Nutrición Hospitalaria

Nutr Hosp. 2012;27(2):504-509
ISSN 0212-1611 • CODEN NUHOEQ
S.V.R. 318

Original

Lactobacillus plantarum CECT7315 and CECT7316 stimulate immunoglobulin production after influenza vaccination in elderly

M. Bosch[1], M. Méndez[2], M. Pérez[3], A. Farran[4,5] , M. C. Fuentes[1] and J. Cuñé[1]

[1]AB-Biotics, S. A. Cerdañola del Vallés. Spain. [2]Serveis Residencials d'estades temporals i respir. Llars Mundet. Barcelona. Spain. [3]Centre Assistencial Dr. Emili Mira i López. Recinte Torribera. Santa Coloma de Gramenet. Spain. [4]Centre d'Ensenyament de Nutrició Humana i Dietètica (CESNID). Santa Coloma de Gramenet. Spain. [5]Departament de Nutrició i Bromatologia. Universitat de Barcelona. Barcelona. Spain.

Abstract

Objective: **The effectiveness of influenza vaccination in preventing illness is lower in the elderly; this is why the ability of *Lactobacillus plantarum* CECT 7315/7316 to stimulate the response to influenza vaccination in elderly was evaluated.**

Research methods and procedures: **A randomized, double-blind, placebo-controlled human trial including 60 institutionalized volunteers aged 65-85 years was performed. All the volunteers were vaccinated with a trivalent influenza vaccine (A/Wisconsin/67/2005 NYMC X-161B (H3N2), A/Solomon Islands/3/2006 (H1N1) and B/Malaysia/2506/2004) for the Spanish vaccine campaign 2006/2007. The consumption of the probiotic began between three and four months after the vaccination. Volunteers were randomly assigned to one of three following groups: group A (receiving $5*10^9$ cfu/day of *L. plantarum* CECT 7315/7316 in 20 g powdered skim milk), group B (receiving $5*10^8$ cfu/day of *L. plantarum* CECT 7315/7316 in 20 g powdered skim milk) and group C or placebo (20 g powered skim milk). The participants consumed the probiotic during 3 months.**

Results: **The consumption of *L. plantarum* CECT 7315/7316 during 3 months after influenza vaccination increased the levels of influenza-specific IgA and IgG antibodies. Moreover, a trend towards an increase in influenza-specific IgM antibodies was also observed.**

***Conclusion: L. plantarum* CECT7315/7316 has an immunostimulating effect and could be used to improve the response to influenza vaccination in elderly.**

(*Nutr Hosp.* 2012;27:504-509)

DOI:10.3305/nh.2012.27.2.5519

Key words: *Lactobacillus plantarum CECT 7315/ 7316. Immunoestimulation. Influenza. Vaccine. Elderly.*

Correspondence: Jordi Cuñé.
AB-Biotics, S. A.
Edifici Eurela, P1M1.1.
Campus Universitat Autònoma de Barcelona.
08193 Bellaterra, Barcelona, Spain.
E-mail: cune@ab-biotics.com

Recibido: 6-VII-2011.
1.ª Revisión: 26-IX-2011.
Aceptado: 15-XI-2011.

LACTOBACILLUS PLANTARUM CECT7315 Y CECT7316 ESTIMULA LA PRODUCCIÓN DE INMUNOGLOBULINAS TRAS LA VACUNACIÓN CONTRA LA INFLUENZA EN ANCIANOS

Resumen

Introducción y objetivos: **La efectividad de la vacunación contra la influenza es menor en ancianos por lo que en este trabajo se evalúa la habilidad de las cepas de *Lactobacillus plantarum* CECT 7315/7316 para estimular la respuesta a la vacuna contra la influenza en ancianos.**

Métodos: **60 ancianos institucionalizados (65-85 años) participaron en un diseño aleatorizado, doble ciego controlado por placebo. Los voluntarios fueron vacunados con una vacuna trivalente contra influenza (A/Wisconsin/67/2005 NYMC X-161B (H3N2), A/Solomon Islands/3/2006 (H1N1) and B/Malaysia/2506/2004) durante la campaña española de vacunación 2006/2007. El consumo del probiótico empezó entre tres y cuatro meses después de la vacunación. Los voluntarios fueron distribuidos aleatoriamente en tres grupos: grupo A (recibieron $5*10^9$ ufc/día de *L. plantarum* CECT 7315/7316 en 20 g de leche desnatada en polvo), grupo B (recibieron $5*10^8$ ufc/día de *L. plantarum* CECT 7315/7316 en 20 g de leche desnatada en polvo) y grupo C o placebo (recibieron 20 g de leche desnatada en polvo). Los participantes consumieron el probiótico durante 3 meses.**

