

Mucosal bacterial vaccines in clinical practice – a novel approach to an old problem?

 João Neiva Machado¹
 José Coutinho Costa¹
 Teresa Costa¹
 Cidália Rodrigues¹

¹. Unidade de Pneumologia – Universidade e Centro Hospitalar de Coimbra, Coimbra, Portugal.

<http://dx.doi.org/10.1590/1806-9282.66.5.659>

SUMMARY

OBJECTIVES: To evaluate the efficacy of mucosal bacterial vaccines (MBV) in reducing the number of exacerbations in patients with chronic respiratory disease.

METHODS: A prospective cohort study of patients followed at the Pneumology Unit of the University and Hospital Centre of Coimbra, with frequent infectious exacerbations (3 or more) despite the best therapeutic strategies employed. MBV was used as additional therapy. The number of exacerbations 1 year before therapy and 1 year after it were analyzed.

RESULTS: A sample of 11 individuals, 45.5% male, mean age 62.5 years. Eight patients had non-cystic fibrosis bronchiectasis, 2 COPD (1 on long-term oxygen therapy), and 1 patient with Mounier Kuhn's syndrome. Three patients were on azithromycin, 1 on inhaled colistin, and 2 on inhaled tobramycin. Out of the 11 patients, one presented complication (fever), which led to a suspension of therapy (excluded from results). Of the 10 patients who completed treatment, 5 had bacterial colonization and were submitted to a custom vaccine. The remaining 6 completed the standard composition. The average of infectious exacerbations in the previous year was 4.3 (0.7 with hospitalization). In the year after therapy, the mean number was 1.5 (0.5 with hospitalization).

CONCLUSION: The results obtained in this study favor the use of bacterial immunostimulation to reduce the frequency of RRI in patients with chronic respiratory disease.

KEYWORDS: Bacterial vaccines. Administration, sublingual. Respiratory tract infections. Respiratory tract diseases.

INTRODUCTION

Antibiotic resistance has been a focus of concern of public health care, and the problem is such that the World Health Organization (WHO) implemented a Global Action Plan in 2015 to tackle it, which includes increasing investment in new medicines, diagnostic tools, vaccines, and other interventions¹.

Approximately 65% of the agents causing human infections form bacterial biofilms that favor chronic infections¹, and the frequent use of antibiotics favors the risk of antibiotic resistance². Recurrent respiratory

infections (RRI) are characterized by at least three episodes of fever, locoregional inflammation, cough, asthma, and wheezing without severe impairment of respiratory functions yearly³. This syndrome is frequent, therefore RRI are a major health-care problem associated with significant morbidity and mortality. They are also associated with the spread of resistance to antibiotics in patients with deficient humoral and cellular immune functions, such as primary and secondary immunodeficiencies and chronic obstructive

DATE OF SUBMISSION: 15-Dec-2019

DATE OF ACCEPTANCE: 28-Dec-2019

CORRESPONDING AUTHOR: João Pedro Neiva Machado
 Quinta dos Vales, São Martinho do Bispo, Coimbra, Portugal - 3041-801
 E-mail: joaoneivamachado@gmail.com

pulmonary disease⁴. Immunostimulant agents of bacterial origin have been used as an adjunct treatment in these patients because of their immunomodulatory properties, increasing immune responses and boosting the innate immune system. For example, several studies have shown that the oral administration of bacterial immune stimulants ameliorates RRI in adults and children by reducing the number, duration, and severity of infectious clinical episodes⁵. Unfortunately, antigens delivered orally are easily degraded during the passage through the acidic environment of the stomach, while antigens delivered intranasally have the demonstrated potential to migrate through the olfactory bulb to the brain, thereby reducing vaccine safety. The sublingual route has been used for many years as a noninvasive and effective immunotherapy for the treatment of allergies to a variety of allergens and, recently, the sublingual administration of bacterial preparations has been proposed as a safer and effective immunotherapy to stimulate strong and long-lasting systemic and mucosal antigen-specific humoral and cell-mediated immunity⁶. Moreover, the induction of immunological memory to a specific pathogen is critical to maintaining long-lasting immune surveillance upon vaccination using the sublingual immunization strategy⁷⁻¹¹.

