Immunostimulants for the Prevention of Respiratory Infections

Arturo Borzutzky and Rodrigo Hoyos-Bachiloglu

Viral upper respiratory tract infections encompass an enormous public health problem. Only the common cold has an estimated cost of 7.7 billion dollars per year in the United States, making the search of mechanisms to decrease the incidence of upper respiratory tract infections a highly relevant subject. In daily practice, clinicians frequently encounter patients that complain of what they consider an elevated frequency of respiratory infections and seek a way to decrease these by boosting the function of their immune system with an immunostimulant.

In general terms, immunostimulants are defined as natural or synthetic substances that activate the immune system by inducing an increase in the function of any of its components. Immunostimulants can be divided in two categories:

a) Specific immunostimulants are those that create immunity to any given antigen in a specific manner. This class of immunostimulant is mainly composed by immunizations (vaccines) and is strongly backed by scientific evidence that supports its application in the population.

b) Nonspecific immunostimulants are those that boost the activitiy of the immune system in a non-antigen specific manner. A clear example of this group is aluminum hydroxyde, an adjuvant that is widely used in the preparation of vaccines and is capable of triggering a non-specific inflammatory reaction in the inoculation site that potentiates the adaptive immune response. Nonspecific immunostimulants are a heterogenous group of natural and artificial substances that due to their non-antigen specific effects have been studied and used to decrease respiratory infections. This chapter will focus in the revision of this class of immunostimulant.

When seeing patients with recurrent infections, and before prescribing any type of immunostimulant, it is of vital importance to assess environmental risk factors (e.g. cigarette smoke exposure and other indoor air pollution), as well as immune and allergic diseases that may increase susceptibility to infections. It is the role of the physician to actively look for symptoms suggestive of primary immunodeficiencies (**Table 1**), chronic respiratory infections like tuberculosis, asthma and allergic rhinitis. The normal variation in incidence of viral respiratory infections must be taken into account, especially in infants and toddlers in whom between 5 and 10 respiratory infections per year is considered normal. Lastly, universal precautions must never be forgotten in order to reduce the transmission of respiratory viruses, probably being hand washing the most cost-effective measure to prevent viral respiratory infections.

Table 1. Warning signs of primary immunodeficiency for PI children and adults developed by the Jeffrey Modell Foundation.

Children	Adults
Four or more new ear infections within 1 year.	Two or more new ear infections within 1 year.
Two or more serious sinus infections within 1 year.	Two or more new sinus infections within 1 year, in the absence of allergy.
Two or more months on antibiotics with little effect.	One pneumonia per year for more than 1 year.
Two or more pneumonias within 1 year.	Chronic diarrhea with weight loss.
Failure of an infant to gain weight or grow normally.	Recurrent viral infections (colds, herpes, warts, condyloma).
Recurrent, deep skin or organ abscesses.	Recurrent need for intravenous antibiotics to clear infections.
Persistent thrush in mouth or fungal infection on skin.	Recurrent, deep abscesses of the skin or internal organs.
Need for intravenous antibiotics to clear infections.	Persistent thrush or fungal infection on skin or elsewhere.
Two or more deep-seated infections including septicemia.	Infection with normally harmless tuberculosis-like bacteria.
A family history of PI.	A family history of PI.

In the next sections we will detail the characteristics and available scientific evidence about the natural and synthetic immunostimulants most commonly prescribed in the prevention of respiratory infections.

1. *Echinacea purpurea*:

The term Echinacea corresponds to a group of nine herbaceous and perennial plants native of North America that belong to the Asteraceae family. Within the Echinaceas, *Echinacea purpurea* (EP) has been most commonly used with medicinal purposes. The effects of its root as well as the aerial parts have been studied due to its putative effects on the immune system that would help to fight respiratory viral infections such as the common cold and the flu.

