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Immunostimulants in the prevention of respiratory infections

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Abstract: Immunomodulation promises to be an effective prophylactic and therapeutic modality for chronic and recurrent respiratory infections. As opposed to vaccines, the term Immunostimulant (IS) refers to a compound that produces a state of non-specific immunity. Most IS are oral formulations of bacterial lysates that have been used in clinical practice for decades. One of the main obstacles in the development of immunostimulants is the poor understanding of the mechanism of action. Except for some compounds the mechanism of action of bacterial products is not well understood. Some appear to act through activation of monocytic cells and macrophages and enhancement of polyclonal proliferation of B cells. In general, the use of IS results in beneficial clinical outcome but the quality of some clinical trials for preventing Acute Respiratory Tract Infections (ARTIs) must be improved. In pediatric

population the use of IS for the prevention of ARTIs needs to be limited to children with high susceptibility to ARTIs or overexposed children, while in adults it must be indicated for patients with Chronic Obstructive Pulmonary Diseases (COPD) at high risk of exacerbation.

Keywords: Immunostimulant (IS); mechanism of action; Acute Respiratory Tract Infections (ARTIs); Chronic Obstructive Pulmonary Diseases (COPD); clinical trials.

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1 Introduction

For years immunologists were trying to devise immunomodulators that could be useful for treatment of illness where there are defects in the immunity like in cases of chronic and recurrent infections, cancer, or autoimmune diseases. Immunomodulators may enhance immune response in some but decrease in other cases. The term Immunostimulant (IS), as used in this paper, refers to a compound that produces a state of non-specific immunity that contributes to enhancement of resistance to an infection or malignancy (Bomford, 1989; Hadden, 1993). On the other hand, immunosuppressors interfere in a non-specific way with the immune response, which in some cases can increase the susceptibility to infections and cancer. Nevertheless, as noted by Bomford (1989) and Hadden (1993) it is often difficult to draw a clear line between ISs and immunosuppressors.

The main difference between an IS and a vaccine is that the latter is supposed to produce a protective immune response against a specific microorganism, which is included in the formulation either as a whole organism or a subunit thereof. Many ISs were developed in the preantibiotic era as an option to treat and prevent infectious diseases. During the first part of the 20th century the development of ISs has been ongoing in parallel with that of vaccines, that is, their empirical use preceded the understanding of the mechanism of action. Most of the registered ISs currently employed for the prevention of Acute Respiratory Tract Infections (ARTIs) are bacteria-derived products. Table 1 lists the common IS formulations for ARTIs prevention.

2 Mechanism of action of IS

The immune system is an intricate network of cells and a great variety of signalling molecules. The regulation of the immune system depends on the interaction points between cells and soluble substances that define the immune response (Bomford, 1989; Hadden, 1993).

Table 1 Most common ISs for prevention of acute respiratory infections

<i>Trade name</i>	<i>Common name</i>	<i>Active entity</i>
Adimod	Pidotimod	Pidotimod
Biostim	RU41740	Glycoprotein and membranes of <i>Klebsiella pneumoniae</i>
Broncho-Vaxom	OM-85	Lyophilized bacterial lysates
Decaris	Levamisole	Levamisole
Echinacea	Echinacea purpurea,	Extract of <i>Echinacea purpurea</i>
Immunoferon, Inmunol	AM3	Glycophosphopeptical
IRS 19	Not available	Bacterial lysates
Ismigen	Not available	Bacterial lysates
Lantigen B	Not available	Bacterial antigens
Leucotrofina, Leucogen	Thymomodulin	Thymus extract
Luivac	LW50020	Bacterial antigen
Munostin	Not available	Bacterial corpses and lysates
Not available	SLO4	Bacterial extracts
Paspat	LW50020	Autolysate mixture of bacterial antigens for parenteral application
Pulmotabs	Not available	Bacterial lysates
Pulomonarom	Not available	Bacterial lysates
Ribovac, Ribomunyl, Immucithal	D53	Proteoglycans of <i>K. pneumoniae</i> plus bacterial ribosomes
Umckaloabo	<i>Pelargonium sidoides</i>	Alcohol extract from the roots of <i>Pelargonium sidoides</i>