These bacterial preparations also present a higher likelihood to generate the necessary B and T cell responses for optimal protection at the point of entry of infectious agents, in addition to promoting the levels of systemic immunity generally achieved by conventional injected vaccines⁸.

Mucous membranes are the main route of entry of infectious and environmental agents. Mucosal anti-infective vaccines are combinations of inactivated whole bacteria or fungi or of bacterial lysates that stimulate the immune system, which according to numerous studies, reduce the number of reinfections by the same pathogens and others. The mechanisms underlying this collateral benefit may be due in large part to “trained immunity” rather than the specific wing, which has also been found to contribute to the protective effect².

For mucosal vaccines to be effective, it is desirable that secretory IgA antibody (the hallmark of immune responses at mucosal surfaces) responses should persist, or at least be very rapidly recallable in the face of a pathogenic attack to forestall infection at the mucosal surface. However, it appears that secretory IgA (S-IgA) antibodies do not always persist for long

after the removal of the inducing antigenic stimulus, meaning that these vaccines require repetitive stimulation to reach their full potential¹².

Therapeutic interventions with mucosal anti-infectious vaccines have different advantages over conventional parenteral vaccines: a) they carry out their effects directly at the mucosal infection site and can prevent infection and colonization by pathogens³; b) mucosal immunization may result in the secretion of antibodies in other, more distant mucous membranes, and also systemically¹³; c) they do not require medical personnel for administration and can be used in mass vaccinations and disease prevention campaigns, and d) they are painless and simple to administer. Also, taking into account that gram-positive bacteria stimulate the production of IL-12 and that gram-negative bacteria stimulate the production of IL-10 by monocytes, the combination of gram-positive and gram-negative bacteria in infectious mucosal vaccines seems to provide a synergistic immunologic response that could be greater depending on the pathology involved¹⁴. These preparations for mucosal administration with completely inactivated bacteria are presented to the immune system in a more natural way, maximizing their full potential as immunogens¹⁵ stimulating different immune mechanisms, which have been proven to be very important for complete cellular activation, such as phagocytosis¹⁶.

Besides these advantages, formulations for sublingual immunotherapy have a lower cost of manufacture since non-sterile products can be delivered by this route and endotoxic shock is not a concern¹⁷.

In the present study, we evaluated prospectively whether the daily administration of a polyvalent bacterial preparation via the sublingual route could reduce the number of infectious exacerbations of patients with RRIs.

METHODS

This prospective cohort study aimed to assess whether the daily sublingual administration (4.5 - 6 months, depending on manufacturer instructions) of a polyvalent bacterial preparation could impact on the clinical outcome of patients with RRIs. The study was conducted between January 2016-January 2019, including the treatment and follow-up period. We used a convenience sample of 11 patients affected by RRIs and followed-up at the subspecialty (patients with chronic obstructive diseases and/or

respiratory failure) Pneumology consultation of the Pneumology Unit of the University and Hospital Center of Coimbra.

All patients had suffered RRIs during the 12 months prior to the study and were required to meet the following criteria: 3 or more respiratory infections in the previous 12 months; chronic pulmonary disease (COPD, bronchiectasis, other); recurrent need for oral and/or intravenous antibiotic courses and/or hospitalizations to clear infections in the previous 12 months. The exclusion criteria were: treatment with immunosuppressants (prednisone 10mg/day or equivalent), immunostimulants, gamma globulins within the previous 12 months; patients who had laboratory or clinical criteria for lymphoproliferative disorders or non-respiratory chronic infections. All patients eligible started the immunizations with Bactek® (Immunotek) or Vacinas Bacterianas Diater® when they had asymptomatic clinical status.

