

RESPIRATORY IMMUNITY AND THE RATIONALE FOR IMMUNOMODULATION IN THE PREVENTION OF RESPIRATORY TRACT INFECTIONS

A narrative summary of selected presentations given at the second Encuentro Latinoamericano de Infecciones Respiratorias Recurrentes (ELAIR), an educational summit held in Buenos Aires, Argentina, 4th–5th July 2015

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ABSTRACT

This educational summit, supported by an independent grant from OM/Vifor Pharma, brought together physicians specialising in pulmonary medicine, otolaryngology, surgery, immunology, and paediatrics. Delegates from the USA, Europe, and Latin America met and discussed the current unmet needs of patients suffering respiratory tract infections (RTIs). The meeting included plenary lectures, workshops, and interactive sessions, allowing delegates and presenters to debate the most pressing local and international issues in the field.

INTRODUCTION

The immune system may be thought of as a battleship providing both defensive armour and offensive firepower against infections. These elements do not always fully protect against pathogens, however, and it is the physician's role to understand the root cause of these failures in order to facilitate both the prevention and cure of infection. The immune response can be divided into innate immunity, which is non-specific and has no memory, and acquired immunity, which is specific and retains a memory to fight future infections. These two aspects can be further divided into systemic and mucosal immunity, the latter of which is the major barrier against respiratory infection.

The mucosal membranes of the human body represent a large surface area. The intestinal mucosa constitutes the largest subdivision of the mucosal membranes, followed by the respiratory mucosa

and the urogenital mucosa. Of the three mucosal membranes, the respiratory mucosa has the most regular direct contact with the exterior environment, via the act of breathing, and is therefore a key front in the battle against infection.

ORGANISATION OF THE RESPIRATORY MUCOSA

The innate immune system contributes a first and second line of defence in the respiratory mucosa. The first line is composed of the mucous covering the epithelial cells, which both traps foreign particles and contains antimicrobial molecules (defensins), and the epithelial cells themselves, which are linked to one another by tight junctions and which act as a further mechanical barrier to foreign particles/molecules. Trapped particles are removed by mucociliary clearance, the most primitive and general respiratory defence

mechanism. The second line of innate defence is represented by phagocytic neutrophils and macrophages, the cytotoxic natural killer (NK) cells, and the antigen-presenting dendritic cells (DCs). Antigen-presenting DCs migrate through the epithelial layer and act as a bridge between the innate system and the downstream effectors of the third line of defence, adaptive immunity.

The seat of the adaptive immune response is the mucosa-associated lymphoid tissue (MALT), represented by inductive sites where antigen sampling and recognition occurs, and diffuse effector sites found throughout all mucosal tissue.¹ The effector cells of the adaptive immune system are the T and B cells. T cells can be further subdivided into, amongst others, the large subclass of CD4⁺ T helper cells (Th) and the CD8⁺ cytotoxic T cells (Tc). Th cells, including Th1, Th2, and Th17 subclasses, support other immune cells through the release of cytokines that stimulate and promote cell proliferation and survival. Cytokines produced by Th cells include Th1-related interferon gamma (IFN γ), which supports the inflammatory immune response and activates macrophages, and Th2-related interleukin (IL)-4, which stimulates activated B cells and promotes naïve Th cell (Th0) maturation. Tc cells act similarly to NK cells in destroying virus-infected, tumoural, and other damaged cells. A third class of regulatory/suppressor T cells (Treg) can express both CD4 and CD8, and are key to homeostasis in the immune system.

B cells generate various classes of antibody: IgG, IgA, and IgM. IgA, specifically secretory IgA (sIgA), is the most important immunoglobulin for mucosal immunity. IgA makes up approximately 70% of immunoglobulins in the respiratory mucosa, with the remainder being IgG. sIgA forms in plasma cells, a differentiated form of B cell, with IgA monomers being linked by joining chain (J chain) molecules. Following this, the linked IgA molecules are absorbed by epithelial cells and secreted, along with the secretory component acquired during absorption/secretion, into the respiratory lumen. sIgA is active at all levels of the respiratory system from the nostril to the alveoli, and acts through an immune exclusion mechanism: binding bacteria in the lumen and excluding them from entering the deeper layers of the respiratory system.²

The MALT contains both inductive sites where the initial adaptive immune response begins through antigen sensing, represented by Waldeyer's ring at the oropharyngeal level in the respiratory system and by Peyer's patches in the gut-associated lymphoid tissue (GALT), and numerous effector sites that execute the immune response. A key aspect of the integrated immune response is that inductive and effector sites may be remote from one another. In fact, they may be located in distinct subsections of the immune system, enabling activation of inductive sites within the GALT to confer increased resistance to infection in the respiratory mucosa.

