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Case Report

Mucosal Bacterial Immunotherapy in Solid Organ Recipients with Recurrent Respiratory Tract Infections: Case Report

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ABSTRACT

Bacterial infections are the most frequent infectious complications among solid organ recipients. These complications are associated with a high morbidity and mortality, despite recent advances in antimicrobial prophylaxis in the transplant setting. New therapeutic modalities are warranted. We present here a retrospective study based on medical records review of 2 solid organ recipients that were treated with mucosal bacterial immunotherapy because of recurrent bacterial respiratory infections long time after transplantation. A successful decrease of the frequency of bacterial respiratory infections during a period of up to 8 years was observed in one of the patients. We suggest that clinical trials in this field are warranted.

Introduction

It is well known that in 2050 infections by resistant bacteria will be one of the first causes of death in the world. Taking this into account all diagnostic improvements and new anti-bacterial therapeutic strategies warrant to be evaluated. Globally, community-acquired respiratory tract infections account for a large proportion of antibiotic prescriptions and visits to family practitioners. Bacterial infections are the most frequent infectious complications among solid organ recipients. This complication is associated with a high morbidity and mortality, despite recent advances in antimicrobial prophylaxis in the transplant setting.

Bacterial infections are predominant during the first two months post-liver transplantation and affect patient and graft survival [1]. They might cause surgical site infections, including deep intra-abdominal infections, bacteremia, pneumonia, catheter-related infections and urinary tract infections. Recently, the emergence of multi-drug resistant bacteria is of concern in liver transplantation. To prevent post-transplant bacterial infections, proper strategies need to be addressed based upon center-specific information. Beyond 12 months, the risk of opportunistic infections wanes as immunosuppression is reduced. Recipients continue to be at risk for community acquired infections including upper and lower tract respiratory infections. Infections with viruses, bacteria, and fungi have all been associated with the development of bronchiolitis obliterans syndrome (BOS, chronic allograft rejection) in lung transplant recipients [2, 3]. Lung transplant recipients have a higher risk of infectious complications than recipients of other solid organs. After the first 6 months following transplantation, lung recipients are frequently affected by viral and bacterial respiratory tract infections. Presentation of late viral and/or bacterial infections may be associated with a secondary decline in respiratory function [4].

The objective of this study was to preliminary evaluate in a small case series if mucosal bacterial immunotherapy can produce clinical improvement in solid organ recipients with community acquired recurrent respiratory tract infections long time after transplantation.

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Methods

This was a retrospective study based on medical records review of 2 solid organ recipients that were treated with mucosal bacterial immunotherapy because of recurrent bacterial respiratory infections long time after transplantation. These patients were from of our clinical immunology unit in Madrid, Spain. The medical records were evaluated. There was no loss of follow-up.

Mucosal bacterial immunotherapy (Bactek, Inmunotek, Madrid, Spain) consisted of a suspension of inactivated whole bacteria (equivalent to 10^9 bacteria/mL), containing a mixture of selected strains of bacteria frequently present in infected tonsils and in the oropharyngeal mucosa *Staphylococcus aureus* (15%), *S. epidermidis* (15%), *Streptococcus pneumoniae* (60%), *Klebsiella pneumoniae* (4%), *Branhamella catarrhalis* (3%) and *Haemophilus influenzae* (3%). The route of administration was sublingual by means of spraying 2 puffs of 100 µl each/puff) daily, avoiding the concomitant intake of beverage or food. The delivered dose was maintained in the oral mucosa for a period of 2 minutes and then swallowed. The following schedule was used: days 1 and 2, one and two sublingual puffs, respectively, and thereafter 2 puffs daily up to day 90 [5].

Case Description

Case 1

A 61-year-old white woman. No previous allergic diseases. First liver transplantation was performed in 1987. Etiology of transplantation was autoimmune liver disease associated with antinuclear antibodies. In 1996 a re-transplantation was necessary due to de novo autoimmune disease mediated by anti-GSTT1 antibodies. Recurrent bronchitis was persistent after liver transplantation. At least 3 episodes per year required antimicrobial therapy. Quality of life of this patient was affected by the recurrence of respiratory infections. Comorbidities of transplantation included chronic anaemia, arterial hypertension, renal failure due to toxicity of calcineurin inhibitors. Due to persistent bacterial respiratory infections the patient was referred to the clinical immunology unit for an extended immunological evaluation that was performed in 2012. IgG hypogammaglobulinemia was demonstrated (447 mg/dL, normal range 650-1610 mg/dL). IgG2 (0.386 g/L, normal range 1.47-6.29 g/L) and IgG4 (0.01 g/L, normal range 0.015-0.959 g/L) were low as well as anti-pneumococcal polysaccharide antibody titers (0.8 md/dL, normal range >1 mg/dL). Anti-pneumococcal antibodies remained low despite repeated vaccination (Table 1).