Studies in murine models have suggested that EP extracts administered orally increase white blood counts and concentration of interleukin 2 (IL-2) in peripheral blood,[1] increase phagocyte activity in spleen and lung, and production of IL-2, tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) upon non-specific costimulation with ionomycin.[2] The intraperitoneal administration of EP extracts in murine models have also demonstrated an increase in circulating white blood cells, phagocytic activity and serum immunoglobulins.[3] The effects of EP on dendritic cells have been evaluated *in vitro* with contradictory results, finding immunostimulant or immunosuppressive effects depending on whether root or leaf EP extracts are used.[4]

Multiple studies in humans have been recently performed to evaluate the efficacy of *Echinacea purpurea* to prevent viral infections like the common cold. However, these studies largely differ on which *Echinacea purpurea* is used, the part of the plant used, extraction methods and extract administration regime. To date, two systematic reviews (SR) have been performed on this topic that have shown discordant results.

Shah *et al* performed a meta-analysis of 9 randomized controlled trials (RCTs) tested against placebo that evaluated the efficacy of *Echinacea purpurea* extracts to decrease the incidence of the common cold.[5] The authors showed that the use of *Echinacea purpurea* decreased the risk of having a common cold in 58% (OR 0.42, IC95% 0.25-0.71) with a number needed to treat of 6. Despite the fact that the studies included in this SR had significant heterogeneity, all agreed in the positive effect of *Echinancea purpurea* in decreasing the incidence of common cold, but not as much in the magnitude of the effect. However, a recent Cochrane systematic reviews by Karsch-Völk *et al* did not find a significant effect of the use of *Echinacea purpurea* when analyzing trials that used the same *Echinacea purpurea* component. Nonetheless, when analyzing together all trials that used *Echinacea purpurea* preventively, a significant decrease in the episodes of common cold was observed compared with placebo (RR 0.83, IC95% 0.75-0.92), with a risk reduction of 10% and number needed to treat of 9.[6] These differences could be explained by the varying quality and high heterogeneity of studies included in both systematic reviews. **Thus, new studies of good methodological quality and with comparable extracts of *Echinacea purpurea* are required before stating that this can be recommended for the prevention of the common cold.**

2. Garlic:

Garlic or *Allium sativum* is a plant of the Amaryllidaceae family that originated in Asia and is widely used in naturopathic medicine. Multiple antibacterial, antiviral and antifungal properties have been attributed to garlic.[7-9] In murine models it has been described that garlic extracts are capable of stimulating the production of IL-2, TNF-α and IFN-γ by splenic cells, as well as boosting natural killer (NK) cell activity and phagocytic activity of macrophages.[10]

A randomized placebo-controlled trial recently published by Nantz *et al* evaluated the effect of supplementation with garlic extracts aged for three months on the function of the immune system and the frequency of common cold on 60 healthy adults.[11] Immune function evaluated by production of IL-2 and TNF-α did not vary significantly between treatment and placebo groups, but, an increased proliferation of NK cells and γδ+ T cells were reported in the garlic extract supplemented group. However, this study did not show a significant difference in the incidence of common cold between groups, although the supplemented group reported a lower number of days and shorter duration of symptoms of common cold.

Lissiman *et al* performed a serial of systematic reviews that evaluated the use of garlic for prevention of the common cold. Unfortunately, only one study fulfilled the proposed inclusion criteria.[12] In this study, 146 volunteers were randomized to receive garlic supplements or placebo and were evaluated for the

number of comon cold episodes as well as severity and duration of symptoms. The participants thar received garlic supplements had significantly less episodes of common cold (OR 0.06, IC95% 0.02-0.15, NNT 1.78) and reported a shorter duration of symptoms compared with the placebo group.[13]

Although results appear promising, in light of the available information to date, additional studies are needed before recommending the use of garlic extracts for the prevention of the common cold.

3.- Zinc:

Zinc (Zn) is a metallic chemical element of great importance for metabolism, acting as a cofactor or as part of the active site of numerous enzymes both in animals and humans. It plays a fundamental role in diverse cellular processes such as signal transduction, transcription and replication.[14] Zn is obtained from diet by intake of protein-rich foods such as meats and seafood.