The vast majority (80–85%) of the body's 10¹² lymphocytes are found within the GALT, which contains 10¹¹ lymphocytes in total. Each Peyer's patch constitutes a follicle-associated epithelium containing M cells, a sub-epithelial area or dome containing DCs, a lymphoid follicle containing B cells, and finally an extra-follicular area containing T cells.³ Antigens that enter the gut are captured by the M cells, which are closely associated with sub-epithelial lymphocytes and DCs. These absorbed antigens are subsequently released by the M cells and taken up by DCs that proceed to stimulate cellular constituents of the adaptive immune system.

In addition to antigens, particular structural elements of pathogens known as pathogen-associated molecular patterns (PAMPs) are important in alerting the immune system. The peptidoglycan constituent of the cell wall of gram-positive bacteria and the lipopolysaccharide (LPS) constituent of the external membrane of gram-negative bacteria represent important PAMPs for these two groups of pathogens. Host recognition of PAMPs is mediated by a number of receptor classes, including the Toll-like receptors (TLRs).⁴

TLRs are present on many cell types, including macrophages and DCs. Binding of different TLRs results in the activation of various intracellular transcription pathways. One major consequence of TLR activation is the production of cytokines via two alternative pathways mediated by either the transcription factor NF- κ B or by mitogen-activated protein (MAP) kinases that bind the transcription factor AP1. Cytokines are the major effector molecules in the immune system and are potent, low-molecular-weight proteins that act upon many

cell types with differing effects depending on both the cytokine type and its concentration. The cytokines produced via TLR activation can be subdivided into classes including chemokines, cellular chemo-attractants, and adhesion molecules that mediate cell adhesion to the endothelium.⁵ TLR-induced activation of DCs lies at the crux of the immune response and leads to activation of both the innate and adaptive components.⁶ As well as directly activating innate immune cells through cytokine release and adaptive immunoglobulin-producing B cells through antigen presentation, DC cells are capable of activating Th0. These activated CD4⁺ Th cells then release further cytokines that stimulate and potentiate the innate immune response, as well as support immunoglobulin-producing B cells.

The final element in the integrated GALT-respiratory immune response is the process of immune cell homing, which allows the DC-mediated immune response in the inductive Peyer's patches to result in immune activity at the effector sites of the respiratory mucosa. Following activation of Th cells, innate immune cells, and B cells within Peyer's patches, the cells migrate via the thoracic duct and bloodstream to the respiratory mucosa in response to inflammation and immune challenge. Once in

the effector site of the respiratory mucosa, the CD4⁺ T cells further stimulate the innate immune response through cytokine release and induce B cell differentiation into plasma cells. Mature plasma cells then produce IgA dimers that are released into the respiratory mucus following binding with the secretory component. Therefore, PAMP-induced TLR activation of DCs in the GALT results in both innate and adaptive immune response in the respiratory system (Figure 1).

ORAL IMMUNOMODULATORS AND RESPIRATORY IMMUNITY

Relapse and recurrence driven by immune dysfunction are common in RTIs. Immunomodulators may enhance both regulation and expression of an integrated response from the immune system that protects against RTI. Bacterial immunomodulators come in different forms, including bacterial lysates and ribosomal extracts. In all cases these are formed from dead microorganisms with novirulence but retained immunogenicity. The lyophilised and standardised bacterial lysate OM-85, formed from 21 strains of 8 bacterial species and sub-species (5 genera), is the most studied of the bacterial immunomodulators.

