VIA MEDICA

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Bacterial immunostimulants — mechanism of action and clinical application in respiratory diseases

Immunostymulujące preparaty pochodzenia bakteryjnego
— mechanizm działania i zastosowanie w chorobach układu oddechowego

Streszczenie

Odporność przeciw chorobom zakaźnym powstaje w wyniku procesów naturalnych (zakażenie) lub w wyniku interwencji medycznej (szczepienia, podanie immunoglobulin, bakteryjnych preparatów immunostymulujących).
Wykazano, że immunostymulatory bakteryjne (ISs) zawierające lizaty bakterii (OM-85 BV, LW 50020) lub elementy ich komórek (ekstrakt rybosomalny) indukują zarówno niespecyficzną, jak i swoistą (komórkową, humoralną) odpowiedź immunologiczną organizmu. Dwoistość ich aktywności immunomodulacyjnej naśladuje lub w pewnym stopniu powtarza odpowiedź immunologiczną rozwijającą się po wniknięciu patogenu do organizmu człowieka, która początkowo jest nieswoista i stopniowo nabywa cech odpowiedzi swoistej. Jednak kliniczna skuteczność ISs w zapobieganiu infekcjom dróg oddechowych (RTI) jest wciąż dyskutowana. W niniejszej pracy omówiono mechanizm działania immunomodulatorów i przeanalizowano wyniki dostępnych danych klinicznych u dzieci i dorosłych.

Słowa kluczowe: immunostymulacja, lizat bakteryjny, ekstrakt rybosomalny, infekcje dróg oddechowych

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Abstract

Immunity towards bacteria might be achieved as a result of natural processes following infection, or as a consequence of medical intervention including vaccination, administration of immunoglobulins or therapy with immunostimulants derived from bacteria.
Bacterial immunostimulants (ISs) containing bacterial lysate (OM-85 BV, LW 50020) or components of bacterial cells (ribosomal extracts) were shown to induce a non-specific response (i.e. intensification of phagocytosis) but also to orchestrate both cellular (B, T cell stimulation) and humoral responses (antibodies and proinflammatory cytokines production). Therefore, the duality of their immunomodulatory activity mimics or, to a certain extent, repeats the immune response evoked by the intrusion of a pathogen into the human body, which is initially non-specific, but subsequently becomes specific. However, their clinical efficacy in the prevention of respiratory tract infection (RTI) is still debated. This article reviews their mechanism of action, as well as the available clinical data, discussing the pros and cons of their use in the prevention of RITs in children and adults.

Key words: immunostimulation, bacterial lysate, ribosomal extracts, respiratory tract infections

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Introduction

Immunity towards bacteria might be achieved as a result of natural processes following infection, or as a consequence of medical intervention including vaccination, administration of immunoglobulins or therapy with immunostimulants derived from bacteria.

While vaccination initiates a response similar to natural contact with an antigen, i.e. activation of immunocompetent cells, production of cytokines and specific antibodies, administration of preformed immunoglobins provides a direct, though passive and relatively short-lived, form of defence. Interestingly, bacterial immunomodulators (IMs) that contain killed bacteria, their lysate or components of bacterial cells were proved to increase the efficiency of immune system response, via both a specific as well as a non-specific effect on the cellular and humoral mechanisms [1–3]. Thus, the duality of their immunomodulatory activity mimics or, to certain extent, repeats the immune response evoked by the intrusion of a pathogen into the human body, which is initially non-specific, but subsequently becomes specific.

Since the 1970s, when the concept of the bacteria-derived immunomodulators appeared, various products were developed and accepted mostly for the supplementary treatment of recurrent respiratory tract infections. Here, we revisit the mode of their action in the context of the most recent data and hypotheses on the mechanism of antibacterial innate and adaptative immune responses.

Mechanism of action

The main gateways for micro-organism penetration into the human body are epithelial surfaces and mucosa. This explains why over 50% of lymphoid tissue is strategically located in this area, as so-called MALT (Mucosal-Associated Lymphoid Tissue). MALT consists of: NALT (Nasal-Associated Lymphoid Tissue), BALT (Bronchus-Associated Lymphoid Tissue), GALT (Gut-Associated Lymphoid Tissue) and urogenital system lymphoid tissue, covering therefore most of the internal body surfaces accessible for microorganisms [4].