The role played by Zn in the immune system has been previously described,[15] being immunity highly dependent on this element for its normal function. The molecular mechanisms through which Zn affects the immune system *in vitro*, have been reviewed by Rink *et al*.[16]

Acrodermatitis enteropathica, a disease characterized by a specific malabsorption of Zn is a clear clinical example of how Zn is involved in immune system physiology. Patients with this disease present thymic atrophy and high rates of bacterial, viral, and fungal infections that can be reverted by Zn supplementation.[17]

Due to the actions of Zn on the immune system, the use of Zn supplements as an immunostimulant has been studied to decrease the rate of upper respiratory infections. Unfortunately, the studies performed to date have shown conflicting results. Sing *et al* perforned a systematic reviews of randomized trials that evaluated the effect of Zn supplementation for 5 months on the risk of having a common cold.[18] In this systematic reviews, two clinical studies with a total of 394 participants were included, finding an incidence rate ratio of developing a common cold of 0.64 (IC95% 0.47-0.88), as well as a significant reduction in school absenteeism and antibiotic prescription in the Zn group. **However, differences exist in formulations and dosing of Zn, which need to be standardized before the widespread use of Zn as an immunostimulant in clinical practice.**

4.- Vitamin C:

Vitamin C (VC) or ascorbic acid, is a nutrient that is essential for multiple metabolic processes. This vitamin cannot be synthesized by the human body, so it must be obtained from dietary sources such as citric fruits. The main biological actions of VC are as antioxidant and enzymatic cofactor.

The use of VC to prevent the common cold is rooted in the general public since the publication of the studies of the Nobel laureate Linus Pauling in 1970. Numerous clinical trials have evaluated the role of VC supplementation both in the prevention and the symptom severity of common cold, giving rise to multiple reviews on the subject; all presenting conflicting results.

Hemiliä *et al* performed systematic reviews that compared VC supplementation in doses higher than 0.2g with placebo.[19] This systematic reviews that included 29

trials and 11,306 participants was not able to demonstrate that the use of VC supplements decrease the incidence of the common cold in the general population (RR 0.97, IC95% 0.94-1.0). However, studies performed in marathon-runners, skiers and soldiers showed a lower incidence with VC than placebo (RR 0.48, IC95% 0.35-0.64). Therefore, in the general population the effect of VC to prevent common colds would at best a reduction in the annual incidence of common cold from 20 to 19 episodes per year, an effect so insiginficant that the use fo VC supplements for this purpose does not seem to be justified.

In light of the available evidence to date, it is not possible to recommend the routinary use of VC as an immunostimulant to prevent respiratory infections, but it could be useful in selected populations subjected to highly demanding physical exercise for limited periods of time.

5.- Vitamin D:

Vitamin D (VD) is a steroidal hormone that can be synthesized naturally by the organism in presence of solar light and that plays a fundamental role in bone metabolism and other biological systems. In humans, the most relevant forms of VD are cholecalciferol (VD3) and ergocalciferol (VD2). About 90% of VD is synthesized in the skin in the form of VD3 from 7-dehydrocholesterol in presence of ultraviolet B rays, while VD2 is obtained from dietary sources. Subsequently, VD2 and VD3 must be hydroxylated in carbon 25 in the liver and in carbon 1 in the kidney giving rise to the active metabolite known as 1,25 dihydroxyvitamin D or calcitriol. The intermediate metabolite generated by hydroxylation in the liver, 25 hydroxycholecalciferol (25OHD), can be measured in plasma and is used internationally to classify individuals as VD sufficient (25OHD >30ng/ml), insufficient (25OHD 20-30ng/ml) or deficient (25OHD < 20ng/ml).