Except for the compound RU41740, the mechanism of action of bacterial products is not well understood, as their chemical nature has not been fully elucidated. RU41740 is an organic extraction of *Klebsiella pneumoniae*, it consists of two repetitive glycoprotein subunits: P1 from bacterial capsule and F1 from external bacterial membrane. It has been suggested that RU41740 induces activation of macrophages as well as polyclonal activation of B cells (Boissier, 1988; Chaumet and Boissier, 1988). Vacheron et al. (1989) and Miller et al. (2005) reported that Lipopolysaccharide (LPS) fraction F1 acts on LPS-receptors TLR4-MD2-CD14.

D53 contains proteoglycans from *Klebsiella pneumoniae* combined with ribosomal fractions from four different bacterial strains. D53 stimulates polymorphonuclear cells and macrophages, which leads to increased phagocytosis and proinflammatory cytokines. Also, ribosomal fractions induce formation of specific antibody-forming B cells, but response on T-cell has not been reported (Bene and Faure, 1997; Clot, 1997).

Another IS, OM-85, acts on macrophages and monocytic cells through the increase of intracellular calcium, glucose-regulated protein 7815 and *c-fos*/serum response element protein. These secondary messengers induce expression of pro-inflammatory interleukins IL-1a, IL-6, IL-8, and tumor necrosis factor-alpha (Broug-Holub et al., 1995; Keul et al., 1996). Other investigators reported that OM-85 BV induces nitric oxide and oxygen radicals in phagocytic cells (Broug-Holub et al., 1995; Lusuardi et al., 1993). In addition, the upregulation of adhesion molecules has been described by Marchant and Goldman (1996) and Jacquier-Sarlin et al. (1996). Humans who received OM-85 BV displayed enhanced cellular immune responses (Girard and Fleury, 1979; Maestroni and Losa, 1984); increase in secretory IgA (Emmerich et al., 1990; Lusuardi et al., 1993); serum IgA (Cvoriscec et al., 1989); serum IgG and IgM (Puigdollers et al., 1980); and activated phagocytic cells (Emmerich et al., 1990; Lusuardi et al., 1993).

The IS based on thymic extracts has not been well characterised, but their effect appears to be mainly on T-cell response. The use of thymic extract has been banned by the World Health Organization (WHO fact sheet 113, 2005) because of the risk of spongiform encephalopathy.

One of the main obstacles in the development of ISs is the poor understanding of the mechanism of action. It has been particularly challenging to identify a receptor or molecular target that would be associated with prevention of ARTIs. However, we have a better knowledge of synthetic ISs. For instance, tucaresol acts by forming covalent Schiff bases between ligands on APC and T-cells. Schiff bases are an essential element for providing a costimulatory signal to T-cell and increasing the synthesis of IL2 and gamma IFN (Rhodes, 1996). The imidazolic compounds, imiquimod and resiquimod appear to act through Toll Like Receptor 7 (TLR 7) and TLR 8 (Spaner et al., 2005; Weeratna et al., 2005). Other functions of levamisole and pidotimod have also been described. For example, levamisole enhances cellular immunity by enhancing *in vitro* T-cell proliferation as induced by antigens and mitogens (Amery and Bruynseels, 1992; Van Wauwe and Janssen, 1991) This action is mediated by a metabolite called OMPI (dl-2-oxy-3-(2-mercaptoethyl)-5 phenylimidazolidine (Hanson and Heidrick, 1991) Yet, the use of levamisole should be restricted because of the risk of agranulocytosis (Symoens et al., 1978) Pidotimod is another imidazolic compound that acts by enhancing cellular immunity (Benetti et al., 1994; Coppi and Manzardo, 1994). Thus, despite intensive research on immune function of IS we still do not know what is the ultimate mechanism of action either for bacterial IS or synthetic IS.