Consequently, bacterial immunomodulators follow the route of naturally evoked immune response in a way of being administered orally (not parenterally, as typical vaccines). They are absorbed in the intestine, triggering GALT stimulation and subsequently generating the immune response within mucosal tissue in other organs including BALT [2, 5]. The key factors in this chain-like reaction are reactive Peyer's patches of GALT, re-

sponsible for the alien antigen identification and subsequent generation of the adequate response. It should be mentioned that 3 structurally and functionally different regions have been distinguished in the Peyer's patches structure: lymph nodules — mostly formed by the cluster of B cells, internodule areas — generally containing T cells and dome-like structures consisting of M cells, and follicle-associated epithelium — marked by the extraordinarily porous basal membrane. The M cells are characterized by the presence of microfolds, pocket-like structures where lymphocytes, mostly T and B memory cells (so called M-cell associated T-, B-cells) rather than macrophages and neutrophils, are located. It is now believed that the most important function of M cells is the trapping of micro-molecules from the intestinal lumen followed by their transfer to the subepithelial region, in which they are processed by mucosal membrane macrophages or dendritic cells (DCs) and presented to the lymphocytes. As a result, antigen-specific T and B cells are generated in the Peyer's patches as well as a considerable number of lymphoblasts, mostly IgA$^+$ precursors of the IgA-producing plasmocytes. Subsequently, lymphocytes and lymphoblasts are transported with the lymph from Peyer's patches into the mesenteric lymph nodes to mature. From the lymph nodes, activated lymphocytes get into the blood stream via the thoracic duct and finally return to all MALT-associated structures in different organs, e.g. airways [5].

Therefore, the protective effect of bacteria-derived immunomodulators is particularly related to memory cells, both T and B, enabling quick and specific cellular and humoral responses as a result. It is believed that antigen-defined induction of immunoglobulin (Ig) synthesis, mostly A isotype (secretory IgA – sIgA), is the most important protective activity against respiratory tract infection, providing help in ongoing and future contact with the same antigen. Both an increased number of IgA$^+$ plasmocytes as well as IgA in the upper and lower respiratory tract have been observed. Observations concerning the corresponding changes in the peripheral blood are conflicting.

The cellular-specific response is also modified simultaneously to the humoral response. IMs inducing interferon gamma (IFN-γ) and interleukin-12 (IL-12) production by some immunocompetent cells might allow preferential stimulation of the Th1 response. Besides, some studies demonstrated an increased ratio of CD4$^+$/CD8$^+$ cells in the local airways following treatment with IMs.

As well as antigen-specific defensive mechanisms, immunomodulators also evoke a non-specific response enhancing the activity of the main
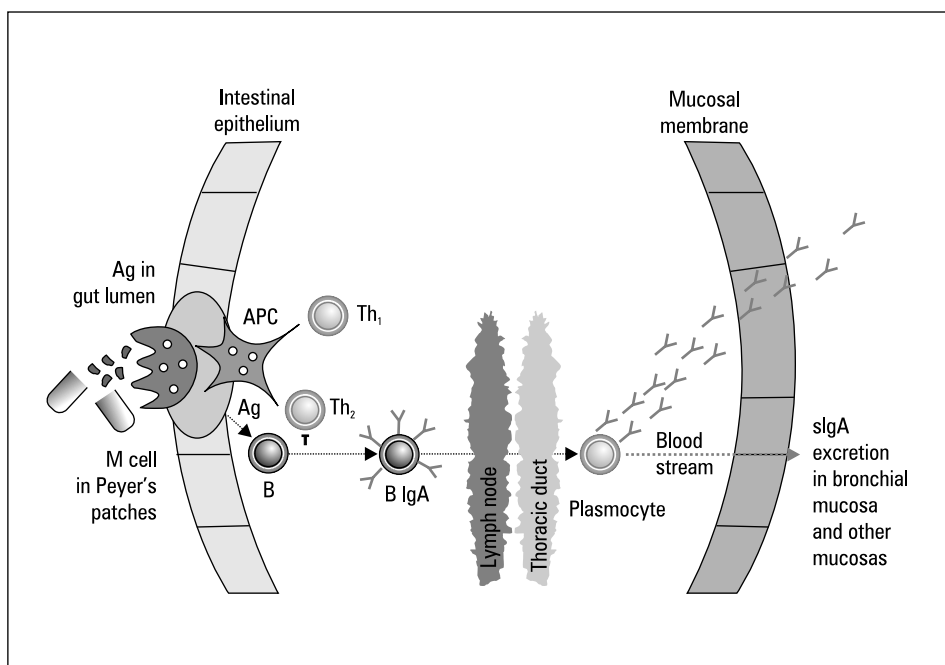
Figure 1. Mechanism of action of the oral immunostimulators [51]
Rycina 1. Mechanizm działania doustnego immunostymulatora [51]

