Transplant International

LETTER TO THE EDITORS

Autoantibodies against granulocyte macrophage colony-stimulating factor and *Nocardia* infection in solid organ transplant recipients

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Dear Editors,

Nocardiosis is a rare but potentially severe bacterial opportunistic infection that may occur after solid organ transplantation (SOT), typically among thoracic transplant recipients and/or in recipients with a high degree of immunosuppression due to anti-rejection therapy [1]. However, nocardiosis may also occur late after transplantation among minimally immunosuppressed patients, suggesting that additional risk factors exist [1]. Recently, autoantibodies against granulocyte macrophage colony-stimulating factor (GM-CSF) have been identified among five previously healthy patients with disseminated nocardiosis [2]. These autoantibodies likely promote nocardiosis by reducing neutrophil and macrophage activation, phagocytosis, and bactericidal activity [2]. We hypothesized that anti-GM-CSF autoantibodies might be involved in post-SOT nocardiosis, especially in patients who apparently have a relatively low degree of immunosuppression.

We retrospectively analyzed sera from SOT recipients included in a European case–control study and compared the prevalence of anti-GM-CSF autoantibodies between SOT recipients with nocardiosis (cases) and SOT recipients without nocardiosis (controls) [1]. Cases were SOT recipients who had a diagnosis of nocardiosis between 2000 and 2014. Matched control patients were SOT recipients who (i) had received the same type of transplanted organ in the same institution as the case; (ii) had received their transplant at about the same time as the case; (iii) had no evidence of nocardiosis after

doi:10.1111/tri.13727

Table 1. Clinical and biological characteristics of 22 nocardiosis cases and 45 matched controls for whom at least one serum sample has been stored and analyzed to investigate the presence of anti-GM-CSF autoantibodies.

Characteristics	Cases (n = 22)	Controls (n = 45)
Clinical characteristics		
Age at diagnosis (years; mean ± SD)	57 ± 16	50 ± 14
Male	16 (70)	29 (60)
Donor age (mean ± SD) n = 63	48 ± 19	46 ± 18
Length of stay in the intensive care unit after transplantation (days; mean ± SD)	6 ± 10	6 ± 9
Transplanted organ		
Kidney	17 (77)	34 (75)
Heart or lung	1 (5)	3 (7)
Chronic obstructive pulmonary disease after transplant	2 (10)	1 (2)
Acute rejection episode in the 6 months before diagnosis of nocardiosis	4 (20)	4 (10)
Cytomegalovirus infection in the 6 months before diagnosis of nocardiosis	3 (10)	1 (2)
Cytomegalovirus disease in the 6 months before diagnosis of nocardiosis	1 (5)	1 (2)
Bloodstream infection before diagnosis of nocardiosis	3 (10)	5 (10)
Therapeutic characteristics		
Dose of corticosteroids at diagnosis of nocardiosis (mg*) (mean ± SD)	7 ± 3	5 ± 3
High-dose steroids in the 6 months before diagnosis of nocardiosis	4 (20)	4 (10)
Cyclosporin at diagnosis of nocardiosis	3 (10)	10 (20)
Tacrolimus at diagnosis of nocardiosis	19 (90)	30 (70)
High calcineurin inhibitor blood level in the month before diagnosis of nocardiosis[†]	8 (40)	5 (10)
Use of antiproliferative agents (AZA or MMF) at diagnosis	18 (82)	40 (89)
Use of triple immunosupression[‡] at diagnosis	17 (77)	26 (58)
Cotrimoxazole prophylaxis at diagnosis of nocardiosis	5 (20)	5 (10)
Biological characteristics		
WBC count 1 month before diagnosis of nocardiosis (×1000/µl; mean ± SD) n = 64	10.0 ± 4.2	7.2 ± 2.5
Lymphocyte count at 2 months post-transplant (×1000/µl; mean ± SD) n = 59	0.9 ± 0.7	1.0 ± 0.7
Lymphocyte count 1 month before diagnosis of nocardiosis (×1000/µl; mean ± SD) n = 60	0.8 ± 0.5	1.6 ± 1.1
Neutrophil count 1 month before diagnosis of nocardiosis (×1000/µl; mean ± SD) n = 60	8.3 ± 4.1	5.2 ± 2.0
Nocardiosis characteristics		
Time from transplantation to diagnosis of nocardiosis (months; median, range)	17 [1–171]	NA
Involved organs		
Lung	19 (90)	NA
Skin and soft tissue	8 (40)	NA
Brain	4 (20)	NA
Disseminated infection	9 (40)	NA
Patient outcomes		
Relapse of nocardiosis	2 (10)	NA
Alive 12 months after diagnosis of nocardiosis	21 (95)	NA

AZA, azathioprine; MMF, mycophenolate mofetil; NA, not applicable; SD, standard deviation; WBC, white blood cell.