Resultados: **El consumo de *L. plantarum* CECT 7315/7316 durante tres meses después de la vacunación contra influenza incrementó los niveles de anticuerpos IgA y IgG específicos contra la influenza. Además, se observó una tendencia hacia un incremento en los niveles de anticuerpos IgM específicos contra la influenza.**

Conclusiones: **Las cepas de *L. plantarum* CECT 7315/7316 tienen un efecto inmunoestimulador y podrían utilizarse para mejorar la respuesta a la vacuna contra la influenza en ancianos.**

(*Nutr Hosp.* 2012;27:504-509)

DOI:10.3305/nh.2012.27.2.5519

Palabras clave: *Lactobacillus plantarum CECT 7315/ 7316. Inmunoestimulación. Vacuna. Influenza. Ancianos.*

Introduction

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit to the host.[1] The potential benefits of probiotics have been studied extensively for a variety of systemic indications and medical disorders, such as gastrointestinal and cardiovascular disorders, respiratory infections, allergies, gynecology and atopic eczema.[2-8] Initially, it was thought that this beneficial effect was a consequence of improvements in the intestinal microbial balance. However, there is now substantial evidence that probiotics can also provide benefits by modulating immune functions.

Influenza is an acute viral respiratory infection caused by RNA viruses of the family Orthomyxoviridae. The most common symptoms of the disease are fever and coughs but in most serious cases, influenza causes pneumonia, which results in high morbidity and significant mortality especially for children and elderly. To control influenza, protective immunity must be induced in advance by the administration of a vaccine. Vaccination of children, healthy younger adults, elders and both children and adults with high-risk medical conditions provides substantial benefits, although the types of benefits vary with age.[9] The effectiveness of influenza vaccination in preventing illness ranges from 70 to 90% in healthy persons younger than 65 years but only from 30 to 40% in the elderly residing in nursing homes,[10] although these values varies among studies. To improve the effectiveness of the vaccine, co-administration of the inactivated virus with adjuvants such as cholera toxin or heat-labile enterotoxin has been used. However, the addition of these kinds of adjuvants may not be clinically safe.[11] In these sense, the use of a probiotic with the status QPS (Qualified Presumption of Safety) by EFSA (European Food Safety Authority) able to stimulate the immune system could be a perfect alternative to increase the effectiveness of the influenza vaccine.

Lactobacillus plantarum is extensively used as a probiotic due to its beneficial effects on human health. *Lactobacillus plantarum* is a nonspecific stimulator of the immune response. In fact, it has been identified as the major determinant of the adjuvanticity of a mistletoe preparation, which in *in vitro* models promotes the secretion of TNF-α (tumor necrosis factor-alpha) and IL-12.[12] Intriguingly, *L. plantarum* may induce innate or adaptive immune responses, dependent on the viability of the bacteria.[13] Recently, Mañé et al.[14] demonstrate that *L. plantarum* CECT 7315 and CECT 7316 dietary supplementation results in overall activation of the immune system, as well as in a decrease of TGF-β release, which inhibits dendritic cell maturation and natural killer activity. Moreover, Bosch et al.[15] demonstrate that *L. plantarum* CECT 7315 and CECT 7316 also helps to regulate intestinal transit and improves the nutritional status in elderly. The aim of the present work is to evaluate the effect of the consumption of the probiotic *L. plantarum* CECT 7315 and CECT 7316 on the immune response induced by an influenza vaccine in elderly individuals, who tend to immune decline and are at high-risk of developing serious influenza infections.

Materials and methods

Study design

Sixty institutionalized volunteers aged 65-85 years were recruited to participate in the study. The exclusion criteria were presence of serious acute illness; supplementation with vitamin/oligoelements, probiotics or antibiotics within one month previous to the study; advanced neoplasic disease; intolerance to dairy products; and swallowing disorders. A written consent was obtained from all the subjects prior to their enrolment. The study was carried out according to the Helsinki Declaration. The study protocol was approved by the Ethical Committee of the Health Sciences Research Institute of the "Germans Trias i Pujol" Foundation (IGTP, Badalona, Spain) and the participating institutions.