phagocytic cells constituting innate immunity in the lungs: monocytes, alveolar macrophages and granulocytes. Innate or natural immunity is the principal way for the effective elimination of bacterial organisms that have reached bronchioles and alveoli. The effectiveness of this first-line response is increased by the immunomodulators by means of enhanced expression of adhesion molecules on monocytes (lymphocyte function-associated antigen-1 (LFA-1, CD11a/CD18), Mac-1 (CD11b/CD18), intercellular adhesion molecule-1 (ICAM-1)) and neutrophils (Mac-1, ICAM-1) important for their transendothelial migration from the blood stream into the respiratory tissues. Similarly, the stimulation of alveolar macrophages and granulocytes phagocyte activity allows efficient annihilation of the foreign organisms that reach distal airways [4, 6]. Mauel et al. demonstrated that bacterial immunomodulators enhance "respiratory burst" — superoxide and nitrite production by alveolar macrophages, therefore increasing their microbicidal and cytolytic activities. Likewise, their stimulatory effect on the production of key proinflammatory cytokines (tumour necrosis factor α (TNF-α), IL-8, IL-6, monocyte chemotactic protein-1 (MCP-1)) has been demonstrated [7].

In addition to the effects listed above, it has been suggested that bacterial immunomodulators might induce a considerable positive effect on the antigen-presenting cells (APCs) and DCs functional activity. Accordingly, enhanced expression of CD83, CD86

and class II human leukocyte antigen (HLA) molecules markers of DCs maturation has been observed following IM application, while increase in IL-12 production demonstrated in DC cultures in vitro might be important for preferential triggering of a Th1-type response (fig. 1).

Bacterial immunomodulators

OM-85 BV (Broncho-Vaxom)

OM-85 BV preparation contains lysates of eight bacterial pathogens (in equal parts), the most often encountered microorganisms in respiratory tract infections: *Haemophilus influenzae, Streptococcus pneumoniae, Klebsiella pneumoniae, Klebsiella ozaenae, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus viridans, Neisseria catarrhalis* [3, 8].

As a typical bacterial immunostimulator (IS), OM-85 BV was proved to affect both innate immunity influencing macrophages, neutrophils activity and proinflammatory cytokines production, as well as acquired immune response regulated by lymphocytes and synthesis of immunoglobulins [3, 7]. Several i*n vitro* studies demonstrated significant up-regulation of the oxidative metabolism, superoxide anion and nitric oxide production, and release by macrophages due to OM-85 BV administration. *In vivo*, OM-85 BV effectively boosted pathogen destruction by increasing macrophage activation (phagocytosis and antigen presentation) and

natural killer (NK) cell migration, activity and antigen recognition. Moreover, OM-85 BV stimulated the expression of proinflammatory cytokines in macrophages and monocytic cells, mainly of IFN-γ, IL-2, IL-1, IL-6, IL-8 and TNF-α, therefore affecting indirectly natural and acquired immunity by stimulation of T and B lymphocytes, granulocyte migration and macrophage phagocytic activity [3, 8–10]. OM-85BV was shown to increase expression of adhesion molecules on circulating monocytes and granulocytes — LFA-1, MAC-1, protein p150, intercellular adhesion molecule-1 (ICAM-1) mediating T cells APC interaction and immune cell migration, resulting in their increased activity [8].