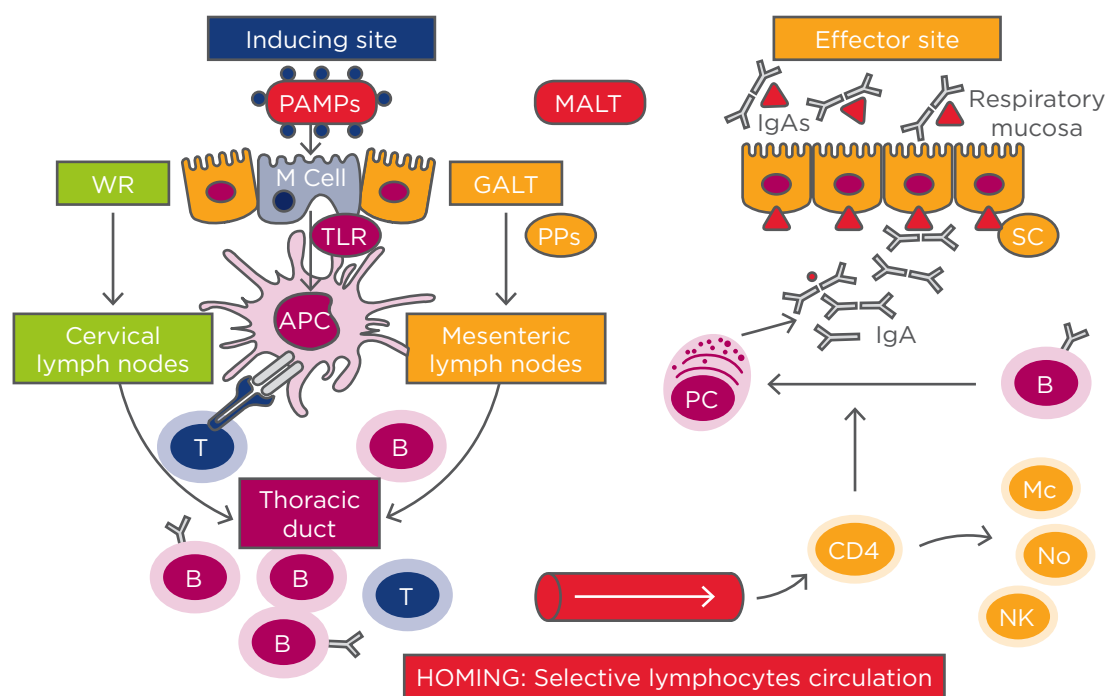


Figure 1: Immune responses in the mucosa-associated lymphoid tissue.

APC: antigen-presenting cell; GALT: gut-associated lymphoid tissue; MALT: mucosa-associated lymphoid tissue; Mc: monocyte; NK: natural killer; No: neutrophil; PAMP: pathogen-associated molecular pattern; PC: plasma cell; PPs: Peyer's patches; SC: secretory component; WR: Waldeyer's ring.

OM-85 and Innate Immunity

OM-85 has a number of effects on the innate immune system derived from modulation of human DCs. DC activation occurs in a modulated manner and is of a lower intensity, with slower kinetics, than that produced by LPS. Stimulation of DCs by OM-85 *in vitro* results in the release of chemokines (CCL2 and CCL3) that act on monocytes and NK cells; pro-phagocytic chemokines CXCL1, CXCL6, and CXCL8; and the chemotactic factors CCL20 and CCL22. OM-85-derived DC maturation leads to production of cytokines, chemokines, and adhesion molecules. The resulting putative increase in non-specific phagocytosis at the mucosal level may promote non-specific respiratory immunity. OM-85 also promotes the production of interferon alpha by DCs, which is the most important cytokine for defence against viral infections. These data suggest that, despite its bacterial constituents, OM-85 stimulates a basal antiviral state in the human immune system. The central message from these data is that OM-85 modulates DC activity, creating a mild activation that promotes downstream immune activity resulting in a pre-alert state protecting against future bacterial and viral infections.⁷

A recent *in vivo* study confirmed the ability of OM-85 to stimulate antiviral innate immunity and to protect against primary viral infections and secondary bacterial infections, in this case by *Streptococcus pneumoniae* or *Klebsiella pneumoniae*, which often follow a primary viral infection. Mice received OM-85 or placebo daily for 10 days before infection with the H1N1 influenza virus, with a bacterial agent being introduced 1 week after viral infection. Markers of DC activation and maturation were increased following OM-85 treatment. OM-85 enhanced the innate immune response, resulting in more rapid control of the infection and a reduced viral H1N1 load in the lungs. The innate response was also faster in OM-85-treated mice as shown by the lower number of neutrophils ($p < 0.05$) in the lungs 5 days post-H1N1 infection, which is indicative of a quicker resolution of the immune response. These OM-85-induced improvements in immune responses against influenza virus protected against the effects of secondary bacterial infections, including bacteraemia, weight loss, and temperature increase.⁸

