

Position paper

Microbial products in allergy prevention and therapy

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Premise

Epidemiological and experimental studies have led to the hypothesis that stimulation of the immune system by certain microbial products may prevent or treat allergic diseases (1–5). There are also bacterial products on the market which have been proposed for treatment of allergic diseases but their clinical efficacy is not well documented, however.

The European Academy of Allergology and Clinical Immunology (EAACI) appointed a Task Force to critically evaluate available information on the potential use of microbial products in allergy prevention and therapy and to discuss guidelines for future research. This first position paper is separately assessing the effects of bacterial extracts, probiotics, mycobacteria, oligodesoxynucleotides (ISS-ODN), and lipopolysaccharide derived molecules in allergic diseases. The section for each of the listed products is structured to present the alleged rationale that would justify their use against allergy and experimental data supporting it. Then, the outcome of representative clinical trials are summarized with regard to efficacy and safety (Table 1). Finally, conclusions are made regarding their potential role in allergic diseases and the eventual need for further research.

As this is a very dynamically evolving field, the EAACI-Task Force will keep monitoring the new developments and will upgrade this position paper at regular intervals.

Bacterial extracts

Bacterial extracts are made from common pathogenic bacterial species, mostly those involved in upper and lower airway and/or urinary tract infections, with some million bacteria of different species/ml. Some recent studies have used bacterial lysate or bacterial surface layer proteins. While early preparations were administered subcutaneously, newer ones are given orally.

Experimental data

Oral bacterial extract enhanced natural killer activity and increased spontaneous and PHA-induced production of TNF-alpha, IL-2 and IFN-gamma (6) in peripheral blood mononuclear cells in vitro and the expression of adhesion molecules (LFA-1, MAC-1, ICAM-1) in phagocytes (7). Transcription and synthesis of IL-6 and IL-8 have been observed in human lung fibroblasts cultured in the presence of oral bacterial extracts (8). Bacterial extracts inhibit serum-induced IL-12 expression in peripheral blood lymphocytes (9) and were proposed to interfere with other treatments, such as allergen-specific immunotherapy (10).

In human volunteers, oral bacterial extracts induced an increase of IFN-alpha, IgA, and IL-2 concentrations in the bronchoalveolar lavage, increased serum total IgG levels and decreased serum total IgE levels (8). In addition, bacterial lysate induced the appearance in the

Table 1. Clinical trials using microbial products in allergy prevention and therapy