It should also be mentioned that in newborn animals OM-85 BV encouraged preferential development of the Th1-type immunity characterized by amplified IFN-γ and decreased IL-4 production [11]. Interestingly, it was also found to act as a maturation-inducing factor for human DCs, as potent as TNF-α [12]. In human studies, an increased content of secretory IgA in bronchoalveolar lavage fluid in chronic bronchitis patients and healthy controls saliva as well as serum IgG, IgA and IgM levels were observed upon OM-85 BV treatment, reflecting up-regulation of specific immune response towards bacterial pathogens included into the lysate [13, 14].

LW 50020 (Luivac)

LW 50020 contains bacterial lysate of a slightly different profile, consisting of: *Staphylococcus aureus, Streptococcus mitis, Streptococcus pyogenes, Streptococcus pneumoniae, Klebsiella pneumoniae, Moraxella (Branhamella) catarrhalis, Haemophilus influenzae.*

LW 50020 has been demonstrated to considerably stimulate lymphocytes proliferation as well as T cell activity in the mesenteric lymph nodes model [15, 16]. Accordingly, up-regulated local production of IL-2 and IFN-γ was observed in the mesenteric lymph nodes and BALf from experimental animals [15–17], while IL-5 and IL-6 secretion was enhanced in cultured animal pulmonary lymphocytes [18]. In healthy volunteers treated with LW 50020, up-regulation of T lymphocytes proliferative activity was noted, as well as increased relative numbers of CD4, CD8 and memory cells (CD45RO^{+}) in bronchial lamina propria. Moreover, a raise in serum IL-1β, IL-6 and sICAM-1 levels was observed [19, 20].

Animal studies also demonstrated that oral administration of LW 50020 resulted in plasmocytes proliferation within Peyer's patches and in the BALT system, consequently leading to the incre-

ased production of specific IgA, mostly sIgA [21, 22]. An increased density and percentage of secondary lustre lymphoid cells containing specific antibodies producing cells in the tonsils of children treated with LW 50020 [23]. Accordingly, clinical trials showed higher levels of specific IgA and sIgA in the saliva of patients with recurrent respiratory tract infections subjected to immunostimulation with LW 50020, compared to the control group [24, 25]. The blocking of adhesion of bacteria to the nasal mucosa epithelial cells as a result of specific antibody presence was proven [26].

It has also been documented that the overexpressed intrapulmonary response to *Streptococcus pneumoniae* infection was reduced in animals orally immunized with the LW 50020 bacterial lysate, mainly by suppression of exaggerated PMN-elastase synthesis [17]. Simultaneously, enhancement of neutrophils, alveolar macrophages and peripheral blood cell phagocytic activity [27] as well as intensification of their oxidative capacity were observed as well [16, 28].

Ribosomal extract (Ribomunyl)

Ribosomal extract contains the immunogenic cellular components, i.e. ribosomes of bacteria, most often responsible for recurrent respiratory tract infections: *Klebsiella pneumoniae, Streptococcus pneumoniae, Streptococcus pyogenes* and *Haemophilus influenza*, together with the proteoglycans from cell membrane of *Klebsiella pneumoniae* strain [29, 30]. It has been suggested that strong nonspecific immune response evoked by this particular extract in experimental studies is mostly due to its unique content, particularly proteoglycan A protein (KpOmpA) derived from *K. pneumoniae* cell membrane. Interestingly, KpOmpA has been described as a potential toll-like Receptor-2 (TLR-2) inducer, acquiring, therefore, attributes adjuvant in vaccination [29]. Moreover, it was demonstrated that, similarly to other immunomodulators of bacterial origin, dynamic nonspecific response in subjects treated with ribosomal extract preparation depends on NK, lymphocyte, DCs and phagocyte stimulation [31] and on induced polyclonal nonspecific lymphocytes activation. Importantly, non-specifically activated B and T cells also engage in specific actions leading to up-regulated antibody production by plasmocytes or their increased ability to recognize and respond to antigens [32]. That goes along with the above-mentioned ability to induce TLR-2 receptors but also up-regulated expression of certain DCs surface molecules (CD83, CD86, HLA II) that actively participate in antigen presentation. Ribosomal extracts