All the volunteers were vaccinated with a trivalent influenza vaccine (A/Wisconsin/67/2005 NYMC X-161B (H3N2), A/Solomon Islands/3/2006 (H1N1) and B/ Malaysia/2506/2004) for the Spanish vaccine campaign 2006/2007. The consumption of the probiotic began between three and four months after the vaccination. Therefore, at the moment of supplementation with the probiotic the response to the vaccine —it means the induction of memory cells— was already produced. During the intervention time, the

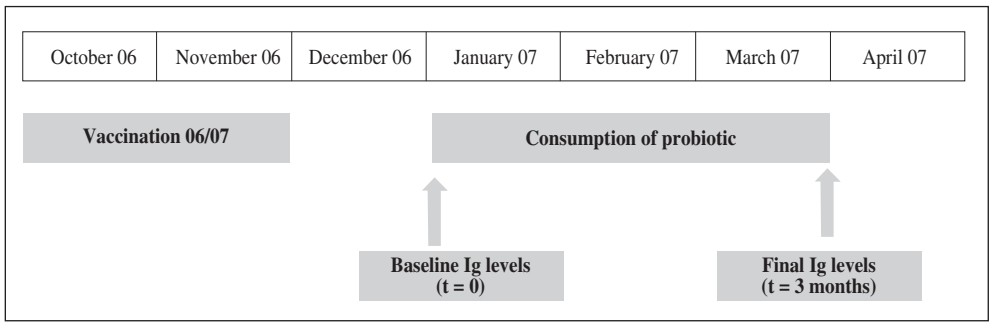

Fig. 1.—Diagram of the study design.

Table I
Influenza-specific immunoglobulin concentrations

Volunteer	IgA (ng/µl)		IgG (ng/µl)		IgM (ng/µl)	
	t = 0	t = 3 months	t = 0	t = 3 months	t = 0	t = 3 months
Probiotic high dose						
A1	11.29	13.81	7.96	8.21	4.97	5.30
A2	4.08	7.17	9.11	9.22	12.39	13.66
A3	3.93	4.75	9.45	9.86	5.87	6.65
A4	3.98	3.49	7.80	7.95	3.31	3.04
A5	5.27	6.27	7.41	7.57	3.75	3.85
A6	4.66	4.88	7.85	8.00	4.37	4.82
A7	5.93	6.67	14.56	16.00	11.22	11.98
A8	5.68	4.90	16.18	18.34	8.93	9.54
A9	4.77	5.45	7.89	7.80	7.38	7.48
A10	4.37	4.50	8.18	8.01	3.82	4.19
A11	3.64	3.59	7.55	7.73	3.23	3.23
A12	3.74	3.68	7.95	8.16	2.94	2.96
A13	3.90	3.37	9.29	9.62	4.56	4.06
A14	4.93	5.87	10.66	11.13	5.01	4.54
A15	3.98	4.81	8.93	9.23	6.30	6.33
A16	4.41	5.40	7.60	7.69	7.80	6.97
A17	3.77	4.01	9.29	9.79	5.00	5.34
A18	3.76	3.31	8.19	7.81	5.79	6.03
A19	4.00	4.70	7.70	7.58	4.07	4.46
Probiotic low dose						
B1	4.85	5.23	7.74	7.83	4.39	4.81
B2	4.08	4.73	7.85	7.98	4.97	4.77
B3	5.20	5.52	7.77	7.81	4.51	4.75
B4	3.33	3.43	7.62	7.78	4.89	5.15
B5	4.93	5.24	8.99	8.62	4.47	4.17
B6	6.87	7.08	7.67	7.73	4.37	3.79
B7	6.70	7.01	9.95	10.38	4.61	5.11
B8	8.42	9.21	7.93	8.01	5.15	5.18
B9	3.39	3.45	7.65	7.53	5.15	6.00
B10	6.40	5.63	8.32	8.12	4.95	4.29
B11	4.30	4.50	7.91	7.92	4.49	4.67
B12	5.16	5.48	8.08	8.15	3.79	3.79
B13	8.42	7.40	11.30	10.64	4.35	4.11
B14	6.11	7.18	8.99	9.33	3.48	3.30
Placebo						
C1	6.15	5.32	7.99	8.03	5.44	5.23
C2	7.04	7.26	8.03	7.97	5.19	5.34
C3	7.34	6.67	7.68	7.67	4.39	4.27
C4	9.07	11.40	7.97	7.99	3.80	4.01
C5	6.36	6.76	7.68	7.83	4.83	4.96
C6	8.37	8.89	7.75	7.61	4.71	4.58
C7	4.41	4.27	8.16	8.21	5.13	4.86
C8	6.61	7.13	7.91	7.78	4.57	4.69
C9	4.97	5.20	9.00	9.07	4.87	4.93
C10	7.96	8.84	7.64	7.63	5.91	5.36
C11	7.83	7.12	8.04	8.13	4.57	5.19
C12	7.69	7.21	7.92	7.97	4.69	4.81
C13	7.74	8.10	8.14	7.98	5.24	5.03
C14	10.03	9.57	8.06	8.08	5.76	5.38
C15	10.03	9.92	8.37	8.27	5.68	6.22