The functions and studies of VD have been traditionally centered in calcium metabolism, however, VD is now understood as a pleiotropic subsance, with functions in several different systems such as the cardiovascular and immune systems.[20] Regarding its immunomodulatory properties, it has been shown that VD can affect the incidence of respiratory infections as it has biological effects on the innate immune system by inducing production of antimicrobial peptides such as cathelicidin as well as effects on B and T lymphocyte function.[21, 22]

The role of VD on the immune system has also been documented in regards to infection susceptibility. Evidence has shown that low levels of VD increase the risk and severity of infections due to *Mycobacterium tuberculosis*, and in addition, supplementation with VD in association with standard antibiotic treatment may facilitate the resolution and negativization of sputum in patients with tuberculosis.[23] In terms of the role of VD in the susceptibility to viral respiratory infections, observational trials have associated a higher incidence rate of respiratory infections with the seasons that have lower ultraviolet radiation available for VD synthesis,[24, 25] and it has been demonstrated that patients that suffer rickets have a higher rate of respiratory infections.[26] A large study that has shown an association between VD status and the frequency of respiratory infections derives from a secondary analysis of the third National Health and Nutritional Examination Survey in the United States (NHANES-III).[27] In this

study, after adjusting for clinical factors (season, body mass index, smoking, asthma and chronic obstructive pulmonary disease) patients with 25OHD concentrations < 10ng/ml had a respiratory infection OR of 1.36 (IC95% 1.01-1.84) and patients with 25OHD between 10 and 30ng/ml an OR of 1.24 (IC95% 1.07-1.43) compared with those with 25OHD ≥ 30ng/ml.

Due to the associations found between VD deficiency and respiratory infections, randomized placebo-controlled trials have been performed to evaluate the effect of VD supplementation on the incidence of respiratory infections. The studies performed to date vary in terms of the VD dose and supplementation regime. However, two studies have been performed that support the use of VD to prevent respiratory infections: Charan *et al*[28] report an OR of 0.58 (IC95% 0.41-0.81) and Bergman *et al*[29] report an OR of 0.64 (IC95% 0.49-0.84). The evidence contributed by these trials must be evaluated with caution, due to the heterogeneity of the studies that were included and the possibility of publication bias. Mao *et al*[30] recently performed another systematic reviews that did no find benefit of VD supplementation in the general population for prevention of respiratory infections.

Considering current available evidence, **additional randomized controlled trials are needed to clarify target population, dosing and supplementation regimes, before widespread recommendation can be made on VD supplementation to prevent respiratory infections.** However, its use may be recommendable in populations at high risk of VD deficiency.

6.- Probiotics:

Probiotics are live non-pathogenic microorganisms that can be taken orally as a food supplement, can be recovered alive from feces, as well as adhere and reside in the intestinal mucosa. These microorganisms, administered in sufficient quantities, can have beneficial effects for the human organism.[31]

Experimental murine models have suggested that the use of probiotics has immunostimulant effects and could confer the host resistance against respiratory infections.[32, 33] Probiotics can have immunstimulant actions on the innate immune system, boosting phagocytic activity, expression of complement receptors, and the activity and number of circulating natural killer cells.[34-37]

Hao *et al* performed a systematic reviews of 14 controlled trials with a total of 3451 participants, that evaluated the effect of the use of any probiotic (single strain, strain mix, any dose, any route of administration) for more that 7 days against placebo or nothing on the number of upper respiratory infections and their duration.[38] In this systematic reviews the use of probiotics was shown to be better than placebo to prevent upper respiratory infections with an incidence rate ratio of 0.88 (IC95% 0.81-0.96), but no differences were found regarding disease duration. **Due to differences in probiotic strains used and the methodological differences among the studies that were included in the systematic reviews of Hao *et al,* the results must be interpreted with caution. More trials are needed to discern which probiotic strains, dose and treatment duration are useful before probiotics can be strongly recommended for the prevention of respiratory infections.**

7.- Bacterial lysates:

Bacterial lysates are immunostimulant agens that contain lyophilized fractions of bacteria. The most common bacterial lysate is OM-85V (Bronchovaxom®) that contains lyophilized fractions of *Haemophilus influenzae, Diplococcus pneumoniae, Klebsiella pneumoniae, Klebsiella ozaenae, Staphylococcus aureus, Streptococcus pyogenes, Streptococus viridans* and *Moraxella catarrhalis*.