Bacterial preparations Bactek® (Immunotek Laboratories, Madrid, Spain) and Vacinas Bacterianas Diater® (Diater Laboratories, Madrid, Spain) are commercially available polyvalent bacterial preparations. Bactek® contains different species of inactivated bacteria at 10^9 bacteria/ml which are frequently present in the respiratory tract: *S. aureus* (15%), *S. epidermidis* (15%), *S. pneumoniae* (60%), *Klebsiella pneumoniae* (4%), *Branhamella catarrhalis* (3%), and *Haemophilus influenzae* (3%). Vacinas Bacterianas Diater® contains *S. pyogenes* (25%), *S. pneumoniae* (25%), *Klebsiella pneumoniae* (25%), and *Haemophilus influenzae* (25%). We established the composition of the patient bacterial preparation based on the most common bacteria causing respiratory infections, combined with sputum analysis, to address for either colonization or the most common infectious pathogen. We found 5 patients had bacterial colonization; they were submitted to a custom vaccine composition with a higher percentage of the colonizing agent (minimum of 10% in the composition) associated with at least 50% of the standard composition.

The preparation was delivered sublingually, two sprays, each day, for 4,5 to 6 months.

Patients were assessed every 3-6 months and every time they had respiratory tract symptoms. RRIs were defined by the presence of diagnostic symptoms for at least 48–72 h. Multiple illnesses were counted only if the patient had been without symptoms for at least 72 h between the end of one episode and the beginning of another.

The clinical status and total number of infectious respiratory episodes that had occurred in the year previous to immunization were recorded for each patient by their attending physician during the 12 months that followed immunization. The number of infectious respiratory episodes prior and after the treatment was considered the main variable for the clinical outcome. Patients were treated with antibiotics for controlling their RRIs at physician criteria.

The study was conducted according to the declaration of Helsinki. Data were obtained through a chart review and analyzed using SPSS® v23. Data were presented as mean and standard deviation (SD) for continuous variables and percentages for categorical variables. Data analysis demonstrated a non-normal distribution, and non-parametric tests (Wilcoxon signed-rank test and Mann-Whitney U test) were therefore employed in the statistical analysis. The significance level of 0.05 was used.

RESULTS

The sample contained 11 patients, 45.5% (n=5) males, with a mean age of 62.5 years (± 9.8). Their clinical characteristics are presented in table 1. One COPD patient (#6) and one NCFB (#10) were on long-term oxygen therapy. Three patients were or used to be on long-term therapy with azithromycin (#5, #9 and #11), 1 patient was on inhaled colistin (#10), and 2 on inhaled tobramycin (#3 and #4). Out of the 11 patients, only one (patient with NCFB) presented a possible complication (fever), which led to the suspension of therapy at the end of the first month its exclusion from the results (table 2). Of the 10 patients who completed the treatment, 5 had bacterial colonization, all of them with *Pseudomonas aeruginosa*, and one also with *Haemophilus influenzae*; they were submitted to a custom vaccine composition (table 2). The remaining patients completed the standard composition.

The mean respiratory infectious episodes in the previous year were 4.3 (± 1.3). Clinical assessment throughout the study showed a significant reduction of the total number of respiratory tract infection episodes after the treatment compared with the number of RRIs scored throughout the 12 months prior to the treatment ($Z=-2.871$; $p = 0.004$) (figure 1). There was no statistically significant difference regarding the laboratory manufacturing the vaccines ($U=8.000$; $p>0.05$). Anecdotally, four patients reported major clinical improvement with therapy.

DISCUSSION

In this pilot study, we observed a remarkable reduction in the frequency of respiratory tract infectious episodes in a cohort of patients with RRIs treated with mucosal bacterial vaccines over a 12-month period after initiation of therapeutic immunization, in comparison to the number of RRIs prior to the treatment and using personalized formulations based on previous sputum bacterial results, aiming to achieve better clinical outcomes by exploring the advantages of the personalized therapy that these vaccines offer. Our results agree with other studies showing an association between immunization with polyvalent bacterial preparations and clinical improvement of infectious respiratory diseases^{5-7,9-11}.

Several clinical studies have shown that the oral administration of a polyvalent bacterial preparation in patients with COPD is capable of improving impaired immune functions, such as alveolar macrophage activity and interferon-gamma production¹⁰. The preparations used by our patients differ from others in their formulation, concentration, and/or route of administration (sublingual), and delivers whole inactivated bacteria instead of the most common bacterial lysates.