volunteers were exposed to influenza virus, since some cases of influenza infections in the institutions where they resided were recorded. This fact leads us to evaluate the effect of the consumption of the probiotic on the modulation of the activity of memory cells.

Volunteers were randomly assigned to one of three following groups: group A (receiving $5*10^9$ cfu/day of

L. plantarum CECT 7315/7316 in 20 g powdered skim milk), group B (receiving 5*10^8 cfu/day of *L. plantarum* CECT 7315/7316 in 20 g powdered skim milk) and group C or placebo (20 g powered skim milk). For the purposes of the present trial both strains were mixed at a 1:1 ratio. The participants consumed the probiotic during 3 months. Each dose was packed in a vacuum sealed envelope to be dissolved in 200 ml of water or other cold drink. Forty-eight volunteers finished the study, 19, 14 and 15 of groups A, B and C, respectively. Blood samples were taken immediately before (t = 0) and after the consumption of the probiotic (t = 3 months) (fig. 1).

Immunoglobulin measurements

The primary endpoint of the study was to evaluate the modulation of the immune response induced by influenza vaccination by the consumption of *L. plantarum* strains. In concrete, differences in immunoglobulin levels in blood before and after the consumption of probiotic strains were determined.

Influenza-specific IgA, IgG and IgM concentrations in plasma were measured by enzyme-linked immunosorbent assay (ELISA) quantification kits (Bethyl Laboratories Inc, Montgomery, TX, USA) as published.[16] The measurements were performed in triplicate.

Statistical analysis

In order to compare the immunoglobulin levels within each group before and after the consumption of the probiotic (t = 0 and t = 3 months) the non-parametric Wilcoxon test was performed. The difference in the concentration of each immunoglobulin in each group was calculated using the GraphPad Insat program. The non-parametric Mann-Whitney and Spearman tests were used to compare the immunoglobulin levels between individuals infected and non-infected by influenza during the course of the study. A p-value of < 0.05 was regarded as statistically significant.

Results

Influenza-specific immunoglobulin levels were measured before and after the consumption of the probiotic. Significant differences in the probiotic but not in the placebo group would indicate that the probiotic modulates the immune system stimulating the production of immunoglobulins. It was observed a significant increase in influenza-specific IgG only in the group receiving the high dose of *L. plantarum* CECT 7315/7316 (p = 0.023). Regarding influenza-specific IgA, the increase in concentration was observed in both probiotic groups but not in the placebo (p = 0.008, 0.039 and 0.1 for groups A, B and C, respectively). Finally, the influenza-specific IgM concentration was not significantly increased in any group but there was a trend towards an increase in IgM levels in the probiotic group A (p = 0.054) (table I).

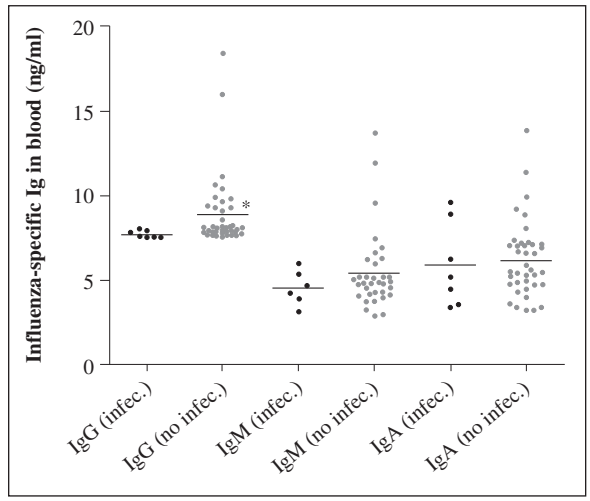

Fig. 2.—Immunoglobulin levels in blood comparing infected and non-infected individuals. (*) shows that IgG levels of non-infected individuals are statistically higher than those of the infected indivuals ($p < 0.05$, Mann-Whitney test). No differences between groups were found in IgA or IgM levels.