The mechanism of action of these immunostimulants appears to be related to the stimulation of the innate immune system, secretion of proinflammatory cytokine and production of reactive oxygen species *in vitro*.[39] *In vivo* studies have demonstrated that OM-85V is capable of stimulating macrophage actions, recruiting NK cells and stimulating production of proinflammatory cytokines IFN-γ, IL-2, IL-1, IL-6, IL-8 y TNF-α by monocytes and macrophages, as well as boosting IgA secretion by resident cells of the respiratory mucosa.[40]

Multiple clinical trials have evaluated the effectiveness of the administration of bacterial lysates on the incidence of respiratory infections. However, **great heterogeneity exists between the different bacterial lysates' composition, administration regimes and duration of treatment, that difficult the application of their results in daily clinical practice,** despite the evidence in favor of their potential benefits. Del Río Navarro *et al*[41] performed a systematic review to study the effect of bacterial lysates on the prevention of respiratory infections. This systematic reviews showed that bacterial lysates such as OM-85V reduced the number of infections in 40% (IC95% 48.78-31.67) compared to placebo with an acceptable heterogeneity among studies.

8.- Isoprinosine:

Isoprinosine is a combination of inosine, acetamidobenzoic acid, and dimethylaminoisopropanol that acts as a stimulant of lymphocytes, NK cell activity and phagocytes *in vitro*. Due to its immunostimulant actions *in vitro*, isoprinosine has been used in some countries as prophylaxis of viral respiratory infections. Litzman *et al* performed a randomized double-blind placebo-controlled trial of the use of isoprinosine for reduction of the number of respiratory infections in children with recurrent viral respiratory infections.[42] This study included children that in the previous winter season had at least 5 episodes of viral respiratory infection. Prior to the start of the trial primary immunodeficiencies and autoimmunity were ruled out. The participants received a daily dose of 50 mg of isoprinosine or placebo for 6 weeks and subsequently 50 mg of isoprinosine or placebo twice per week. At the end of the study, the number of respiratory infections was registered and changes in lymphocyte populations and lymphocyte activiation markers were evaluated. After 12 weeks, no significant differences were found between groups in terms of number of respiratory infections or immunological parameters. **The use of isoprinosine as an immunostimulant to prevent respiratory infections is not currently recommended.**

Conclusions

In conclusion, despite a great interest in finding substances that aid in reducing the rate of respiratory infections in the population and diverse alternatives that ara available in the market, most of these do not yet have solid scientific evidence to back up their putative clinical effects. Many caveats exist about the studies on immunostimulants to prevent respiratory infections that difficult their comparison: many have methodological errors, differ in immunostimulant type or characteristics, treatment regime or the population under study. Thus, high quality randomized placebo-controlled double-blind trials are needed before a clear recommendation can be given about the use of immunostimulants in the prevention of respiratory infections of the general population. However, studies of several immunostimulants such as garlic, zinc, VD, probiotics and bacterial lysates show promising that need to be further confirmed. It remains essential for the clinican, before supplementing with any immunostimulant, to rule out the presence of diseases that could explain recurrent respiratory infections, and upon suspicion of immunological abnormalities to promptly refer the patient to a specialist in allergy and immunology.