Preparation	Route	Reference	Disease	Design	Patients examined	Clinical outcome measures	Results	Ref
Autologous bacterial extract (ABE) + <i>Pneumococcus</i> , Friedlander's bacillus, <i>Staphylococcus</i> , <i>Bacillus septus</i> , H influenzae, <i>Streptococcus</i> , <i>Micrococcus catarrhalis</i> ,	Subcutaneous	Frankland AW et al. Autogenous bacterial vaccines in the treatment of asthma. BMJ 1955;2:941.	"infectious" asthma	Open, PC	100 treated vs 84 controls; adults and children	Clinical score	No difference ABE vs saline	16
<i>Staphylococcus aureus</i> , <i>Staph albus</i> , <i>Streptomyces viridans</i> , <i>Streptococcus haemolyticus</i> , <i>Pseudomonas aeruginosa</i> , <i>Proteus mirabilis</i>	Subcutaneous	Fontana VJ et al. Bacterial vaccines and infectious asthma. JAMA 1965;193:123.	"infectious" asthma	DBPC	15 treated vs 15 controls; children	Symptom and medication score	Less reduction of symptoms among treated v placebo group; no difference treated v placebo in antiasthmatic medication	17
<i>Staphylococcus aureus</i> , <i>Staph albus</i> , <i>Streptococcus viridans</i> , <i>Streptococcus haemolyticus</i> , <i>Pneumococcus</i> , <i>Neisseria catarrhalis</i> , Haemophilus influenzae + <i>Candida albicans</i>	Subcutaneous	Koivikko A. Bacterial vaccine in childhood asthma. Acta Allergol 1973;28:202-210.	"infectious" asthma	PC	16 treated vs 18 controls; children	Symptom score	No difference BE vs saline	18
<i>Staphylococcus aureus</i> , <i>Staph albus</i> , <i>Streptococcus viridans</i> , <i>Streptococcus haemolyticus</i> , <i>Pneumococcus</i> , <i>Neisseria catarrhalis</i> , <i>Haemophilus influenzae</i> , <i>Klebsiella pneumoniae</i> ,	Subcutaneous	Mueller HL, Lanz M. Hyposensitization with bacterial vaccine in infectious asthma. A double-blind study and a longitudinal study. JAMA 1969;26:1379-83.	"infectious" asthma	Open;	62 children	Number of asthma attacks associated with infection episodes	Significant decrease in frequency and severity of attacks of infectious asthma	19
Yogurt containing <i>Lactobacillus acidophilus</i>	Per os	Wheeler JG et al. Immune and clinical impact of Lactobacillus acidophilus on asthma. Ann. Allergy Asthma Immunol. 1997;79:229-33.	Asthma	DB cross-over;	15 adults	PEF; spirometric values; quality of life indices	No significant changes in outcome variables during treatment	27
Cow's milk formula 'fortified' with <i>Lactobacillus GG</i>	Per os (groups A, B), per os to breast-feeding mother (C)	Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. J Allergy Clin Immunol 1997;99:179-85.	Atopic eczema	DBPC + open	A) 13 ch. LGG treated + B) 14 ch. receiving placebo + C) 10 breast-fed ch. whose mothers received LGG	Clinical score	Similar and significant improvement of clinical score at 1 month among groups A and C; no improvement in group B	41

When formula with <i>Bifidobacterium lactis</i> (Bb-12) or with <i>Lactobacillus</i> GG (LGG)	Per os	Isolauri E, et al. Probiotics in the management of atopic eczema. Clin Exp Allergy 2000;30:1604–1610	Atopic eczema	DB	when formula with Bb-12 (group A, n = 9), with LGG (group B, n = 9), or without probiotics (group C, n = 9).	Severity of eczema (SCORAD)	42	Significant improvement of SCORAD in 9/9 subjects of groups A, 9/9 of group B and 4/9 of group C
<i>Lactobacillus</i> GG	Per os	Helin T et al. No effect of oral treatment with an intestinal bacterial strain, <i>Lactobacillus rhamnosus</i> (ATCC 53103), on birch pollen allergy-A placebo-controlled double-blind study. Allergy 2002;37:243-6	Allergic rhinitis and asthma in birch sensitized subjects	DBPC	38 adolescent/ adults	Symptom-medication score; open oral challenge test;	43	No differences between treatment and placebo receiving groups
<i>Lactobacillus</i> GG	Per os	Kalliomaki M et al. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. Lancet. 2001; 357:1076-9	Atopic eczema;	DBPC	A) 36 ch. LGG treated + B) 28 breast-fed ch. whose mothers received LGG + C) 68 ch. placebo group	Presence and severity (SCORAD) of atopic eczema;	44	Atopic eczema diagnosed in 9/36 of group A, 6/28 of group B, and 31/68 of group C
Delipidated, deglycolipidated M vaccae (DDMV) or autoclaved M vaccae (HKMV)	Intradermally in the deltoid region	Shircliff PM et al. The effect of delipidated deglycolipidated (DDMV) and heat-killed Mycobacterium vaccae in asthma. Am J Respir Crit Care Med 2001 May;163:1410-4	Asthma with atopy	DBPC	42 adults; 16 to 60 years; divided in 3 groups (DDMV; HKMV; placebo)	Mean morning PEF (MMPEF); symptom-medication score; asthma exacerbations	50	No differences in outcome variables between either the DDMV or the HKMV group and the placebo group
Heat killed M vaccae (SRL172)	Intradermally in the deltoid region	Arkwright PD, David TJ. Intradermal administration of a killed Mycobacterium vaccae suspension (SRL 172) is associated with improvement in atopic dermatitis in children with moderate-to-severe disease. J Allergy Clin Immunol 2001;107:531-4	Atopic dermatitis	DBPC	41 children; 5 to 18 years;	Skin surface area affected; dermatitis score; potency of topical steroid prescribed	51	Significant reduction of the surface area affected and of dermatitis score only in the group treated with SRL172;
Monophosphoryl lipid A (MPL) adjuvant + grass pollen extract	Subcutaneous	Drachenberg KJ et al. A well-tolerated grass pollen specific allergy vaccine containing a novel adjuvant, Monophosphoryl Lipid A reduces allergic symptoms after only four pre-seasonal injections. Allergy 2001;56:498-505	Seasonal allergic rhinoconjunctivitis in grass-pollen sensitized subjects	DBPC (multicentre)	81 receiving MPL-grass pollen vaccine; 60 receiving placebo	Symptom-medication score; titrated skin prick tests with grass pollen	72	Treatment compared to placebo receiving patients had significant improvement of symptom-medication score and significant reduction of degree of skin sensitization to grass pollen