The mechanisms behind the efficacy of this novel approach, despite not fully understood, are being studied in order to clarify all its potential. For example, a prospective observational clinical study evaluated the clinical and immunological effects of the treatment with sublingual vaccines of bacterial combinations in antigen-specific responses to bacteria responsible

TABLE 1. CLINICAL CHARACTERISTICS OF THE PATIENTS (N=11). VALUES REPRESENT FREQUENCIES OTHERWISE STATED IN CONTRARY

Patient	Diagnosis	Episodes before vaccination (total)	Episodes before vaccination without hospital stay	Episodes before vaccination with hospital stay	Episodes after vaccination (total)	Episodes after vaccination without hospital stay	Episodes after vaccination with hospital stay
1	NCFB	5	4	1	1	1	0
2	NCFB	3	3	0	2	1	1
3	NCFB	3	3	0	1	1	0
4	COPD	6	4	2	1	1	0
5	Mounier Kuhn's Syndrome	6	6	0	4	3	1
6	NCFB	5	4	1	2	2	0
7	NCFB	3	3	0	-	-	-
8	NCFB	3	3	0	1	1	0
9	NCFB	6	4	2	1	1	0
10	NCFB	3	2	1	1	1	0
11	COPD	3	3	0	1	1	0

NCFB (Non Cystic Fibrosis Bronchiectasis); COPD (Chronic obstructive pulmonary disease)

TABLE 2. MUCOSAL BACTERIAL VACCINATION INFORMATION

Patient	Diagnosis	Formulation	Duration of therapy (months)	Complications
1	NCFB	100% Polibacterial	6	0
2	NCFB	100% Polibacterial	6	0
3	NCFB	100% Polibacterial	4,5	0
4	COPD	100% Polibacterial	4,5	0
5	Mounier Kuhn's Syndrome	100% Polibacterial	4,5	0
6	NCFB	100% Polibacterial	4,5	0
7	NCFB	50% Polibacterial + 50% PA	1*	Fever
8	NCFB	50% Polibacterial + 50% PA	6	0
9	NCFB	50% Polibacterial + 50% PA	6	0
10	NCFB	50% Polibacterial + 50% PA	4,5	0
11	COPD	75% Polibacterial + 10% HI + 15% PA	4,5	0

NCFB (Non Cystic Fibrosis Bronchiectasis); COPD (Chronic obstructive pulmonary disease); PA (Pseudomonas aeruginosa); HI (Haemophilus influenzae)

*Did not complete treatment

for respiratory tract infections⁶. Daily immunization with a multivalent bacterial sublingual preparation over a period of 6 months was studied in a cohort of 17 patients with RRIs, and the number of respiratory infections in immunized patients decreased significantly compared to the previous year. Besides, an increase in the proliferative capacity of CD4+ and CD8+ T lymphocytes specific for influenza virus antigen after 6 months of treatment (not contained in the vaccine) was evidenced⁶.

Another study described the clinical evolution of 88 patients, diagnosed with recurrent tonsillitis and chronic inflammation¹⁸. The authors observed that the majority of the patients (82%) who received immunotherapy experienced clinical improvement, avoiding tonsillectomy, whereas in the other group the percentage of patients was 51%. It is emphasized that purified components from bacteria or bacterial lysates selectively activate specific Toll-Like Receptors (TLR), leading to shared and unique responses in innate immune cells, whereas whole bacteria contain multiple agonists for multiple TLR, eliciting a potent and robust response. None of the patients reported any local (at the site of administration) or systemic side effects¹⁸.

A prospective observational study conducted on 50 patients with inflammatory diseases (RA, systemic lupus erythematosus, and mixed connective tissue disease), most of whom were undergoing treatment with biological and/or non-biological disease-modifying anti-rheumatic drugs and glucocorticoids at low doses, and who presented recurrent urinary and/or respiratory infections, evaluated treatment with two different polybacterial sublingual formulations

depending on the type of infection¹⁹. Vaccines were administered in cycles of 3 months per year and the clinical response was recorded at 6 months and a year. The paired comparison of the number of infectious events in both groups showed a significant decrease in the rate of repeated urinary tract infections and in the rate of recurrent respiratory infections in the previous year compared to the year after the vaccine¹⁹.