The immunoglobulin levels between individuals infected and non-infected by influenza were compared. Individuals infected by influenza showed significantly lower concentration of influenza-specific IgG, demonstrating that IgG production could be a major defense mechanism against influenza infections. In contrast, no difference between groups was observed in IgA and IgM levels (fig. 2).

Discussion

Defense against influenza infections involves innate and adaptive immune responses. After infection, most influenza viruses are detected and destroyed within few hours by innate immune mechanisms, but if the viruses escape, they are detected and eliminated specifically by adaptive immune mechanisms. Among adaptive immune mechanisms, cytotoxic T lymphocytes and antibodies (IgA and pre-existing IgG) play the major role in combating influenza infections.[17] To control influenza, protective adaptive immunity must be induced in advance by the administration of a vaccine. However, the effectiveness of the vaccine is limited in some cases showing the need of developing strategies to improve the response of host to vaccination.

It is generally accepted that the gut microflora could have an influence on the host's immune system. The modification of this microflora could, therefore, help to improve the response of the host to vaccination. In this sense, some probiotic strains have been administered to stimulate the response of the organism to the vaccina-

tion increasing the immunoglobulin titers against the bacteria or virus pathogen.[18-22] Davidson et al.[23] concluded that *Lactobacillus* GG improved vaccine immunogenicity for the H3N2 strain but not for H1N1 and B strains in a double-blind placebo controlled pilot study. Recently, Olivares et al.[14] and Boge et al.[24] showed that oral administration of *Lactobacillus fermentum* CECT 5716 and *Lactobacillus casei* DN-114001 potentiate the immunologic response of an anti-influenza vaccine. While *L. casei* DN-114001 increases influenza-specific antibody titers and seroconversion after vaccination,[24] *L. fermetum* CECT 5716 increases both T-helper type I response and virus-neutralizing antibodies.[16] Regarding humoral effects, *L. fermetum* CECT 5716 induces an increase in specific anti-influenza IgA antibodies in plasma whereas no increase was observed in influenza-specific IgG or IgM antibodies.[14] In the present work, it has been demonstrated that *L. plantarum* CECT 7315/7316 increases both influenza-specific IgG and IgA antibodies and a trend towards an increase in influenza-specific IgM antibodies levels is also observed. Moreover, the same strains have shown a stimulatory effect of the innate and acquired immunity, by promoting and activating natural killer and antigen presenting cells from the innate immunity system as well as increasing the number of activated B and cytotoxic T cells from the adaptive system.[14]

Lactobacillus fermentum CECT 5716, *L. casei* DN-114001 and *L. plantarum* CECT 7315/7316 are able to stimulate the response of the host to influenza vaccine. *Lactobacillus casei* DN-114001 is excluded from the comparison among strains since the effect of this probiotic on the induction of the different types of Igs is not described.[24] It seems that *L. plantarum* CECT 7315/7316 potentiates the response to influenza vaccination in a broader way than *L. fermentum* CECT 5716. Therefore, to our knowledge this is the first time that the ability of probiotic strains to significantly increase the production of influenza-specific IgG antibodies, which are key factors in the defense against influenza infections[17] has been demonstrated. However, the differences between *L. fermentum* CECT 5716 and *L. plantarum* CECT 7315/7316 could not be only attributed to the use of different strains. There are some issues in the design of both studies that could contribute to explain this different behavior. First, in this work the probiotic was administered during 3 months while in Olivares et al.[14] the administration of the probiotic was during 28 days. However, in this shorter period, Olivares et al.[14] were able to detect an increase in total IgG levels, showing that the difference in the administration period seems not so important as to explain the differences observed in both studies. Second, while Olivares et al.[14] vaccinated the volunteers during the period of probiotic supplementation, in the present study the probiotic was administered between three and four months after influenza vaccination. Therefore, what it is really measured is the effect of the probiotic on the response of previous created memory cells to a re-infection with influenza virus. Our results show that in order to improve the response to influenza vaccine is not necessary the consumption of the probiotic before or at the same time as vaccination. On the contrary, the stimulating effect of the humoral immune system is also present even four months after influenza vaccination. Finally, the age of the integrants of both studies is also different. Whereas the medium age in Olivares et al.[14] was 33 years, in this study the integrants are elder people (between 65 and 85 years). The trial was performed in elders for two reasons: they are among the high-risk groups of developing serious influenza infections and it is well-characterized that increasing age involves a thymus involution and immunosenescence.[25] The immunosenescence has been related to a decrease of mature T lymphocytes numbers, of natural killer and dendritic cell numbers, and the loss of the diversity of β cells population in the blood of elders. Moreover, aging causes declines in cell-mediated cytotoxic and phagocytic responses, and increases circulating levels of pro-inflammatory cytokines. These alterations of both innate and acquired immunity in elder people result in decreased capacity to mediate effective immune responses to vaccination and invading pathogens increasing susceptibility to infectious diseases, and inflammatory conditions.[25] For these reasons, the need of improving the response to influenza vaccination is even more urgent in this group. The results obtained in this study demonstrate that *L. plantarum* CECT 7315/7316 is able to stimulate the immune system even in those individuals who tend to immune decline.