Table 1: Immunological parameters in a liver recipient treated with mucosal bacterial immunotherapy.

Parameter	06.12	03.13	11.13	10.14	04.18	02.19
IgG mg/dL	447	592	675	799	769	652
IgG2 mg/dL	0.386	-	-	-	0.77	0.456
IgG4 mg/dl	0.01	-	-	-	0.014	0.01
Anti-PPS mg/dL	0.8	0.6	1.7	0.5	0.3	1
C3 mg/dL	78	80	82	87	80	93
CD4 cells/uL	480	439	381	441	546	586
NK cells/uL	8	7	7	26	44	56
PPS vaccine, type	-	PS-23	-	CONJ-13	-	PS-23
MBI, months	3	3	3	3	3	3

Anti-PPS: anti pneumococcal polysaccharide IgG titers; CONJ-13: 13 serotypes conjugated pneumococcal vaccine; MBI: mucosal bacterial immunotherapy; PPS: pneumococcal polysaccharide; PPS vaccine: pneumococcal vaccine; PS-23: 23 serotypes pneumococcal polysaccharide vaccine.

Immunosuppressive drugs at the time of this immunological evaluation included mofetil mycophenolate at a dose of 250 mg bid and prednisone 5 mg bid. Retrospective clinical history was compatible with a primary antibody deficiency with recurrent respiratory infection since she was 4-year-old including otitis requiring drainages, sinusitis and recurrent bronchitis. Unfortunately, there not available baseline levels of IgG subclasses or specific anti-pneumococcal antibodies before transplantation, so the antibody deficiency was defined as a secondary antibody deficiency after liver transplantation. At the time of the immunological evaluation, due to IgG hypogammaglobulinemia and specific antibody failure to pneumococcal polysaccharide and conjugated vaccines, intravenous immunoglobulins was a therapeutic option but the patient was reluctant to start this therapy at this time.

Between 2012 and 2018 the patient was treated with mucosal bacterial immunotherapy as outpatient monotherapy for recurrent respiratory infections without antimicrobial therapy. The patient received treatment for a period of 3 months once a year during 7 years. During the last two years (2018-2010) she was changed to mucosal bacterial immunotherapy including V104 S Pneumoniae 50%, V193 Neisseria sp 50%. These microbial agents were recovered after nasopharyngeal swab and culture.

During follow up there was a period of 3 months with otitis requiring oral antibiotics. This period developed as a single episode between immunotherapy cycles. There was no recurrence of bronchitis requiring antibiotics. In (Table 1) a summary of immunological data is disclosed. Improvement of the patient coincided with restoration of IgG levels and CD4 counts. During follow-up there was no evidence of other infections or immunotherapy related complications. The allograft is doing well. There were no episodes of rejection.

Case 2

A 48-year-old white male patient with bronchiectasis secondary to common variable immune deficiency (CVID) receiving lung transplantation. The patient had been diagnosed with CVID in 1988. Past history included recurrent pneumonia and frequent chest infections. He also associated toxoplasmosis in 1981 and pulmonary tuberculosis in 1988. Other CVID related complications included chronic

thrombocytopenia, lymphoid hyperplasia with splenomegaly (> 20 cm) and chronic hepatopathy. His sister had a diagnosis of CVID. CVID diagnosis was defined by low IgG, IgA and IgM levels. Immunoglobulin subclasses revealed IgG subclass 1 to 4 deficiency. He had low antibody levels to the recall antigens of tetanus toxoid and pneumococcal polysaccharides, and both failed to normalise after vaccination. There were low numbers of B CD19+ lymphocytes (1.39%, normal range 6-19%) with CD4 level (< 350 cells/uL, normal range 300-400 cells/uL), CD8 (< 200 cells/Ul, normal range 200-1200 cells/uL) and a reversed CD4/CD8 ratio (0.96, normal range 1-3.60). CD19+CD27+IgM-IgD- memory class-switched B-cells were low (1.63%). Increased T cells activation was demonstrated, CD4+CD38+DR+ 28.74%, CD8+DR+ 72%. The lymphocytes also showed poor *in vitro* functional activity with subnormal lymphocyte proliferative responses to phytohaemagglutinin, concanavalin A and pokeweed mitogen. A sweat sodium and chloride test to exclude cystic fibrosis was normal.