Cells from patients with chronic bronchitis ($n=28$) treated with OM-85 were compared with those from healthy controls. Bronchoalveolar lavage cell counts revealed an increase in the CD4/CD8

(helper/suppressor) cell ratio in favour of CD4 in patients with chronic bronchitis following treatment ($p=0.04$). In addition, both macrophage activity ($p=0.03$) and concentration of IFN γ ($p=0.03$), a promoter of NK cell activity, were increased following OM-85 therapy.⁹ *In vitro* data from human peripheral blood mononuclear cells demonstrates that OM-85 also stimulates NK cell activity, as well as increasing the concentration of the related cytokine IL-2.¹⁰ In human lung fibroblasts, OM-85 also induces increased expression of IL-8, the major chemotactic factor for neutrophils, in a concentration-dependent manner.¹¹ Three studies from the early 1990s, summarised in a 1994 review by Jacques Mauël, demonstrated increases in adhesion molecules, polymorphonuclear leukocytes, and phagocytosis.¹² In addition, a recent Chinese study on children with concomitant asthma and recurrent RTI demonstrated an increase in the neutrophil, macrophage, and epithelial cell-derived antimicrobial human beta-defensin 1 (h β D-1) following treatment with OM-85.¹³

In summary, OM-85 acts on the primary sentinel cells of the innate immune system, DCs. Numerous downstream immune processes indicative of DC activation and immune system activity have been demonstrated. OM-85 promotes CD4⁺ T cells, leading to an increase in the innate immunity-related cytokines IFN γ and IL-2, which promote activity of lymphocytes, including NK cells. OM-85-associated increases in adhesion molecules and IL-8 further promote innate immune cell activity, in particular that of neutrophils. In turn, these cells promote increased phagocytosis and production of the antimicrobial h β D-1. Therefore, OM-85 promotes innate immune responses via multiple pathways.

OM-85 and Adaptive Immunity

The cytokines IL-6, IL-10, IL-11, and B cell activating factor (BAFF) are involved in B cell maturation and activation. OM-85 has been shown to activate DCs, leading to the production of IL-6, BAFF, and IL-10.⁷ OM-85 also induces increased expression of IL-6 and IL-11 in human lung fibroblasts *in vitro*.^{11,12,14} Puigdollers and colleagues¹⁵ demonstrated increased sIgA in the saliva of healthy volunteers following oral administration of OM-85, and increases in serum (IgM and IgG) and intestinal (IgA and IgG) immunoglobulins were demonstrated in immunosuppressed mice.¹⁶ Three further studies have demonstrated increased bronchial sIgA9 and serum IgA and IgG in response to OM-85.^{13,17}