have been also proven both *in vitro* and in *vivo*, to boost IL-12 production by DCs that could trigger Th type 1 (Th1) response [33, 34]. Their down--regulatory effect on the CD4$^+$cell population in atopic children has been also suggested [35]. Consecutively, mounting non-specific macrophage stimulation with the intensification of phagocytosis and increased production of pro-inflammatory cytokines TNF-α, MCP-1, IL-6, IL-8 results in intensive, more effective combined specific and non-specific resistance to infection [36, 37].

Apart from the cellular mechanisms, ribosomal extract, as other bacterial preparations, evokes specific immune response increasing antibody levels in peripheral blood as well as those produced by MALT — mainly of IgA isotype.

Clinical effects

Respiratory tract infections (RTIs) in adults and children are one of the primary causes of missed school days and absenteeism from work, as well as morbidity and mortality in children and particular groups of adults, mostly with other chronic co-morbidities of the respiratory and cardiovascular system.

Though the therapeutic use of ISs derived from bacteria is common in some European countries, the clinical efficacy of these medications in the prevention of RTIs is still debated. Also, most acute RTIs have a viral origin, and it is not entirely clear how a bacterial immunostimulant might prevent these infections.

It is usually suggested that highly susceptible patients have at least some immune disturbances. It has been shown that 57% of children with recurrent RTIs (3 or more episodes a year during at least 2 years) were deficient in one of the IgG subclasses while 17% were IgA deficient [38]. Similar data are available for the adult population [39]. Besides, selective IgA deficiency is known to be linked with frequent bacterial and viral respiratory infections [1]. Hence, the protective effect of bacterial immunostimulants is attributed to the enhancement of non-specific immunity as well general activation of the cellular and humoral responses, mostly increased secretory IgA levels.

The clinical usefulness of bacterial immunomodulators in preventing respiratory tract infections has been evaluated in a variety of individuals.

Several controlled studies have demonstrated their significant beneficial effect in children with RTIs, both young (< 6 yrs) as well as school children, although some groups published totally negative results concerning the preventive effect of bacterial immunotherapy [41].

Generally, in most IS treated groups, the number of acute upper RTI episodes was significantly lower and their duration shorter. Interestingly, some authors have observed that younger children and children with higher incidence of acute RTIs benefited more than others. Accordingly, Paupe et al. showed that in 116 children aged 0.6–19 years treated for 90 days with OM-85 BV, the number of upper RTIs diagnosed over a 6-month period of observation was 39.5% in comparison to 16.5% in a placebo treated group. These differences were even greater in the subgroup of children aged 6 years and less (34% vs. 3.5%) [40]. However, Schaad et al. did not manage to reproduce these data in younger subjects. Instead, they demonstrated that in a group of 170 children aged 36–96 months treated with three consecutive courses of OM-85 BV, upper RTIs reduction was significantly greater mainly in those with a history of frequent RTIs (reduction of up to 22% in comparison to a placebo group vs. 16% in the whole studied group) [41]. Still, no differences were observed in the number of lower RTIs and antibiotic treatment between evaluated subsets, while local antiseptic, anti-inflammatory and mucolytic products were significantly less frequently used by the OM-85 BV treated patients. Additionally, the immunomodulatory effect of bacterial extracts was prone to wear off gradually after cessation of treatment, although Paupe et al. did not report any significant deterioration during the 90 days of follow--up period [40]. Two other placebo-controlled, double-blind studies demonstrated even greater reductions in the frequency and duration of upper RTIs in highly susceptible children (by 52% and 38%) treated with IS. However, both should be very cautiously interpreted as the recruited groups were prone to unusually frequent RTIs (5 episodes in 6 months prior to inclusion) due to the highly polluted environment (Mexico City) or to the social conditions favouring microbial contamination (orphanage) [42, 43]. It is highly possible that the immune status of the evaluated subjects differed from the normal population as well as the children evaluated in other studies. Therefore, it is debatable to what extent the mentioned results and conclusions might be extrapolated into other populations.

