Death	17
	18	40/male	Disseminated	NA	Presence	No evidence of PAP	AmphoB + 5-FC, relayed by FLC	Severe sequelae	
	19	59/female	Ocular	NA	Presence	No evidence of PAP	Intraoculaire AmphoB relayed by voriconazole	Full recovery	
	20	37/male	Meningitides	NA	Presence	No evidence of PAP	AmphoB + 5-FC 2 weeks, relayed by FLC	Full recovery	

Abbreviations: AMC, amoxicillin/clavulanate; AmphoB, amphotericin B; CNS, central nervous system; FLC, fluconazole; GM-CSF, granulocyte macrophage colony-stimulating factor; IgG, immunoglobulin G; IV, intravenous; LP, lumbar puncture; MXF, moxifloxacin; NA, not available; PAP, pulmonary alveolar proteinosis; Ref, reference; TMP-SMX, trimethoprim-sulfamethoxazole, 5-FC, 5-flucytosine.

whole-lung lavage is still the gold standard for autoimmune PAP, subcutaneous and inhaled GM-CSF supplementations were reported to be beneficial [3]. In prospective studies, daily injection of GM-CSF was effective in 43% to 75% of patients at 1 year and 12 weeks, respectively. In addition, inhaled GM-CSF presents several advantages: reduced cost, reduced side effects, and 66% efficiency at 3 years. Two clinical trials evaluating the effect of inhaled GM-CSF on PAP patients are ongoing: IMPALA and PAGE [3]. More recently, rituximab has been proposed as a therapeutic option for the treatment of autoimmune PAP with controversial results. Some series of patients treated with rituximab showed PaO_2, pulmonary function test, and chest CT scan lesion amelioration, whereas retrospective reports on 13 PAP patients did not support rituximab as a second-line therapy [3, 18]. Plasmapheresis has not shown promising results, and few cases of lung transplantation to treat severe PAP have been reported [3].

Granulocyte-macrophage colony-stimulating factor, a cytokine produced by T cells, B cells, macrophages, endothelial cells, and fibroblasts, is involved in proinflammatory functions such as the differentiation, adhesion, chemotaxis, and activation of inflammatory and immune cells such as monocytes, macrophages, neutrophils, microglia, and dendritic cells [19]. Granulocyte-macrophage colony-stimulating factor is also a hematopoietic growth factor that activates the proliferation of myeloid cells from bone marrow progenitors [19, 20]. Although overproduction of GM-CSF is associated with rheumatoid arthritis, multiple sclerosis, juvenile myelomonocytic leukemia, and chronic myelomonocytic leukemia, GM-CSF deficiency induces a lack of maturation of alveolar macrophages and accumulation of surfactant in the alveolar space, leading to PAP [3]. Anti-GM-CSF antibodies neutralize and clear GM-CSF in cases of PAP, which could induce an immune deficiency favoring opportunistic diseases [21]. Indeed, GM-CSF deficiency causes impaired antigen presentation, and reductions in dendritic cell numbers in nonlymphoid tissues, as well as in phagocytosis and bactericidal activities of neutrophils, promoting immunodeficiency [21]. Thus, the role of GM-CSF is not limited to the lungs and seems to be decisive in the host's defense against pathogens, especially against opportunistic infections such as nocardia. On the basis of our experience and the research developed here, we therefore propose to test all patients with cerebral or disseminated nocardiosis for immunodeficiency with at least serum protein electrophoresis, immunophenotyping of circulating lymphocytes, presence and neutralizing activity of anti GM-CSF antibodies, and chest CT scan to diagnose asymptomatic pulmonary alveolar lipoproteinosis.

In conclusion, the presence of anti-GM-CSF autoantibodies should be considered an underdiagnosed immunodeficiency. Systematic screening of these autoantibodies in patients with nocardial, fungal, or mycobacterial infection will allow us to characterize this immunodeficiency and prevent the outbreak of disseminated infectious and pulmonary diseases.

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