Data are n (%) unless otherwise indicated. n: number of data analyzed (when <67).

*All the corticosteroid doses are expressed in milligrams (mg) of methylprednisolone equivalent per day.

[†]High calcineurin inhibitor level was defined as a trough blood level >10 µl/ml for tacrolimus and >300 ng/ml for cyclosporine.

[‡]Tripe immunosuppression was defined as the combination of corticosteroids, calcineurin inhibitor, and antiproliferative agent.

transplant; and (iv) had survived as long as the case had prior to the diagnosis of nocardiosis. For cases, the date of diagnosis of nocardiosis was defined as the day on which the first clinical sample (e.g., sputum) yielding *Nocardia* spp. was collected. For control patients, a corresponding date was chosen on the basis of their matched case's date of diagnosis, in order to obtain a similar period of time from transplantation.

Specifically, we analyzed 56 sera sampled around the date of transplantation (±1 month; representing 17 cases and 39 controls) and 39 sera sampled around the date of *Nocardia* infection (±6 months; representing 14

Transplant International 2020; 33: 1827–1829

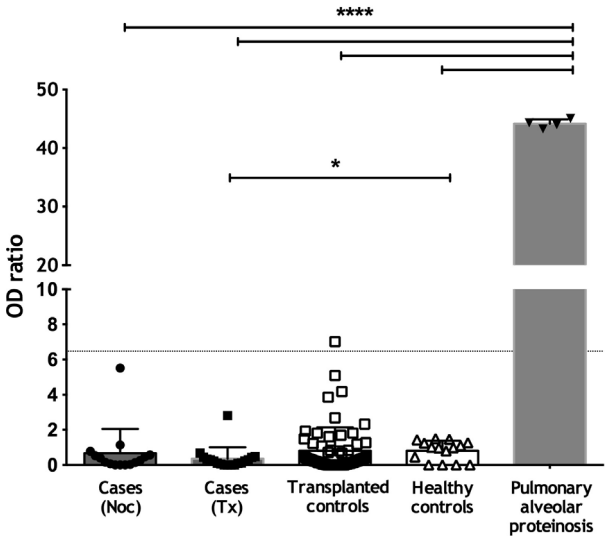

Figure 1 Detection of anti-GM-CSF autoantibodies among nocardiosis cases, matched transplanted controls, healthy controls, and patients with pulmonary alveolar proteinosis (PAP). Sera from nocardiosis cases were sampled around the transplantation date (Tx) or around the date of nocardiosis diagnosis (Noc). Comparisons between groups were performed using Mann–Whitney–Wilcoxon test in Prism® (Graphpad). ***$P < 0.0001$. *$P = 0.034$. OD: Optical density. The presence of anti-GM-CSF antibodies was determined using a previously described ELISA [3]. For controls, we used four sera from patients with acquired PAP—that is, a pulmonary disease induced by anti-GM-CSF autoantibodies—(-positive controls), and ten sera from healthy adult subjects (negative controls). Results were expressed as an OD ratio: mean optical absorbance of the 1/100 diluted sample divided by mean absorbance of a 1/100 diluted negative control. Results for PAP samples were expressed as the mean absorbance of the 1/1000 dilution divided by the mean absorbance of the 1/100 diluted negative control and then multiplied by 10. The dotted line represents the positivity threshold of the assay (6.1 OD ratio) as defined by a receiver operating characteristic (ROC) analysis of values in controls and PAP patients. At this threshold, the sensitivity and specificity of the assay are 100% and 98.7%, respectively.

cases and 25 controls). Characteristics of included cases and controls are summarized in Table 1.

None of the patients exhibited a significant level of autoantibodies against GM-CSF, suggesting that these antibodies are not associated with nocardiosis in SOT recipients (Fig. 1). The physiopathology of post-transplant nocardiosis remains poorly understood, and it is unclear why nocardiosis sometimes occur late after transplantation among minimally immunosuppressed SOT recipients. It remains possible that occult immune defect(s) increase the susceptibility to nocardiosis after transplantation. There is a need for a simple, functional test to predict the risk of nocardiosis (and other opportunistic infections) in SOT recipients. It is also possible that environmental factors play an important role in the occurrence of *Nocardia* infection after SOT, given that *Nocardia* spp. can be found in a variety of environments such as dust, soil, decaying vegetation, and water.

Funding

This work was supported by the "Bourse Junior 2015—Société de Pathologie Infectieuse de Langue Française" (DL).

Conflict of interest

The authors declare no conflicts of interest.

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