tonsil of cells producing antibodies specific for the molecules contained in the lysate itself (11).

No recent experimental studies have been published on bacterial extracts given subcutaneously.

Proposed rationale for use against allergies

Oral bacterial extracts have been primarily proposed as “modulators” of the host local immune response. They were believed to increase the resistance of the respiratory tract mucosa to bacterial infections and consequently to prevent or cure bacterial infections.

Based on the hypothesis that specific IgE antibodies directed towards bacterial antigens contribute the pathogenesis of allergy (12), bacterial extracts have also been proposed many decades ago to be active against airway allergies, particularly rhinosinusitis and asthma, and injected subcutaneously.

Clinical studies

Most clinical trials of bacterial extracts investigated efficacy against respiratory infectious diseases. An analysis of these trials falls beyond the scopes of this position paper and is available elsewhere (12, 13).

A reduction symptoms was reported in 75% of 120 asthma patients treated for 3 years with oral bacterial immunotherapy (14). Another open study from the same group described a favourable response to oral bacterial immunotherapy in 86% of children aged 2–10 years (15). No double blind, placebo controlled studies using oral bacterial extracts against allergies have been published so far.

Bacterial immunotherapy of allergic diseases with subcutaneous injections has been prescribed widely for decades, but its use has shrunk dramatically in the recent years. Double-blind studies using subcutaneous injections with bacterial extracts did not demonstrate any clinical efficacy in asthma (16–18). Notably, these studies were criticized for using insufficient concentration of bacteria and too short treatment period (19). However, only one DBPC study completed in the 1960s showed a reduction in the frequency and severity of asthma attacks in relation to infection episodes (20).

Safety

All of the above mentioned clinical studies did not record any serious side effects, while minor side effects included gastrointestinal symptoms and skin rash (14–20).

Conclusions and perspectives

Bacterial extracts marketed earlier, showing minimal if any effect, contained probably too low amounts of bacterial material (10^{-6} – 10^{-7}) to induce an immune response via injection at weekly or longer intervals. There

is no recently published evidence in favour of a clinical use of so-called bacterial extracts against asthma and allergic diseases. International recommendations formulated in the first half of the 1990s excluded a role for bacterial extracts available in commerce at that time in the treatment of asthma (21–23) and no studies in favour of this treatment have been published recently since then. No studies assessing the preventive effect of oral bacterial extracts on the development of an atopic trait are available.

Probiotics

The term “Probiotics” is referred to living or inactivated organisms that are claimed to exert beneficial effects on health when ingested, and several definitions have been proposed and then changed over time (24). The most commonly used probiotics are lactobacilli and bifidobacteria, but enterococci and *E. coli* have also been proposed as such in the light of the above definition.