Following replacement intravenous immunoglobulin therapy, the frequency of chest infections reduced. He required high doses of IVIG to maintain through IgG levels > 600 mg/dL due to IgG hypercatabolism. Despite replacement IgG therapy, over the next years he progressively deteriorated becoming breathless on minimal exertion and requiring long term 24 hours/day oxygen therapy and cyclical antibiotics. At this stage he was referred for bilateral lung transplantation that was performed in December 2004. Replacement immunoglobulin therapy was adjusted before transplantation to every two weeks with the aim of maintaining IgG levels >1000 mg/dL. He received no induction therapy. Initial maintenance triple immunosuppressive therapy was with cyclosporine, azathioprine and prednisone. Tacrolimus based maintenance was introduced later. Antimicrobial prophylaxis included amphotericin B and IV ganciclovir. GM-CSF therapy was also necessary.

The post-operative course was complicated by acute cellular rejection in 2005, CMV disease in 2007, skin cancer in 2010, bacterial pneumonia in 2013, diabetes mellitus in 2018 and acute myocardial infarction in 2018. He has also developed other long-term transplant related complication such as chronic renal failure. In the early post-transplant period immunoglobulin therapy was adjusted to weekly infusions to guarantee IgG > 1000 mg/dL. For long-term maintenance immunoglobulin therapy continued on a bi-weekly basis. During the last 3 years immunoglobulin therapy is administered each 3 weeks. Periodic spirometric tests at distinct times after transplantation showed that lung function was maintained within normal values and he was back at work. In 2012, despite immunoglobulin replacement and antimicrobial prophylaxis, recurrent upper and lower respiratory tract infections developed again.

Between 2014 and 2016 the patient was treated with mucosal bacterial immunotherapy as outpatient therapy for recurrent respiratory infections combined with levofloxacin and azithromycin. The patient received treatment for a period of 3 months once a year during 2 years. Only a partial improvement of the frequency of respiratory infections was observed. An increase of anti-pneumococcal antibody titers was observed (7.7 to 10 mg/dL, normal range >1 mg/dL). However, the patient continued having recurrent bacterial infections and more than 10 years after lung transplantation he developed bronchiectasis again. A decrease in lung functional test values has been documented during the last 2 years. The last immunological evaluation performed at October 2020 was as follows: IgG 1360 mg/dL (normal range 650-1610 mg/dL), IgA < 7 mg/dL (normal range 85-468 mg/dL), IgM <4 mg/dL (45-276 mg/dl), kappa free light chain <0.05 mg/dL (normal range 0.3-1.9 mg/dL), lambda free light chain < 0.13 mg/dL (normal range 0.6-2.6 mg/dL), complement C3 93 mg/dL (normal range 87-182 mg/dL), complement C4 34 mg/dL (normal range 17-53 mg/dL), ALT 42 U/L (normal range 5-41 U/L), GGT 298 U/L (normal range 10-60 U/L), platelets $48x10^3$/uL (normal range $140-400x10^3$/uL), total lymphocytes $1.2x10^3$/uL (normal range $1.3-3.5x10^3$/uL), CD4 377 cells/uL (normal range 300-400 cells/uL), CD8 475 cells/uL (normal range 200-1200 cells/uL). Up to now this CVID patient has accumulated more than 15 years after bilateral lung transplantation.

Discussion

To the best of our knowledge, this is the first communication of the use of immunotherapy with a sublingual bacterial preparation to treat recurrent respiratory infections in liver and lung recipients. Even if it is limited to two case reports the reported information suggest that mucosal bacterial immunotherapy could be safely administered to liver and lung recipients who have community acquired recurrent respiratory infections long time after transplantation. The use of this immunotherapy approach is in line with the advice of the health organizations (WHO, EMA, FDA) for seeking new treatment alternatives against bacterial diseases, given the rise and spread of antibiotic resistant bacterial infections [6].

One patient had sustained clinical improvement during more than 8 years after introduction of this therapy and there were no complications during follow-up. The fact that in this patient clinical efficacy while on mucosal bacterial immunotherapy was simultaneous with low IgG2, IgG4 and specific antibodies to pneumococcal antigens, highlight the potential impact of this therapy in this patient. Reconstitution of total IgG levels, CD4 and NK counts occurred at the same time mucosal bacterial immunotherapy was administered.

The second patient had a complex primary antibody deficiency but an unusual prolonged survival after lung transplantation [7]. Despite antimicrobials and intravenous immunoglobulin therapy recurrent bacterial respiratory infections developed long time after transplantation. In this patient mucosal bacterial immunotherapy was not useful to prevent recurrences of bacterial respiratory infections and development of bronchiectasis. This observation suggest that this therapy might not be useful in all cases. Even if this report is limited to only 2 cases, the presented information might suggest than mucosal bacterial immunotherapy could be an interesting option for the therapy of selected cases with recurrent respiratory infection long time after solid organ transplantation.