Interestingly, Guiterrez et al. also observed monthly differences in ISs effect (May-August > September-December) and suggested that the best preventive outcome might be expected 2 months after the start of treatment [43].

While the effect of ISs in upper RTIs has been analyzed in a considerable number of studies, their preventive ability towards lower RTIs in children

was not extensively evaluated. Most of the available studies have been conducted in rather small groups or in patients with mixed upper/lower RTIs with no separately reported data for bronchitis. Consequently, most conclusions concerning lower RTIs are of rather speculative character, even though confirming the considerable protective effect (by 48%) of ribosomal extract in a group of 45 children with recurrent acute infections [29]. Importantly, the safety and tolerance of evaluated bacterial ISs in all paediatric trials were good and comparable to placebo.

In adults, ISs have also been extensively evaluated, although mostly in subjects with chronic pathologies of the respiratory tract, both upper and lower, mainly chronic bronchitis, chronic obstructive pulmonary disease (COPD), bronchoectasis and chronic sinusitis. Meanwhile, the ISs effect on the frequency of recurrent acute RTIs episodes in the typical Caucasian population has been poorly analyzed. There has been only one quality (randomized and double-blinded) study with 86 industrial workers treated with OM-85 BV for 10 days repeatedly in 6 consecutive months. Considerably lower incidence and severity of RTIs has been demonstrated, but with no effect on absenteeism at work [44].

In addition, in patients with upper RTIs, bacterial ISs have not been extensively evaluated. Ribosomal extract administered for 6 months were proven to diminish the cumulative number of recurrent acute upper RTIs episodes (–40%) starting from the first month and continuing until the end of the study [45]. Likewise, patients with chronic purulent sinusitis benefited from 3 month treatment with OM-85 BV by means of the number and severity of exacerbations [46]. As in paediatric trials, the final conclusions concerning the clinical usefulness of bacterial extracts in lower RTIs in adults are greatly limited due to the rather low quality of trials.

It should be strongly emphasized that only a minority of the studies conducted in adults is of high quality. Steurer-Stay et al. systematically reviewed 71 manuscripts concerning ISs effectiveness in the lower RTIs, of which 25 were excluded due to the lack of randomization, primary and secondary end--points and duplicated data, while a further 33 did not contain clinical data or included no COPD/bronchitis patients. Out of 13 studies included in the review, only 1 trial received the highest global quality score of 6, while 2 trials scored 4 each, on the basis of multi-component methodological quality assessment. The median score was 2, proving the poor general quality of the evaluated studies [47].

The PARI-IS study, which scored the best rating in the mentioned systematic review and was conducted as a large double-blind placebo-control-led randomized trail in mild to moderate COPD patients, clearly suggested the beneficial effect of the ISs on reduction of hospitalization rate due to severe exacerbations, but not on the prevention of severe respiratory events. Meanwhile, another large trial of acceptable quality showed a significantly lower likelihood of hospitalization [48]. A reduction in the average duration of exacerbation of approximately 3 days, as well as an improvement in symptoms as rated by patients and observers, were also reported, although the pooled results came from studies of limited methodological quality [14, 49, 50]. As in the paediatric studies, side effects were as common in the treated group as in controls.

Also, it should be clearly articulated that the majority of available studies were conducted in mild-to-moderate COPD/chronic bronchitis patients and apply to this category of subjects only. ISs effectiveness in reducing exacerbations in severe patients needs to be evaluated separately. Due to the complex pathophysiology of severe COPD and multi-system involvement (including the immune system), the above-mentioned results cannot be directly extrapolated from severe patients. Additionally, nothing is known about possible additive or synergistic effects with recommended treatment strategies.

Conclusions

In conclusion, strong evidence of the beneficial effects of bacterial extracts on the lower RTIs is still missing. However, their favourable effect on certain clinical aspects, such as severity of symptoms, seems to be reasonably documented. Obviously, more carefully designed studies are needed to answer many questions concerning the clinical usefulness of bacterial extracts.

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