Experimental data

Several studies in human volunteers report that ingestion of probiotic bacteria or fermented milk products triggers the production of interferon-gamma by blood leukocytes (25–27). Increased IL-2 responses after stimulation by T cell mitogens was also observed in blood T cells from people ingesting probiotic bacteria (27–28). Ingestion of probiotic bacteria increased the phagocytic capacity of blood polymorphonuclear leukocytes (28–30), triggered respiratory burst (26, 29) and increased expression of complement receptor 3 (CR3) on these cells (31). Lactobacilli stimulate ample IL-12 production in human macrophages in vitro (32, 33).

Proposed rationale for use against allergies

Considerable circumstantial but little direct evidence support a relationship between the composition of the intestinal flora and allergies. The gut of infants born in poor areas of developing countries, where allergy prevalence is rather low, is colonized earlier by enterobacteria (34), enterococci, lactobacilli and eubacteria (35, 36) and displays a higher turnover of different *E. coli* strains in the intestinal microflora (37) compared to the gut of infants born in developed countries, where allergies are more frequent. In a prospective study among Swedish and Estonian infants, the counts of coliform bacteria were higher in the atopic children while bifidobacteria were more prevalent in controls by 2 years (38). In a prospective study in Finland infants developing atopic sensitisation at 12 months had more clostridia and fewer bifidobacteria in the first 3 weeks of life than infants who did not develop allergy (39). Similarly, lower prevalence rates of bifidobacteria were reported through the first

year of life in infants who developed allergy during their first two years of life than in those who did not (40); the real relevance of the observed differences on the local immune system and their impact on the development of atopic responses is still to be demonstrated. Moreover, lower counts of lactobacilli have not been reported so far in allergic infants.

Clinical studies

A few studies have examined so far the effect of probiotics on asthma and allergic symptoms. An extensively hydrolyzed formula containing *Lactobacillus rhamnosus* GG (LGG) was given to infants with atopic eczema and cow's milk allergy (41). The SCORAD rate decreased in both groups, however, and the two groups were not compared statistically after treatment. In the LGG group, fecal alpha-1-anti-trypsin concentrations and TNF-alpha concentrations decreased, whereas serum ECP was unaltered. This is an interesting observation, but it needs to be shown that the presence in the intestinal contents of lactobacilli have not changed the milieu in a way that would decrease the survival of these molecules (e.g. by lowered pH, increased water content, increased content of organic acids).

In a second study, the same group gave extensively hydrolysed whey formulas supplemented with *Bifidobacterium lactis* Bb-12, or with *Lactobacillus rhamnosus* GG, or not supplemented with probiotics to three groups of 9 infants each (overall mean age 4.6 months) who manifested atopic eczema during exclusive breast feeding. Both formulas supplemented with probiotics produced a significant decrease in SCORAD values in all the infants after two months of treatment, while only 4/9 children had a similar improvement in the group fed with the unsupplemented formula (42).

In a double-blind cross-over study, 15 adults with moderate asthma received for one month yoghurt with live lactobacilli and yoghurt without lactobacilli for another month; no differences in spirometric functions were detected between the two groups (27). In a DBPC study, no reduction of the symptom-medication score was found in 18 teenagers and adults treated with *L. rhamnosus*, as compared with controls, nor was sensitisation to birch pollen and apple reduced by the treatment, as assessed by challenge test (43). It was not stated however whether *L. rhamnosus* colonized or induced any changes in the intestinal microflora.