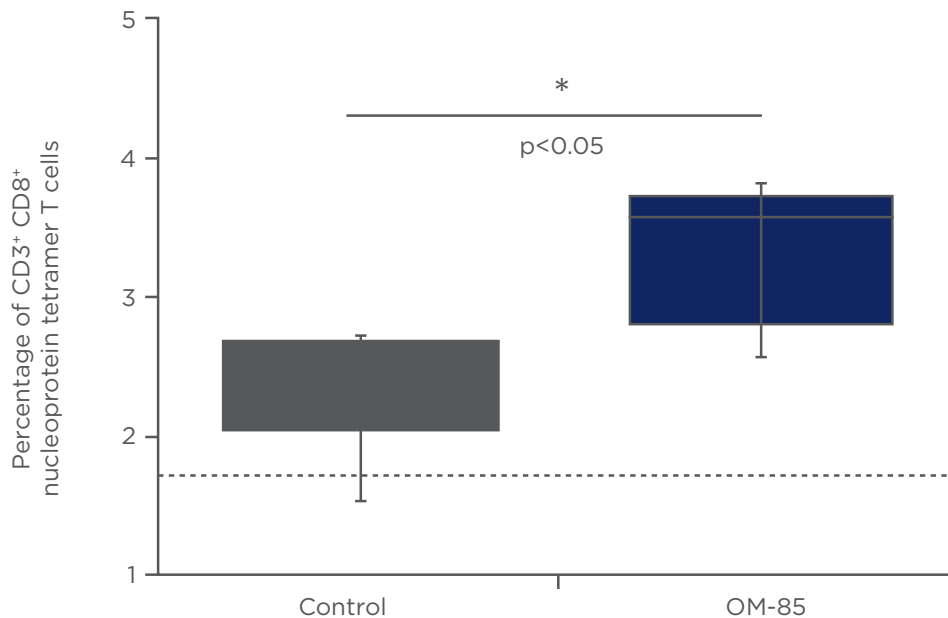


Figure 2: Cytotoxic T cell response in mice treated with OM-85 versus placebo.⁸

OM-85 administration has shown a protective effect in mouse models of both systemic bacterial and respiratory H1N1 viral infection. All mice infected with *Salmonella typhimurium* or H1N1 and treated with OM-85 survived, compared with 58% and 70%, respectively, of those treated with placebo. In addition, the appearance of clinical signs was delayed and their intensity and duration were reduced. Treatment-induced marked increases in serum levels of antibodies recognising all 21 strains of pathogen used in OM-85's manufacturing process. The authors suggest that activity of anti-*Klebsiella* immunoglobulins against the related species *S. typhimurium* may allow an adaptive response against this infection.¹⁸

The adaptive cellular immune response by cytotoxic CD8⁺ T cells was significantly increased in mice who received OM-85 or placebo daily for 10 days before infection with the H1N1 influenza virus followed by introduction of *S. pneumoniae* or *K. pneumoniae* after 1 week ($p < 0.05$; Figure 2). In non-infected mice, OM-85 caused an increase in influenza-specific IgA in both serum and bronchoalveolar lavage. Polyclonal IgG in the serum, and a trend towards increased polyclonal IgA and IgG in the airways, were also found in OM-85-treated mice, which is indicative of B cell activation leading to the release of antibodies active against multiple infectious agents.⁸

In summary, OM-85-induced increases in both cytokines (IL-6 and IL-11) and immunoglobulins have

been demonstrated in the gut (IgA and IgG), in serum (IgA and IgG), and in secretory form (sIgA) within the respiratory system. A putative pathway leading to production of sIgA within the respiratory system derives from the previously mentioned activation of CD4⁺ Th cells leading to release of cytokines (IL-6 and IL-11), which in turn induces B cell maturation into plasma cells. Plasma cells then go on to release sIgA dimers that are released into the respiratory mucosa through epithelial cells. In addition, the increase in CD8⁺ cells and polyclonal IgG and IgA production offer a mechanism for protection against subsequent infections.

OM-85 and Allergy

When discussing any immunomodulatory therapy, such as OM-85, it is important to investigate any possible relationship with immune dysfunction that may affect allergy. Individual Th cell phenotypes have different cytokine profiles. Th1 cells produce IFN γ , which acts against intracellular pathogens, whereas the Th2 phenotype produces IL-4, IL-5, IL-6, and IL-13, which are related to allergy. In addition to the role of Th2 cells in allergy, recent data indicate that Type 2 innate lymphoid cells also have a key role in allergy.¹⁹ The Treg phenotype produces transforming growth factor beta (TGF β), IL-10, and IL-35, which act to regulate the immune response. Regulation is essential in order to control the balance between other Th cell phenotypes and avoid chronic inflammatory activity.