Mucosal bacterial immunotherapy offers potential advantages to conventional systemic vaccination, such as higher levels of antibodies and protection at the airway surface. Previous studies have evaluated the role of nasal, oral and sublingual vaccines against bacterial respiratory pathogens, such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Moraxella catarrhalis*, *Bordetella pertussis*, *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* [8]. Experimental studies have demonstrated that mucosal bacterial vaccine

preparation endorses human dendritic cells with the capacity to generate Th1/Th17 and IL-10-producing T-cells by mechanisms depending on spleen tyrosine kinase and myeloid differentiation myeloid differentiation primary response MyD88-mediated pathways [9].

We have previously reported using the same mucosal bacterial immunotherapy preparate that in patients with recurrent bacterial infections there was a significant increase in the proliferative response of CD3+CD4+ T cells specific to the mucosal vaccine antigens at month 6 in comparison to baseline and a significant increase in total CD3+ T cells. In this study no differences were observed between baseline and month 6 in levels of total immunoglobulins, specific antibodies and B or NK cell subsets [5]. The increase in IgG, CD4 and NK counts observed in patient 1 could be associated with individual reconstitution after long time after liver transplantation but coincided with the long term mucosal bacterial vaccine immunotherapeutic period of up to 8 years. There was otherwise no evidence of rejection during this period. If long term repeated mucosal vaccine immunization might be associated with the observed reconstitution must be evaluated in future studies. Taking into account the observations in these cases we suggest clinical trials evaluating the safety and efficacy of mucosal bacterial immunotherapy are warranted in solid organ recipients having recurrent bacterial infections late after transplantation.

Patient Perspective

Patient 1: This therapy has improved significantly my quality of life because I am free of respiratory infections; Patient 2: The mucosal vaccine was associated with only a temporal decrease of my respiratory infections.

Contribution to the Field Statement

Recurrent bacterial infections are common late after liver and lung transplantation. These complications are associated with morbidity and mortality and also with an important decrease of quality of life of the patients. The information provided in these case reports describes for the first time the potential role of mucosal bacterial immunotherapy for the treatment of recurrent respiratory bacterial infections that developed in 2 solid organ recipients long time after liver and lung transplantation, respectively. These observations focused on clinical and immunological data. Mucosal bacterial immunotherapy might be helpful for the control of these infectious complications in solid organ recipients. We propose that clinical trials are warranted in this field.

Consent

Written informed consent was obtained from the individuals for the publication of any potentially identifiable data included in this article.

References

1. Kim S (2014) Bacterial infection after liver transplantation. *World J Gastroenterol* 20: 6211-6220. [Crossref]
2. Witt CA, Meyers BF, Hachem RR (2012) Pulmonary infections following lung transplantation. *Thorac Surg Clin* 22: 403-412. [Crossref]
3. Gagliotti C, Morsillo F, Moro ML, Masiero L, Procaccio F et al. (2018) Review. Infections in liver and lung transplant recipients: a national prospective cohort. *Eur J Clin Microbiol Infect Dis* 37: 399-407. [Crossref]
4. Dubert M, Visseaux B, Birgy A, Mordant P, Metivier A et al. (2020) Late viral or bacterial respiratory infections in lung transplanted patients: impact on respiratory function. *BMC Infect Dis* 20: 176. [Crossref]
5. Alecsandru D, Valor L, Sanchez Ramon S, Gil J, Carbone J et al. (2011) Sublingual therapeutic immunization with a polyvalent bacterial preparation in patients with recurrent respiratory infections: immunomodulatory effect on antigen-specific memory CD4+ T cells and impact on clinical outcome. *Clin Exp Immunol* 164: 100-107. [Crossref]
6. Vivas R, Barbosa AAT, Dolabela SS, Jain S (2019) Multidrug-Resistant Bacteria and Alternative Methods to Control Them: An Overview. *Microb Drug Resist* 25: 890-908. [Crossref]
7. Burton CM, Milman N, Andersen CB, Marquart H, Iversen M (2007) Common variable immune deficiency and lung transplantation. *Scandin J Infectious Dis* 39: 362-367. [Crossref]
8. Baumann U (2008) Mucosal vaccination against bacterial respiratory infections. *Expert Rev Vaccines* 7: 1257-1276. [Crossref]
9. Benito Villalvilla C, Cirauqui C, Diez Rivero CM, Casanovas M, Subiza JL et al. (2017) MV140, a sublingual polyvalent bacterial preparation to treat recurrent urinary tract infections, licenses human dendritic cells for generating Th1, Th17, and IL-10 responses via Syk and MyD88. *Mucosal Immunol* 10: 924-935. [Crossref]