Lactobacillus rhamnosus GG has been tested for its ability to prevent occurrence of allergic diseases if administered from birth and to pregnant mothers (44). In a DBPC trial comprising 132 Finnish children with a family history of allergy *Lactobacillus rhamnosus* GG or placebo were given daily for 2–4 weeks preceding delivery to the mothers. After birth LGG or placebo life breast-fed infants was given orally to the babies or to the

mothers if breast feeding. "Atopic eczema" was diagnosed at 12 months of age in 9/36 (25%) of infants receiving LGG orally, 6/28 of infants whose mothers received LGG during the breast-feeding period, and 31/68 (46%) in controls. Sensitization, i.e. induction of specific IgE as measured by in vitro IgE-test or skin prick test was similar in the active and control groups. Thus, the treatment did not protect against IgE-mediated sensitization. Despite that, the authors concluded that *Lactobacillus rhamnosus* GG was effective in preventing early "atopic disease" in children at high risk not only when ingested by the babies themselves, but also when these babies do not receive *Lactobacillus* GG, provided that it is ingested by their breast-feeding mothers (44). Data demonstrating that all these "treated" infants were actually colonised by lactobacilli were not given in this study nor in the accompanying reference (41).

Safety

The administration of living organisms, even if considered normal and non-pathogenic as probiotics, must be regarded as potentially dangerous in individuals with primary or acquired immunodeficiency. Anecdotal cases of severe infections or fatalities linked to bifidobacteria or lactobacilli have been reported. Thus, meningitis caused by bifidobacterium was described in an infant treated with probiotics (45) and a patient became fatally septicemic with a vancomycin resistant strain of *Lactobacillus rhamnosus* contained in a live yogurth (46). As infants have lower colonization resistance and are immunologically naïve as compared with adults, it is reasonable to be cautious when considering administration of probiotics to newborn infants. It is controversial whether lactobacilli are part of the normal intestinal microflora in early infancy or not: some studies have reported that they are frequently found whereas others described low frequencies (37). The controversy is probably due to methodological differences. Colonisation by bifidobacteria at one week of age was more frequent in infants who later developed allergy, than in infants who remained healthy (40). Since we still do not possess compelling evidence that allergy is a disease caused by lack of bacterial stimulation, nor the mechanisms by which bacteria would prevent against allergy development, basic and clinical studies are required before recommending microbial treatment to infants.

Conclusions and perspectives

Evidence supporting the use of probiotics in the prevention or treatment of allergy or allergic diseases is still preliminary, and although preclinical studies are of interest, clinical studies are not conclusive yet. More studies are therefore essential. Any clinical trials using probiotics in newborns should be carefully monitored.

Mycobacteria

Mycobacteria are ubiquitous in the environment and include more than 80 species in soil and untreated water. Some of these, such as BCG or its components, have been utilised in humans as immunostimulators, but not as therapy against allergic diseases. The non-pathogenic mycobacterium, *M. vaccae*, has been proposed for the prevention and therapy of allergic diseases (47).

Experimental data

Mycobacterium vaccae (*M. vaccae*) has been reported to prevent sensitisation in rodents and inhibit generation of Th2-type cytokines in response to ovalbumin and to reduce allergic inflammation (48). It was also associated with reduced serum IgE and IL-5 synthesis by spleen cells taken from sensitised mice (48).

Proposed rationale for use against allergies

Natural exposure to environmental mycobacteria has been suggested as being able to promote Th1-type cytokine responses and has, therefore, been suggested to possibly prevent allergy (49). It has been hypothesized that administration of saprophytic mycobacteria in childhood could stimulate the generation of Th1 cytokine responses to common allergens and that this would help to prevent or treat allergy (47).

Clinical studies

A limited number of studies have so far been conducted with *M. vaccae*, but only two have been published in peer-reviewed journals.