It is interesting to note that other attempts based on gut microflora modification have been made in order to increase the effectiveness of the influenza vaccine in the elderly. In these sense, prebiotics which stimulate the growth of bifidogenic bacteria in the gut, could have a beneficial effect. However, the administration of a prebiotic mixture (containing raftilose and raftilinae or maltodextrin) during 28 weeks and 2 weeks prior to influenza vaccination to healthy elder people does not increase the production of antibodies against influenza when comparing with the control individuals.[26] On the contrary, the consumption of *L. casei* DN-114001 increases influenza-specific antibody titers and seroconversion after vaccination.[24] These results show that, although the modification of gut microflora could improve the response to influenza vaccine, this effect is strain-dependent.

Conclusion

The consumption of the probiotic *L. plantarum* CECT7315/7316 could be an efficient, safe, and easy method to improve the protective immune response triggered by influenza vaccination in groups, such as old people, at high-risk for developing serious influenza infections.

Acknowledgments

We are deeply grateful to CARINSA for its innovative vocation and financial support, as well as to CIDEM of the Catalonian Government and the Ministry of Industry, Tourism and Trade of the Spanish Government for their financial support. MB, MCF and JC are employed by AB-BIOTICS, SA. The other authors had no conflict of interest.

References

1. Guarner F, Perdigon G, Corthier G, Salminen S, Koletzko B, Morelli L. Should yogurt cultures be considered probiotic? *Br J Nutr* 2005; 93: 783-786.
2. Vanderhoof JA, Whitney DB, Antonson DL, Hanner TL, Lupo JV, Young RJ. *Lactobacillus* GG in the prevention of antibiotic-associated diarrhea in children. *J Pediatr* 1999; 135: 564-568.
3. Fuller R. Probiotics 2: Applications and practical aspects [R Fuller editor]. London: Chapman & Hall; 1997.
4. Hatakka K, Savilahti E, Pönkä A, Meurman JH, Poussa T, Näse L, Saxelin M, Korpela R. Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. *BMJ* 2001; 2: 1318-1319.
5. Bengmark S. Colonic food: pre- and probiotics. *Am J Gastroenterol* 2000; 95: 5-7.
6. Hilton E, Isenberg HD, Alperstein P, France K, Borenstein MT. Ingestion of yogurt containing *Lactobacillus acidophilus* as prophylaxis for candidal vaginitis. *Ann Intern Med* 1992; 116: 353-357.
7. Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001; 357: 1076-1079.
8. Roemo J, Nova E, Wärnberg J, Gómez-Martínez S, Díaz LE, Marcos A. Immunomodulatory effect of fibres, probiotics and synbiotics in different life-stages. *Nutr Hosp* 2010; 25: 341-349.
9. Nichol KL. Efficacy and effectiveness of influenza vaccination. *Vaccine* 2008; 26S: D17-D22.
10. Brydak LB, Machala M, Mysliwska J, Mysliwski A, Trzonkowski P. Immune response to influenza vaccination in elderly population. *J Clin Immunol* 2003; 23: 214-222.
11. Mutsch M, Zhou W, Rhodes P, Bopp M, Chen RT, Linder T, Spyr C, Steffen R. Use of the inactivated intranasal influenza vaccine and the risk of Bell's Palsy in Switzerland. *N Engl J Med* 2004; 350: 896-903.