Table 2 Toll-like receptors and their ligands

<i>Receptor</i>	<i>Ligand or pathogen-associated molecular pattern (PAMP)</i>
TLR 1	Triacyl lipoproteins
TLR 2	Lipoproteins; gram positive peptidoglycan; lipoteichoic acids; fungi; zymosan
TLR 3	Double-stranded RNA (as found in certain viruses)
TLR 4	LPS (endotoxin)
TLR 5	Flagellin
TLR 6	Diacyl lipoproteins, peptidoglycan
TLR 7	Small synthetic compounds; single-stranded RNA
TLR 8	Small synthetic compounds; single-stranded RNA
TLR 9	Unmethylated CpG DNA
TLR 10	Unknown
TLR 11	Proteins expressed by several infectious protozoa

3 The epidemiology of ARTIs

ARTIs are still an important health and economic burden in the world. According to WHO (1998) ARTIs are the leading cause of morbidity accounting for 20% of medical consultations, 30% of lost days to work, and 75% of antibiotic prescriptions. ARTIs are responsible for most sick days at school amongst children (Haskins and Kotch, 1986) and parental absenteeism from work (Bell et al., 1989).

Community health studies in developed countries have provided basic information on the incidence of ARTIs. In the survey conducted in Tecumseh, Michigan, the annual incidence of ARTIs per person between 1965 and 1971, was 6.1 in children less than one year old; 5.7 in children aged from one to two years; 4.7 in children aged from three to four years; 3.5 in children aged from five to nine years; 2.7 in children aged from 10 to 14 years; 2.4 in 15–19-year-old; and from 2.8 to 1.3 in the adults (20 to >60 years) (Monto and Sullivan, 1974). In the second report of the Tecumseh study, which covered two phases comprising a period of 11 years (1965–1971 and 1976–1981), the mean annual number of ARTIs was 4.9 in the group from zero to four years; 2.8 in the group from 5 to 19 years; 2.2 in the group from 20 to 39 years; and 1.6 in the group ≥ 40 years (Monto and Sullivan, 1993). Table 3 and Figure 1 summarise the incidence of ARTIs found in the Tecumseh study. The BOSTID study in Africa, Asia and Latin America surveyed children from zero to 59 months of age (Selwyn, 1990). The incidence rate in six community-based studies ranged from 12.7 to 16.8 ARTIs per 100 child-weeks and the incidence of lower ARTIs was from 0.2 to 0.4 per 100 child-weeks.

In Mexico, the children raised at home had six ARTIs each year, with a median of 40 sick days per year, while children attending the day-care centres had 14 ARTIs per year with a median of 74 sick days (Flores-Hernandez et al., 1999). Another study in Mexico by Nandi-Lozano et al. (2002) found that the incidence of ARTIs in children attending the day-care centres was 10.3 per child/year.

Viruses are the main etiological agents of ARTIs in children at day-care centres and in the community. The most common virus isolates are rhinovirus, respiratory syncytial virus, parainfluenza virus and adenovirus (Denny et al., 1986; Flores-Hernandez et al.,

1999; Monto and Sullivan, 1974; Monto and Sullivan, 1993; Nandi-Lozano et al., 2002; Selwyn, 1990). So, in our opinion, if an IS is to prevent ARTIs, it should prevent the viral ARTIs and not just the bacterial infections, especially against bacteria contained in IS formulas. In the contrary case IS will not be much different from vaccines.