In the first clinical study with *M. vaccae*, 42 patients with stable moderately severe asthma who were skin prick test positive to house dust mite, were randomized to receive two intradermal injections of either phosphate-buffered saline (placebo), heat-killed *M. vaccae* (HKMV) (0.5 mg), or delipidated deglycolipidated *M. vaccae* (DDMV) (0.05 mg). Markers of asthma severity (mean morning PEF, symptom-medication score) were measured for 3 months and blood eosinophils, IgE levels, and T cell proliferative and cytokine responses were monitored. There were no significant differences between either treatment group and the placebo group for any of the outcome variables. There was also no difference between the treatment groups and placebo for eosinophil, IgE levels, or the T cell proliferative and cytokine response. The results indicated no effect of low dose intradermal DDMV or *M. vaccae* on asthma severity in patients with established asthma (50).

In a second study, significant reduction in the extent of eczematous changes was observed in children with atopic dermatitis 3 months after a single intradermal dose of a

preparation of killed *M. vaccae* (SRL-172) (−48%, 95% CI 32% to 65%, $P = 0.001$) but not in those receiving placebo (−4%, 95% CI −29% to 22%). None of the children, however, had complete resolution of their dermatitis (51).

Safety

The safety and tolerability of *M. vaccae* has been examined in both patients with allergic diseases (50, 51) and in patients with melanoma (52). Erythematous subcutaneous lumps were commonly observed at the site of injection in children with eczema; the magnitude of the local reaction was not related to the severity of eczema, prior BCG vaccination, or response to treatment and the effect resolved spontaneously (51). Side-effects observed in trials of patients with cancer included rare cases of confusion, anaemia, small bowel obstruction, suspected pneumonitis, shortness of breath, cellulites and abdominal pain. However, because of the serious nature of the underlying disease, a clear cause-effect relationship between these observations and *M. vaccae* cannot be established (52).

Conclusions and perspectives

Clinical studies of efficacy of existing mycobacterial preparations in the prevention and therapy of allergy and asthma are still in a preliminary phase. The only two trials completed so far have not fulfilled the enthusiastic expectations raised on theoretical grounds (47), showing some efficacy in eczema but little or no efficacy in asthma. Further information on their efficacy may come from other trials currently in progress.

Immunostimulatory Sequences of bacterial DNA (ISS-ODN)

Immunostimulatory sequences DNA (ISS) and their synthetic oligodeoxynucleotides analogs (ISS-ODN, also known as CpG motifs) are derived from bacterial genomic DNA and provide immune activation and Th1 adjuvant activity in mice (53–55). They contain an unmethylated CpG dinucleotides core within a given hexamer occurring at a frequency of 1 in 16 in many prokaryotic genomes but much less frequently, and moreover in a methylated form in eukaryotic genomes (56). The effect of bacterial DNA on human cells is weaker. Recent reports confirm a Th1 stimulating activity of ISS-DNA in various human systems (53, 57), however some others did not identify IL-12 induction by bacterial DNA (58).

Experimental data

In mice, ISS-ODN were found to exert diverse effects on the immune response, whose description falls beyond the

scope of this position paper and that have been reviewed elsewhere (59). Immunization using ISS-ODN as an adjuvant resulted effective in rodent models of allergic disease, including anaphylaxis (60), asthma (61), and allergic conjunctivitis (62). Mice sensitised to an allergen but treated with ISS-ODN alone (as an immunomodulating agent) had attenuated hypersensitivity responses to subsequent allergen challenge (59). ISS-ODN were as effective as corticosteroids in the prevention of the immediate phase hypersensitivity response of allergic conjunctivitis, and more effective than corticosteroids in the prevention of the late phase inflammatory responses seen in murine models of allergic conjunctivitis and asthma (59).