Overall, these preparations have shown very good safety profiles, even in immunosuppressed patients^{19,20}. In our study, only one patient had what could in theory be a side effect from the therapy leading to discontinuation (fever), out of precaution. Despite this, all other patients tolerated very well the sublingual administration.

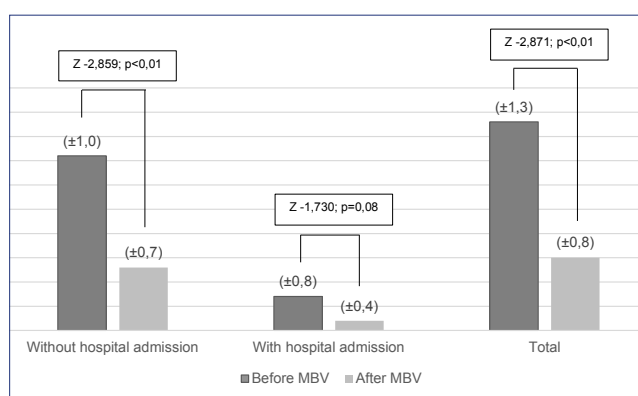
Another concern is the duration of the protective effect since it is known that these therapies require repetitive stimulation. One study demonstrated that the sublingual administration could induce persistent systemic and mucosal immune responses up to 4 months after the last immunization²¹.

The clinical experience with mucosal bacterial vaccines has been increasing not only in the prevention of RRIs but also in recurrent urinary tract infections (RUTI), providing valuable information on how to expand its beneficial effects. In a retrospective observational study of 319 women affected by RUTIs that compared the clinical impact of the prophylactic treatment with a bacterial vaccine (Uromune[®]) and the currently accepted antibiotic therapy, the authors found that the group treated with Uromune[®] experienced a highly significant reduction in the number of infections and none had side effects²².

The positive results obtained with this therapy are probably related to the fact that the sublingual mucosa is a good inductive site for generating a broad spectrum of mucosal and systemic immune responses, with a high degree of efficacy and persistence of the immune response in the respiratory and genitourinary tracts. Furthermore, it has been demonstrated that the sublingual administration of immunogens and whole bacteria activates dendritic cells and induces systemic dose-dependent immune responses, generating Th1, Th17, and IL-10 responses²³.

All things considered, our study demonstrates the effectiveness of a bacterial preparation administered through the sublingual route. We acknowledge that because this study has a very small population and not all the patients were immunized with the same preparation and even the same microorganisms

FIGURE 1. EPISODES OF RESPIRATORY INFECTIONS 1 YEAR BEFORE AND 1 YEAR AFTER TREATMENT (MEAN - SD)



MBV (Mucosal bacterial vaccination)

percentage, it is hard to reproduce. However, we were able to explore one of the advantages of these formulations (personalized formulation) to better suit the immunologic needs of our patients, which possibly helps explain the good results. We were able to reduce the total number of episodes (with statistical significance) and the number of episodes needing hospital stay (without statistical significance probably due to sample size). The data collected from clinical histories of patients provide clinically valuable information of the patients treated under “real-life” conditions. Considering the high prevalence and high cumulative cost of managing respiratory infections, as well as the frequent failure of conventional therapies, bacterial immunostimulation could be an effective management strategy to reduce costs, frequency, severity, and duration of such episodes in adults and children suffering from chronic respiratory tract infections.

CONCLUSION

Limitations of the present study include the relatively small number of patients recruited, in addition

to the lack of a control group. To further progress our understanding, prospective studies involving larger groups of patients, with longer follow-up periods and conducted in a double-blind placebo-controlled manner, are required.

Author's contributions

João Neiva Machado: Conceptualization (Lead); Methodology (Lead); Writing-original draft (Lead); Writing-review & editing (Lead); José Coutinho Costa: Validation (Supporting); Visualization (Supporting); Writing-review & editing (Supporting); Teresa Costa: Methodology (Supporting); Supervision (Supporting); Validation (Supporting); Writing-review & editing (Supporting); Cidália Rodrigues: Conceptualization (Supporting); Supervision (Supporting); Validation (Supporting); Visualization (Supporting); Writing-review & editing (Supporting).