References

1. Cundell DR, Matrone MA, Ratajczak P, Pierce JD, Jr. The effect of aerial parts of Echinacea on the circulating white cell levels and selected immune functions of the aging male Sprague-Dawley rat. Int Immunopharmacol 2003; 3:1041-8.
2. Goel V, Chang C, Slama J, Barton R, Bauer R, Gahler R, et al. Echinacea stimulates macrophage function in the lung and spleen of normal rats. J Nutr Biochem 2002; 13:487.
3. Sadigh-Eteghad S, khayat-Nuri H, Abadi N, Ghavami S, Golabi M, Shanebandi D. Synergetic effects of oral administration of levamisole and *Echinacea purpurea* on immune response in Wistar rat. Res Vet Sci 2011; 91:82-5.
4. Benson JM, Pokorny AJ, Rhule A, Wenner CA, Kandhi V, Cech NB, et al. *Echinacea purpurea* extracts modulate murine dendritic cell fate and function. Food Chem Toxicol 2010; 48:1170-7.
5. Shah SA, Sander S, White CM, Rinaldi M, Coleman CI. Evaluation of echinacea for the prevention and treatment of the common cold: a meta-analysis. Lancet Infect Dis 2007; 7:473-80.
6. Karsch-Volk M, Barrett B, Kiefer D, Bauer R, Ardjomand-Woelkart K, Linde K. Echinacea for preventing and treating the common cold. Cochrane Database Syst Rev 2014; 2:CD000530.
7. Ankri S, Mirelman D. Antimicrobial properties of allicin from garlic. Microbes Infect 1999; 1:125-9.
8. Ruddock PS, Liao M, Foster BC, Lawson L, Arnason JT, Dillon JA. Garlic natural health products exhibit variable constituent levels and antimicrobial activity against *Neisseria gonorrhoeae, Staphylococcus aureus* and *Enterococcus faecalis*. Phytother Res 2005; 19:327-34.
9. Weber ND, Andersen DO, North JA, Murray BK, Lawson LD, Hughes BG. In vitro virucidal effects of *Allium sativum* (garlic) extract and compounds. Planta Med 1992; 58:417-23.
10. Kyo E, Uda N, Suzuki A, Kakimoto M, Ushijima M, Kasuga S, et al. Immunomodulation and antitumor activities of Aged Garlic Extract. Phytomedicine 1998; 5:259-67.

11. Nantz MP, Rowe CA, Muller CE, Creasy RA, Stanilka JM, Percival SS. Supplementation with aged garlic extract improves both NK and gammadelta-T cell function and reduces the severity of cold and flu symptoms: a randomized, double-blind, placebo-controlled nutrition intervention. Clin Nutr 2012; 31:337-44.
12. Lissiman E, Bhasale AL, Cohen M. Garlic for the common cold. Cochrane Database Syst Rev 2009:CD006206.
13. Josling P. Preventing the common cold with a garlic supplement: a double-blind, placebo-controlled survey. Adv Ther 2001; 18:189-93.
14. Coleman JE. Zinc proteins: enzymes, storage proteins, transcription factors, and replication proteins. Annu Rev Biochem 1992; 61:897-946.
15. Wellinghausen N, Rink L. The significance of zinc for leukocyte biology. J Leukoc Biol 1998; 64:571-7.
16. Rink L, Kirchner H. Zinc-altered immune function and cytokine production. J Nutr 2000; 130:1407S-11S.
17. Neldner KH, Hambidge KM. Zinc therapy of acrodermatitis enteropathica. N Engl J Med 1975; 292:879-82.
18. Singh M, Das RR. Zinc for the common cold. Cochrane Database Syst Rev 2011:CD001364.
19. Hemila H, Chalker E. Vitamin C for preventing and treating the common cold. Cochrane Database Syst Rev 2013; 1:CD000980.
20. Wolden-Kirk H, Gysemans C, Verstuyf A, Mathieu C. Extraskeletal effects of vitamin D. Endocrinol Metab Clin North Am 2012; 41:571-94.
21. Wang TT, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 2004; 173:2909-12.
22. Hewison M. Vitamin D and the immune system: new perspectives on an old theme. Rheum Dis Clin North Am 2012; 38:125-39.
23. Coussens AK, Wilkinson RJ, Hanifa Y, Nikolayevskyy V, Elkington PT, Islam K, et al. Vitamin D accelerates resolution of inflammatory responses during tuberculosis treatment. Proc Natl Acad Sci U S A 2012; 109:15449-54.
24. Cannell JJ, Vieth R, Umhau JC, Holick MF, Grant WB, Madronich S, et al. Epidemic influenza and vitamin D. Epidemiol Infect 2006; 134:1129-40.
25. Cannell JJ, Zasloff M, Garland CF, Scragg R, Giovannucci E. On the epidemiology of influenza. Virol J 2008; 5:29.
26. Muhe L, Lulseged S, Mason KE, Simoes EA. Case-control study of the role of nutritional rickets in the risk of developing pneumonia in Ethiopian children. Lancet 1997; 349:1801-4.
27. Ginde AA, Mansbach JM, Camargo CA, Jr. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. Arch Intern Med 2009; 169:384-90.
28. Charan J, Goyal JP, Saxena D, Yadav P. Vitamin D for prevention of respiratory tract infections: A systematic review and meta-analysis. J Pharmacol Pharmacother 2012; 3:300-3.
29. Bergman P, Lindh AU, Bjorkhem-Bergman L, Lindh JD. Vitamin D and Respiratory Tract Infections: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. PLoS One 2013; 8:e65835.
30. Mao S, Huang S. Vitamin D supplementation and risk of respiratory tract infections: a meta-analysis of randomized controlled trials. Scand J Infect Dis 2013; 45:696-702.
31. Reid G, Jass J, Sebulsky MT, McCormick JK. Potential uses of probiotics in clinical practice. Clin Microbiol Rev 2003; 16:658-72.