In a human system, synthetic phosphorothioate ODN were able to induce B cell proliferation and to shift the *in vitro* differentiation of Dermatophagoides pteronyssinus group 1-specific hCD4+ T cells from atopic donors into Th1 phenotype (63). ISS-ODN led also to a significant increase of IFN-gamma production by NK cells through an IL-12-dependent mechanism and increased mRNA expression of IL-12 and IL-18 in hPBMC and monocyte-derived dendritic cells both in atopic and non-atopic individuals. In hPBMC from atopic patients stimulation with ISS-ODN led to a considerable increase of polyclonal IgG and IgM synthesis while the production of total IgE was suppressed. ISS-ODN induced also a significant rise of IgG and IgM specific for allergens to which the patients were sensitised whereas allergen-specific IgE levels remained unchanged (64). ISS-ODN induced IL-12, IFN-alpha, IFN-gamma, IL-10, and IL-6 production from hPBMCs from both non-atopic and atopic donors, increased expression of IFN-gamma receptor and decreased expression of IL-4 receptor on B cells from both atopic and non-atopic donors (65). Furthermore, ISS-ODN inhibited IL-4-dependent IgE production *in vitro*. Neutralization of IL-12, IFN-alpha, IFN-gamma, and IL-10, but not IL-6, attenuated the inhibitory activity of ISS-ODN on IgE production. In contrast to its inhibition of IgE synthesis, ISS-ODN stimulated the production of IgM, IgG, and IgA (65). Amb a 1, the immunodominant allergen in ragweed pollen, was conjugated with ISS-ODN and the activity of this Allergen-ISS conjugate (AIC) was investigated in hPBMC cultures from subjects with ragweed allergy. AIC reversed the dominant allergen-induced Th2 profile and enhanced IFN-gamma production (Th1 profile) (66).

Proposed rationale for use against allergies

The experimental evidence exposed so far has prompted the study of ISS-ODN as an anti-allergic vaccine adjuvant and immunomodulator, on the basis of the hypothesis that TH1 stimulation in humans by this molecule may prevent or reverse allergic immune responses as it was shown in mice.

Clinical studies

ISS-ODN are currently evaluated in clinical trials as vaccine adjuvants for infectious disease, allergy and cancer (67). Only one phase I-II clinical trial using the major ragweed allergen (Amb a 1) conjugated (AIC) to ISS-ODN in allergic patients has been reported so far (68, 69). AIC were less allergenic than allergen extract used for conventional immunotherapy, as measured by skin testing and serum levels of anti-Amba1 IgE antibodies and induced higher serum anti-Amba1 IgG levels than conventional immunotherapy with ragweed extract (66). No data from clinical trials using free ISS-ODN as immunomodulator have been published so far.

Safety

Patients treated with ISS-ODN as a vaccine adjuvant for infectious agents or for cancer did not present any major adverse reactions (67). As mentioned above, patients treated with AIC had less adverse effects than those treated by conventional immunotherapy (68, 69). In principle, a strong Th1 adjuvant might facilitate the development of some autoimmune reactions or autoimmune diseases, but such evidence has not been reported in ISS-treated patients or primates (67–70).

Conclusions and perspectives

Clinical trials using ISS-ODN in allergy prevention and therapy are in progress. It is still uncertain whether ISS-ODN exerts a comparable immunostimulatory activity in humans as observed in mice. The safety, the potency and the immune profile induced by ISS-based immunotherapeutics in allergic patients needs to be assessed.

Lipopolysaccharide derived molecules

Lipopolysaccharide (LPS) derivatives are molecules obtained by modification of LPS isolated by the outer membranes of gram negative bacteria. A molecule originated from LPS of *Salmonella minnesota* R595, the Monophosphoryl Lipid A (MPL), has been proposed as an adjuvant for vaccine antigens (71), and is the only LPS derivative utilized so far in trials against allergic diseases (72).