The authors declare that no sponsorship was received for the work. The authors declare no conflicts of interest.

RESUMO

OBJETIVO: Avaliar a eficácia de vacinas bacterianas de mucosa (MBV) na redução do número de exacerbações de pacientes com doença respiratória crônica.

MÉTODOS: Um estudo de coorte prospectivo incluindo pacientes da Unidade de Pneumologia da Universidade e Centro Hospitalar de Coimbra, com exacerbações infecciosas frequentes (3 ou mais), apesar do uso das melhores estratégias terapêuticas. MBVs foram usadas como terapia adicional. O número de exacerbações 1 ano antes da terapia e 1 ano após ela foram analisados.

RESULTADOS: Amostra incluiu 11 indivíduos, 45,5% do sexo masculino, com média de idade de 62,5 anos. Oito pacientes apresentaram bronquiectasia não relacionada à fibrose cística, 2 DPOC (1 em oxigenoterapia prolongada) e 1 paciente com síndrome de Mounier-Kuhn. Três pacientes estavam sendo medicados com azitromicina, 1 com colistina inalada e 2 com tobramicina inalada. Dos 11 pacientes, um apresentou complicação (febre), o que levou à suspensão da terapia (excluído dos resultados). Dos 10 pacientes que completaram o tratamento, 5 apresentaram colonização bacteriana e receberam uma vacina personalizada. Os 6 restantes foram tratados com a composição padrão. A média de exacerbações infecciosas no ano anterior foi de 4,3 (0,7 com hospitalização). No ano após a terapia, o número médio foi de 1,5 (0,5 com hospitalização).

CONCLUSÃO: Os resultados obtidos neste estudo favorecem o uso de imunoestimulação bacteriana para reduzir a frequência de infecções respiratórias recorrentes em pacientes com doença respiratória crônica.

PALAVRAS-CHAVE: Vacinas bacterianas. Administração sublingual. Infecções respiratórias. Doenças respiratórias.

REFERENCES

- Martín-Rodríguez AJ, Quezada H, Becerril-Aragón G, Fuente-Núñez C, Castillo-Juarez I, Maeda T, et al. Recent advances in novel antibacterial development. [cited 2019 Oct 3]. Available from: https://www.researchgate.net/profile/Alberto_Martin-Rodriguez/publication/301358410_Recent_Advances_in_Novel_Antibacterial_Development/links/57155b5a08ae8a-b56695ac4c.pdf
- Sánchez Ramón S, Manzanera M, Candelas G. Vacunas antiinfecciosas de mucosas en la profilaxis de infecciones recurrentes: más allá de las vacunas convencionales. *Reumatol Clin*. 2020;16(1):49-55.
- Braido F, Tarantini F, Ghiglione V, Melioli G, Canonica GW. Bacterial lysate in the prevention of acute exacerbation of COPD and in respiratory recurrent infections. *Int J Chron Obstruct Pulmon Dis*. 2007;2(3):335-45.