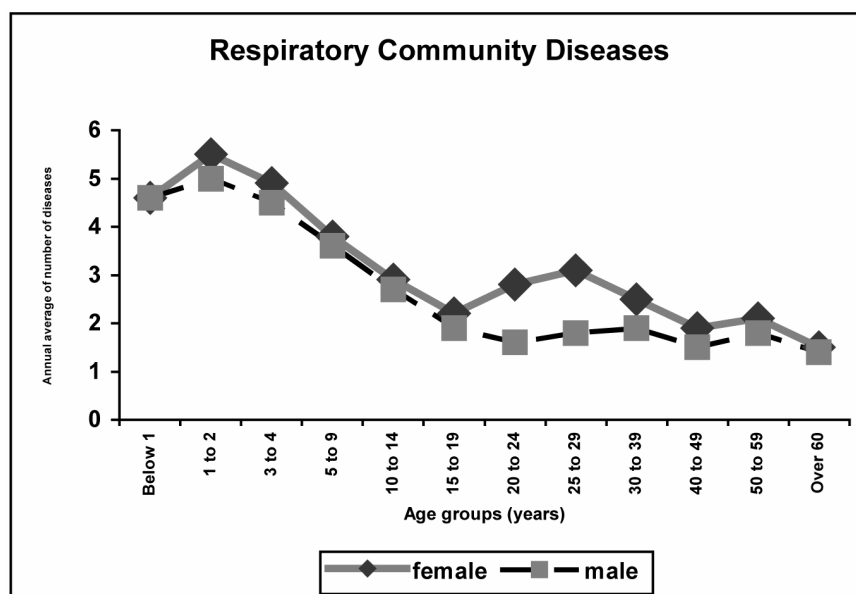
Table 3 The incidence of ARTIs by age groups as found in the Tecumseh study

<i>First phase (1965–1971)^a</i>		<i>Second phase (1965–1971) and (1976–1981)^b</i>	
<i>Percentage</i>	<i>Age group</i>	<i>Percentage</i>	<i>Age group</i>
6.1	0 to 11 months	4.9	0 months to 4 years
5.7	1–2 years	2.8	5–19 years
4.7	3–4 years	2.2	20–39 years
3.5	5–9 years	1.6	≥40 years
2.7	10–14 years		
2.4	15–19 years		
2.8 to 1.3	Adults 20 to >60 years		

^aTotal period of 6 years. Monto and Sullivan (1974).

^bTotal period of 11 years. Source: Monto and Sullivan (1993).

Figure 1 Incidence of ARTIs found in the Tecumseh study



The use of ISs should be appropriate when the number of ARTIs in the target group is higher than the normal incidence in the same age group. Based on our clinical experience in controlled and uncontrolled trials, as well as from general clinical practice, it will be difficult to diminish the number of ARTIs below the number in a normal healthy group.

4 Safety

4.1 Children

The Cochrane review on the use of IS for prevention of ARTIs in children provides data on adverse events revealed during clinical trials. Most of the trials reported a low incidence of adverse events or no adverse events at all. We observed that most frequent adverse events were gastrointestinal complaints such as nausea, vomit, discomfort and diarrhoea, and skin disorders such as rash, urticaria and pruritus (Berber et al., 2005).

According to an expert consensus, the incidence of adverse events in OM-85 BV clinical trials is 3–4% of the treated children. The most frequent adverse events were gastrointestinal complaints (gastric upset, abdominal pain, diarrhoea, nausea, vomit, and appetite loss) and cutaneous alterations (rash, erythema, pruritus). In the pharmacovigilance survey of OM-85 BV the most frequent complaints were fever, diarrhoea, rash, urticaria, abdominal pain, asthma, pruritus, and rhinitis. The risk of an autoimmune disease was pronounced to be minimal (Aiuti, 1993).

4.2 Adults

In adult patients with Chronic Obstructive Pulmonary Diseases (COPD), the bacteria-derived IS may cause adverse reaction in 3.3% of treated population which includes itching and cutaneous eruptions. 8% of the patients presented low urinary tract symptoms. The incidence of other complaints was not statistically different from the placebo group (Steurer-Stey 2004).