12. Heinzerlinh L, von Baehr V, Liebenthal C, von Baehr R, Volk HD. Immunologic effector mechanisms of a standardized mistletoe extract on the function of human monocytes and lymphocytes in vitro, ex vivo, and in vivo. *J Clin Immunol* 2006; 26: 347-359.
13. Bloskma N, De Heer E, Van Dijk H, Willers JM. Adjuvanticity of lactobacilli. I. Differential effects of viable and killed bacteria. *Clin Exp Immunol* 1979; 37: 367-375.
14. Mañé J, Lorén V, Pedrosa E, Lorén V, Gassull MA, Espadaler J, Cuñé J, Audivert S, Bonachera MA, Cabré E. A mixture of *Lactobacillus plantarum* CECT7315 and CECT7316 enhances immunity in elderly subjects. A dose-response, double-blind, placebo-controlled, randomized pilot trial. *Nutr Hosp* 2011; 26: 228-235.
15. Bosch M, Espadaler J, Méndez M, Pérez M, Farrán A, Audivert S, Bonachera MA, Cuñé J. El consumo del probiótico *Lactobacillus plantarum* CECT 7315/7316 mejora el estado de salud general en personas de edad avanzada. *Nutr Hosp* 2011; 26: 642-645.
16. Olivares M, Díaz-Rapero MP, Sierra S, Lara-Villoslada F, Fonollá J, Navas M, Rodríguez JM, Xaus J. Oral intake of *Lactobacillus fermentum* CECT5716 enhances the effects of influenza vaccination. *Nutrition* 2007; 23: 254-260.
17. Tamura S, Kurata T. Denfense mechanisms against influenza virus infection in the respirator tract mucosa. *Jpn J Infect Dis* 2004; 57: 236-247.
18. Isoulari E, Joensuu J, Suomalainen H, Luomala M, Vesikari T. Improved immunogenicity of oral D x RRV reassortant rotavirus vaccine by *Lactobacillus casei* GG. *Vaccine* 1995; 13: 310-312.
19. De Vrese M, Rautengerg P, Laue C, Koopmans M, Herremans T, Schrezenmeir J. Probiotic bacteria stimulate virus-specific neutralizing antibodies following a booster polio vaccination. *Eur J Nutr* 2005; 44: 406-413.
20. Kukkonen K, Nieminen T, Poussa T, Savilahti E, Kuitunen M. Effect of probiotics on vaccine antibody responses in infancy – a randomized placebo-controlled double-blind trial. *Pedriatr Allergy Immunol* 2006; 17: 416-421.
21. Taylor AL, Hale J, Wiltschut J, Lehmann H, Dunstan JA, Prescott SL. Effects of probiotic supplementation for the first 6 months of life on allergen- and vaccine-specific immune responses. *Clin Exp Allergy* 2006; 36: 1227-1235.
22. West CE, Gothefors L, Granström M, Käyhty H, Hammarström ML, Hernell O. Effects of feeding probiotics during weaning on infections and antibody responses to diphtheria, tetanus and Hib vaccines. *Pediatr Allergy Immunol* 2008; 19: 53-60.
23. Davidson LE, Fiorino AM, Snydman DR, Hibberd PL. Lactobacillus GG as an immune adjuvant for live-attenuated influenza vaccine in healthy adults: a randomized double-blind placebo-controlled trial. *Eur J Clin Nutr* 2011; 65: 501-507.
24. Boge T, Rémigy M, Vaudaine S, Tanguy J, Bourdet-Sicard R, van der Werf S. A probiotic fermented dairy iougurt drink improves antibody response to influenza vaccination in the elderly in two randomized controlled trials. *Vaccine* 2009; 27: 5677-5684.
25. Panda A, Arjona A, Sapey E, Bai F, Fikrig E, Montgomery RR, Lord JM, Shaw AC. Human innate immunosenescence: causes and consequences for immunity in old age. *Trends Immunol* 2009; 30: 325-333.
26. Bunout D, Hirsch S, Pía de la Maza M, Muñoz C, Haschke F, Steenhout P, Klassen P, Barrera G, Gattas V, Petermann M. Effect of prebiotics on the immune response to vaccination in the elderly. *J Parenter Enteral Nutr* 2002; 26: 372-376.