4. Felmingham D, Feldman C, Hryniewicz W, Klugman K, Kohno S, Low DE, et al. Surveillance of resistance in bacteria causing community-acquired respiratory tract infections. *Clin Microbiol Infect.* 2002;8(Suppl 2):12-42.
5. Steurer-Stey C, Bachmann LM, Steurer J, Tramèr MR. Oral purified bacterial extracts in chronic bronchitis and COPD: systematic review. *Chest.* 2004;126(5):1645-55.
6. Alecsandru D, Valor L, Sánchez-Ramón S, Gil J, Carbone J, Navarro J, et al. 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* 2011;164(1):100-7.
7. Negri DR, Riccomi A, Pinto D, Vendetti S, Rossi A, Cicconi R, et al. Persistence of mucosal and systemic immune responses following sublingual immunization. *Vaccine.* 2010;28(25):4175-80.
8. Holmgren J, Czerkinsky C. Mucosal immunity and vaccines. *Nat Med.* 2005;11(4 Suppl):S45-53.
9. Passalacqua G, Canonica GW. Sublingual immunotherapy: update 2006. *Curr Opin Allergy Clin Immunol.* 2006;6(6):449-54.
10. Emmerich B, Emslander HP, Pachmann K, Hallek M, Milatovic D, Busch R. Local immunity in patients with chronic bronchitis and the effects of a bacterial extract, Broncho-Vaxom, on T lymphocytes, macrophages, gamma-interferon and secretory immunoglobulin A in bronchoalveolar lavage fluid and other variables. *Respiration.* 1990;57(2):90-9.
11. Cvoriscec B, Ustar M, Pardon R, Palecek I, Stipic-Markovic A, Zimic B. Oral immunotherapy of chronic bronchitis: a double-blind placebo-controlled multicenter study. *Respiration.* 1989;55(3):129-35.
12. Hapfelmeier S, Lawson MA, Slack E, Kirundi JK, Stoel M, Heikenwalder M, et al. Reversible microbial colonization of germ-free mice reveals the dynamics of IgA immune responses. 2010;328(5986):1705-9.
13. Koetz K, Bryl E, Spickschen K, O'Fallon WM, Goronzy JJ, Weyand CM. T cell homeostasis in patients with rheumatoid arthritis. *Proc Natl Acad Sci U S A.* 2000;97(16):9203-8.
14. Hessele C, Andersson B, Wold AE. Gram-positive bacteria are potent inducers of monocytic interleukin-12 (IL-12) while gram-negative bacteria preferentially stimulate IL-10 production. *Infect Immun.* 2000;68(6):3851-6.
15. Kang SS, Yang JS, Kim KW, Yun CH, Holmgren J, Czerkinsky C, et al. Anti-bacterial and anti-toxic immunity induced by a killed whole-cell-cholera toxin B subunit cholera vaccine is essential for protection against lethal bacterial infection in mouse pulmonary cholera model. *Mucosal Immunol.* 2013;6(4):826-37.
16. Ip WK, Sokolovska A, Charriere GM, Boyer L, Dejardin S, Cappillino MP, et al. Phagocytosis and phagosome acidification are required for pathogen processing and MyD88-dependent responses to *Staphylococcus aureus*. *J Immunol.* 2010;184(12):7071-81.
17. Muñoz-Wolf N, Rial A, Saavedra JM, Chabalgoity JA. Sublingual Immunotherapy as an alternative to induce protection against acute respiratory infections. *J Vis Exp.* 2014;(90). doi: 10.3791/52036.
18. García González LA, Arrutia Díez F. Mucosal bacterial immunotherapy with MV130 highly reduces the need of tonsillectomy in adults with recurrent tonsillitis. *Hum Vaccin Immunother.* 2019;15(9):2150-3.
19. Hernández Llano K, Sánchez-Ramón S, Ochoa Grullon J, Macarrón P, Morado C, Martínez-Prada C, et al. Sublingual vaccine: new challenge in the prevention of recurrent infections in autoimmune diseases. *Ann Rheum Dis.* 2017;76(suppl. 2):990.
20. Ochoa-Grullón J, Candelas G, Macarrón P. Polybacterial sublingual vaccines in actively immunosuppressed patients with systemic autoimmune disease. In: *European Congress of Immunology, Berlin, Germany; 2015.*
21. Negri DR, Riccomi A, Pinto D, Vendetti S, Rossi A, Cicconi R, et al. Persistence of mucosal and systemic immune responses following sublingual immunization. *Vaccine.* 2010;28(25):4175-80.
22. Lorenzo-Gómez MF, Padilla-Fernández B, García-Criado FJ, Mirón-Canelo JA, Gil-Vicente A, Nieto-Huertos A, et al. Evaluation of a therapeutic vaccine for the prevention of recurrent urinary tract infections versus prophylactic treatment with antibiotics. *Int Urogynecol J.* 2013;24(1):127-34.
23. Benito-Villalvilla C, Cirauqui C, Díez-Rivero CM, Casanovas M, Subiza JL, Palomares O. 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.* 2017;10(4):924-35.