The systematic review of OM-85 BV revealed that adverse events were mild and similar in frequency to control population (Sprenkle et al., 2005). The most common reported adverse events were headache and gastrointestinal symptoms.

The search of the medical literature identified only two serious adverse event cases associated with IS; one case of bullous pemphigoid (an autoimmune disease associated with autoantibodies directed against the hemidesmosomal antigens BP230 and B180) associated with RU41740 (Chaby et al., 2004), and one case of another rare autoimmune disorder, tubulointerstitial nephritis, associated with D53 (Litwin et al., 2001).

5 Clinical efficacy

5.1 Children

All IS claim to be effective. Unfortunately, many of them failed to show efficacy in randomised, placebo-controlled trials. There are several reviews and meta-analyses of the efficacy of IS for the prevention of ARTIs in children and for the prevention of symptom exacerbations in patients with chronic bronchitis or COPD. The first review in 2001 by Berber et al. (2001) tried to compile and combine the results of randomised placebo-controlled clinical trials in the prevention of respiratory tract infections in children using IS. References of ISs (registered as products for ARTIs prevention) and ARTIs were searched and reports of randomised controlled clinical trials on the prevention of ARTIs in children were selected. Data sources were Medline, EMBASE and Cochrane Acute Respiratory Infection Group's register. The number of ARTIs was extracted as mean and Standard Deviation (SD) for the placebo and treated group.

Among IS, D53 ($n = 2$), LW50020 ($n = 2$), OM-85 BV ($n = 9$), pidotimod ($n = 6$), RU41740 ($n = 4$) and Thymomodulin ($n = 4$) had randomised, controlled trials ($n = 27$). However, only 20 trials reported the number of ARTIs and only 16 reported mean and SD data. The outcome of each trial was expressed as the percent value in comparison to the placebo group considered as 100%. The overall effect of IS in prevention of ARTIs was -42.6% (95% CI -45.2% , -40.1%), meaning that treated group had about 60% of the ARTIs incidence in relation to the placebo group. The quality of most trials was low, but those with higher Jadad's score were indicative of better efficacy (Jadad et al., 1996).

D53 had two extensive reviews of its results in randomised placebo controlled and uncontrolled trials, including results from unpublished trials in children and adults (Boyle et al., 2000). In a three-month period the use of D53 produced a reduction of 1.23 ± 0.45 , while in a six-month period the reduction was 1.51 ± 0.31 (Bellanti et al., 2003).

A comparative meta-analysis by De la Torre et al. (2005) includes the most representative, commercially available IS for ARTIs in children. The study evaluated the effect as the total reduction in number of ARTIs and percentage of reduction considering the mean ARTI number in the placebo group as 100%. Only trials longer than three months were considered and the effect was proportional to six months. The IS considered were D53, OM-85 BV, pidotimod and RU41740; the rest of the IS had no references to randomised, placebo controlled trials. The effects of each IS are listed in Table 4. D53 and OM-85 BV have shown efficacy both in terms of number and percent of ARTIs, while pidotimod had efficacy data expressed as a percent of ARTIs.

Table 4 Clinical effect of IS as a net difference in ARTIs incidence (confidence interval) compared to the mean number of ARTIs incidence in a placebo group considered as 100%

	<i>Number of ARTIs (95% CI)</i>	<i>Percent of ARTIs (95% CI)</i>
D53	$-0.92 (-1.46, -0.39)^*$	$-31.86 (-34.32, -29.40)^*$
OM-85 BV	$-1.20 (-1.70, -0.69)^*$	$-39.28 (-52.58, -25.98)^*$
RU41740	$-0.81 (-2.24, +0.62)$	$-27.60 (-73.88, +18.69)$
Pidotimod	$-0.82 (-1.84, +0.21)$	$-31.26 (-42.51, -20.01)^*$

* $p < 0.05$.