Experimental data

In an animal model, LPS exposure can prevent allergen sensitization and abolish the late phase inflammatory response in already sensitized mice (73). MPL has similar potent adjuvant properties of LPS (71, 74), but is less toxic, as it has a reduced ability (relative to LPS) to induce proinflammatory cytokines (75). Indeed, *in vitro* MPL can induce in mouse peritoneal macrophages

higher levels of IL-10, the anti-inflammatory cytokine which reduces the production of proinflammatory cytokines (75). Accordingly, levels of pro-inflammatory cytokines such as TNF-alpha, IFN and IL6 induced by MPL *in vivo* are much lower than those induced by LPS (76). MPL was also shown to activate *in vitro* antigen-presenting cells and elicit cytokine cascades (71), and to induce in mice systemic antigen-specific antibodies following intranasal administration combined with tetanus toxoid, hepatitis B surface antigen, or Influenza A/HK/68 (77). MPL combined with L-tyrosine is synergistic in enhancing murine antigen specific IgG antibody responses without enhancing antigen specific IgE responses. Furthermore, this adjuvant combination promoted strong IgG2 antigen specific responses indicative of a Th1 directed response. In KLH sensitised rats, treatment with MPL was shown to prevent a secondary IgE antibody response when injected with antigen boosters (78).

Proposed rationale for use against allergies

It was hypothesised that environmental LPS or endotoxin exposure would stimulate in humans TH1 immune responses and that this would protect against the development of atopy and asthma (79, 80). LPS exerts also severe endotoxic effects which limit its potential use as a therapeutic agent (74, 81). On this basis, LPS derivatives that could maintain immunostimulating properties of LPS but not its toxicity were tested *in vivo* as modulators of specific immune responses, including those against allergens (72, 82).

Clinical studies

A vaccine comprising a tyrosine-adsorbed glutaraldehyde-modified grass pollen extract containing MPL was tested in a multicentre, placebo controlled, randomised, double blind clinical study (72). Four subcutaneous injections of the active product were given preseasonally to 81 grass pollen sensitive subjects and 60 received placebo injections (tyrosine alone). Patients under active treatment, compared to patients receiving placebo, reported less nasal and ocular symptoms and combined symptoms, a lower medication score, a reduction of skin sensitivity, and a rise of IgG, but not of IgE antibodies against grass pollen (72). Mechanisms underlying the observed effects were not shown.

Safety

MPL is 100–10,000 times less toxic than LPS (83). It is well tolerated in different populations when given as the

prophylaxis against hepatitis B (84) and malaria (85). Toxicology studies at the Paul Ehrlich Institute, has confirmed the use of this new product in clinical trials (72). Allergic patients treated with MPL combined with grass pollen extracts experienced side effects (redness/swelling and/or pain and/or itching) at the injection site, but no serious or severe systemic reactions (72).

Conclusions and perspectives

The use of LPS derivatives as adjuvants in the control of allergic diseases is potentially of interest as they modulate specific and non specific immune responses (3, 86). Results obtained in the first trial using MPL combined with grass pollen extract on patients with pollenosis provided rather weak, although significant improvements (72).

Concluding remarks and future updates

Advances in molecular mechanisms regulating immune responses against infectious antigens are being extended to vaccines against allergic diseases. Hopefully, this will reduce the gap between the efficacy of vaccines aimed to protect against infectious diseases and those that should prevent or cure allergic diseases. The five strategies discussed in this position paper diverge with regard to scientific rationale and reliability and they are in different stages of research. Even those preparations that have been evaluated in clinical trials have not been conclusively documented and their use should be considered as experimental. Although the issue of using microbial products in allergy prevention and therapies is of great interest and although experimental data are supportive in this respect, the number and quality of clinical studies are insufficient to reach any definitive conclusion about the usefulness of this approach. Actually no level of evidence can be ascribed to the different treatments due to the variable effects or the extremely small number of patients included in the studies. However, performance of studies fulfilling the criteria of evidence based medicine should be strongly encouraged. The rationale proposed in some of the five strategies considered in this position paper were based on the TH1/TH2 paradigm, viewed from the perspective of who is interested mainly in TH2 diseases and looks for up-regulating TH1 effects. However, the effects on T regulatory cells should also be examined in future studies. A wider anti-inflammatory approach where both TH1 and TH2 inflammation are targeted by stimulation of T regulatory cells, in keeping with new explanations for the hygiene hypothesis (87, 88), can be considered an issue of future experimental research.

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