32. Yasui H, Kiyoshima J, Hori T. Reduction of influenza virus titer and protection against influenza virus infection in infant mice fed *Lactobacillus casei* Shirota. Clin Diagn Lab Immunol 2004; 11:675-9.
33. Yasui H, Kiyoshima J, Hori T, Shida K. Protection against influenza virus infection of mice fed Bifidobacterium breve YIT4064. Clin Diagn Lab Immunol 1999; 6:186-92.
34. Donnet-Hughes A, Rochat F, Serrant P, Aeschlimann JM, Schiffrin EJ. Modulation of nonspecific mechanisms of defense by lactic acid bacteria: effective dose. J Dairy Sci 1999; 82:863-9.
35. Schiffrin EJ, Rochat F, Link-Amster H, Aeschlimann JM, Donnet-Hughes A. Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. J Dairy Sci 1995; 78:491-7.
36. Pelto L, Isolauri E, Lilius EM, Nuutila J, Salminen S. Probiotic bacteria down-regulate the milk-induced inflammatory response in milk-hypersensitive subjects but have an immunostimulatory effect in healthy subjects. Clin Exp Allergy 1998; 28:1474-9.
37. Drakes M, Blanchard T, Czinn S. Bacterial probiotic modulation of dendritic cells. Infect Immun 2004; 72:3299-309.
38. Hao Q, Lu Z, Dong BR, Huang CQ, Wu T. Probiotics for preventing acute upper respiratory tract infections. Cochrane Database Syst Rev 2011:CD006895.
39. Mauel J, Van Pham T, Kreis B, Bauer J. Stimulation by a bacterial extract (Broncho-Vaxom) of the metabolic and functional activities of murine macrophages. Int J Immunopharmacol 1989; 11:637-45.
40. Rozy A, Chorostowska-Wynimko J. Bacterial immunostimulants--mechanism of action and clinical application in respiratory diseases. Pneumonol Alergol Pol 2008; 76:353-9.
41. Del-Rio-Navarro BE, Espinosa Rosales F, Flenady V, Sienra-Monge JJ. Immunostimulants for preventing respiratory tract infection in children. Cochrane Database Syst Rev 2006:CD004974.
42. Litzman J, Lokaj J, Krejci M, Pesak S, Morgan G. Isoprinosine does not protect against frequent respiratory tract infections in childhood. Eur J Pediatr 1999; 158:32-7.