A Cochrane systematic review on the use of IS for preventing ARTIs in children is in preparation (Berber et al., 2005). The review considers only randomised (or quasi-randomised), placebo-controlled trials. All kinds of medications have been included. D53 had 17 trials, but six trials were reported only in reviews of the product (Boyle et al., 2000; Bellanti et al., 2003). The duration of eight D53 trials was less than six months and nine trials had duration of six months. In all D53 trials the description of the methodology was not clear and different routes of administration were used (nasal spray or oral). OM-85 BV had 12 trials, all published in journals, but only four were in journals with substantial impact factor (Collet et al., 1993; Gutiérrez-Tarango and Berber, 2001; Jara-Perez and Berber, 2000; Schaad et al., 2002) Four OM-85 trials were of high quality (Collet et al., 1993; Del Rio Navarro et al., 2003; Gutiérrez-Tarango and Berber, 2001; Jara-Perez and Berber, 2000) and in the remaining, the methodology was not very clear. Ten OM-85 trials had a duration of six months and two were longer than

six months. The main end-points were the number of ARTIs during the study period, expressed as a mean and standard error or SD. The outcome was analysed as mean number of ARTIs by group (raw data) and as percentage of ARTIs in the treated groups and compared to placebo, which is considered as 100%. Up to this time only 34 out of 57 randomised, placebo controlled trials had mean number of ARTIs or frequency tables from which means can be deduced. About 23 out of 34 studies showed reduction of ARTIs both as total numbers and as percentage of ARTIs. The effect on the total number of ARTIs was an absolute reduction [-1.27 (95% CI $-1.58, -0.97$)] as well as reduction in percentage of ARTIs [-39.68% ; 95% CI ($-47.27\%, -32.09\%$)]. When results are expressed as absolute number of ARTIs, the data were heterogeneous ($\chi^2 = 579.87$, $p < 0.00001$). The use of percentage values reduced the heterogeneity ($\chi^2 = 185.76$, $p < 0.00001$). The main source of heterogeneity was the mean number of ARTIs in the control group compared to absolute number of ARTIs (Pearson correlation = -0.672 , $p < 0.001$) or mean difference in the percentage of ARTIs related to the mean difference in the number of ARTIs (Pearson correlation = -0.638 , $p < 0.001$).

5.2 Adults

In adults the main application of IS is in prevention of exacerbations in chronic bronchitis or COPD. The use of IS for COPD is mentioned in the guidelines of management of COPD of the Global Initiative for Chronic Obstructive Lung Disease (GOLD group created by the National Heart, Lung, and Blood Institute and the World Health Organisation). (Global Initiative for Chronic Obstructive Lung Disease, 1998) Based on a single OM-85 study by Collet et al. (1997) they rank the use of IS as Type B evidence and do not recommend its regular use.

Yet, there are many other studies on the use of IS for the management of COPD. For instance, there is a systematic review on the use of bacterial IS in COPD (Steurer-Stey et al., 2004) 13 trials were identified: 10 with OM-85 BV, two with LW50020 and one with SL-04. Only three trials reported the number of exacerbations from which it was possible to calculate the relative risk of one exacerbation as 0.66 (95% CI, 0.41, 1.08), $p > 0.05$. Yet the analysis of other outcomes showed significant ($p < 0.05$) observer-assessed improvement of symptoms, patient-assessed improvement, and a short average duration of an exacerbation.

Other reviews dealt with a single type of IS. An OM-85 BV review found 13 randomised placebo controlled trials on COPD and included three in the meta-analysis showing a non-statistically significant trend in favour of OM-85 BV (relative risk of exacerbation 0.83, 95% confidence interval 0.65–1.05) (Sprenkle et al., 2005).

A systematic review of controlled trials of AM3 in patients with COPD by Reyes et al. (2004) cites nine studies. The effect of AM3 translated into a decrease in the average number of exacerbations by 0.31 units ($p < 0.001$; 95% CI 0.20–0.42), significant favourable differences were found in the average length of the exacerbations and the average length of the antibiotic treatment used for the exacerbations ($p < 0.001$).

On the other hand, Dahan et al. (1986) in an early review on the effect of RU41740 (then named C1740) in chronic bronchitis failed to show benefits to the patients. Only four out of eight trials had positive results; the negative results were attributed to the low power of sample size.

IS have been also used to prevent sinusitis (Gomez Barreto et al., 1998; Heintz et al., 1989; Zagar and Lofler-Badzek, 1988), otitis (Giovannini et al., 2000; Mora et al.,

2002; Renzo et al., 2004) and occupational respiratory disease in factory workers (Carmona-Ramirez et al., 2002; De Marco et al., 1984). Although provocative, these uses must have further randomised placebo controlled trials to establish the clinical benefit. Other important point to stress out is that, so far, only one trial has demonstrated effective protection against viral ARTIs as supported by viral culture (Aymard et al., 1994).

6 Discussion

The use of IS is not uncommon in some countries in Europe and America as a means of reducing the incidence of ARTIs in children and number and severity of COPD in adult patients. Yet, as the mechanisms of action are not completely understood and the clinical evidence is not well established, their utility remains controversial. The lack of enthusiasm, particularly among doctors educated in Anglo-Saxon countries, in embracing this kind of medication may have attributed, at least in part, to the deficiencies in the knowledge of mechanism of IS.

The quality of IS clinical trials, in general, is poor, although some of the trials conducted lately have improved. The most common problems in children and adult trials are the failure to select precise endpoints; lack of proper control; poor selection criteria; ill-defined standard operating definitions and proceedings; small sample size; and inapt explanations for dropouts and adverse events. All these deficiencies in the clinical protocol resulted in low power of data to reveal the differences between medication and placebo groups.

Trials in children have demonstrated that the efficacy of IS to prevent ≥ 1 incident is not reliable, but improves with ≥ 2 and ≥ 3 incidents (Del Rio Navarro et al., 2003; Gutierrez-Tarango and Berber, 2001; Jara-Perez and Berber, 2000). We believe that the failure to show significance in COPD is due to reliance on measuring ≥ 1 incident as dichotomous outcome data. We believe that the number of exacerbations (mean and SD) would be a better measure (scaled data). It would be convenient to explore the relative risk of ≥ 2 , ≥ 3 exacerbations in the COPD trials (Del Rio Navarro et al., 2004).

The results of clinical trials have shown that the reduction in the incidence of ARTIs in children and the reduction of exacerbations in COPD patients are real possibilities. Yet, IS protective effect would be noticeable in patients who experience a large number of ARTIs as compared to their normal peers. Therefore, in pediatric population the use of IS for the prevention of ARTIs must be limited to children with proven high susceptibility to ARTIs or overexposed children, while in adults it must be limited to patients with high risk of exacerbation of COPD.

The quality of the IS trials for preventing ARTIs needs to be improved as well as reporting of the results and adverse events (Begg et al., 1996; Collet, 1992; Valleron and Grimfeld, 1992). As a matter of public interest larger clinical trials sponsored by health authorities are desirable to establish the real effect of each individual IS. Additional indications to be explored in adults and children are the prevention of viral ARTIs (including asthma patients) and the prevention of recurrent otitis.

The mechanism of action of the bacterial and synthetic IS needs to be clarified, particularly the site of action or receptor involved, in order to create specific agonists and antagonists for clinical application. For instance, we need to identify active ingredients in bacterial extracts and their affinity receptors in order to create new synthetic entities.

To explore other possible paths is to seek different agonists and antagonists of TLR or to create new series of molecules capable of forming Schiff bases on the surface of immune cells as suggested by Rhodes (2002).

Some years ago, Hadden (1993) stated that the use of ISs was something like 'try to fix a TV set by kicking at it'. Currently, we know that this proverbial 'kick' can fix the TV and we are about to open it with proper tools to figure out what exactly happened.

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