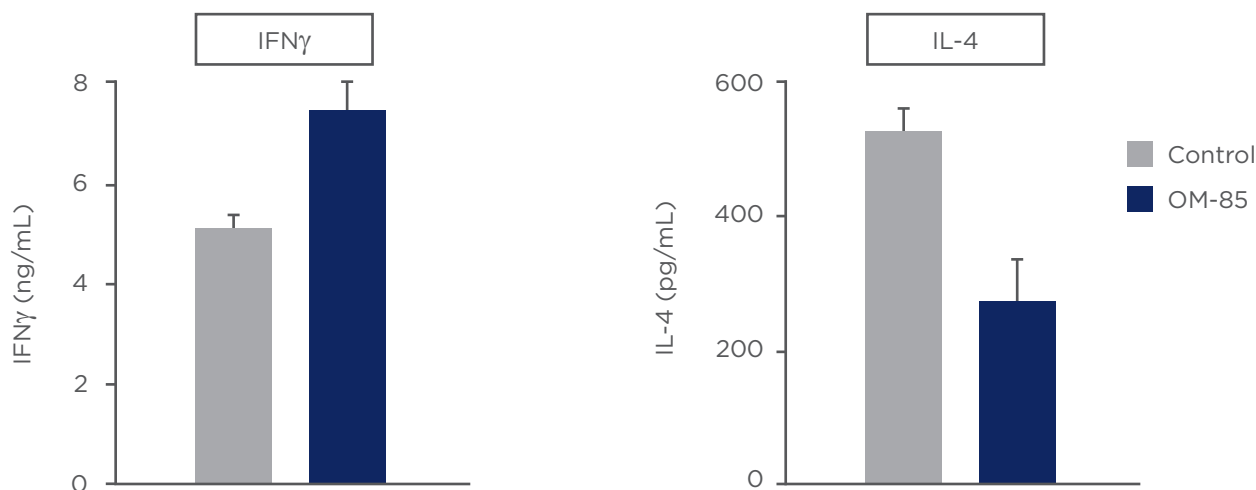


Figure 3: OM-85 favours Th1 activity (IFN γ) over Th2 activity (IL-4) in a mouse model of asthma.²⁷
 IFN γ : interferon gamma; IL-4: interleukin 4.

The balance between Th1 and Th2 cell activity is maintained by two forms of Treg cells, Th3 and Tr1. Th3 cells produce the cytokine TGF β that acts to suppress Th1 activity, while Tr1 cells produce IL-10 that suppresses Th2 cell activity. In addition, the Th1 cytokine IFN γ suppresses Th2 activity, while the Th2 cytokine IL-4 suppresses Th1 activity. An imbalance of activity favouring Th2, and consequent suppression of Th1 activity by IL-4, predisposes toward allergy. Such a Th2 imbalance is important to protect the fetus during gestation but continues into childhood, predisposing towards allergic conditions and infection.

DCs also have a role in allergy as DC activation is key to the maturation of Th cells. The functional characteristics of DCs are adaptive and often change in response to inflammation or infection.⁶ Recent data suggest that dysfunction in the response to allergens and viral pathogens by the DC network of the respiratory mucosa is a primary cause of both disease initiation and progress in asthma.²⁰ As mentioned above, OM-85 activates human DCs, providing a possible pathway via which dysfunction in allergic conditions such as asthma may be modulated.⁷

More direct evidence of a positive immunomodulatory role in allergy comes from the BALB/c mouse model of asthma. OM-85 suppresses airway inflammation via an IL-10 dependent pathway and increases Treg recruitment at the level of the trachea. In addition, transfer of purified CD4⁺ cells from treated mice reduces inflammation in sensitised mice.²¹ These data are supported by similar results from a different mouse model of

asthma, which demonstrated attenuated airway inflammation, reduced Th2 cytokines, and increased Treg expansion and Th1 promoting/Th2 suppressing cytokines.²² Similarly, OM-85 induced IL-10 release from the DCs of both healthy donors and patients with the inflammatory condition chronic obstructive pulmonary disease (COPD) *in vivo*. The increase in IL-10 levels was more pronounced in cells from COPD patients and in the presence of pro-inflammatory cytokines.⁷

There is a prevalence of Th2 signalling in infants that makes allergic conditions more prevalent. Treatment of neonatal rats with OM-85 resulted in the balancing of this Th2 bias, as indicated by upregulation of the Th1-related immunoglobulin isotype IgG2b.²³ In a study using a mouse model of allergic rhinitis, OM-85 reduced Th2-related cytokines (IL-4, IL-5, and IL-13) and immunoglobulins (IgE and IgG1).²⁴

In another study using an asthma model, BALB/c mice were orally immunised with OM-85 and subsequently sensitised with ovalbumin. Those mice pretreated with OM-85 showed a decrease in both total and ovalbumin-specific IgE indicative of a reduction in allergic response. OM-85 favours the Th1 immune response in mice by upregulating Th1-specific IFN γ and downregulating Th2-specific IL-4 in spleen cell supernatants (Figure 3).²⁵ Similarly, in a study in children aged 13-14 years with RTIs (N=20), OM-85 stimulated T cell activity and IFN γ production compared with placebo, thus upregulating the cell-mediated immune response and promoting anti-viral and anti-bacterial activity.²⁶ The IFN γ /IL-4 balance is

important in forms of asthma associated with allergic reactions, suggesting a possible protective mechanism. Finally, OM-85 reduced both the frequency of asthma attacks and RTIs, as well as the use of antibiotics, in a human study. These clinical improvements occurred in conjunction with pro-Th1/anti-Th2 changes in cytokine levels (increased IFN γ and IL-10, reduced IL-4), with these changes likely mediated through an increase in NK T cells.²⁷

The above evidence from multiple sources suggests that OM-85 promotes pathways leading from DC activation to Th cell maturation favouring Th1 activity via increased IFN γ and decreased IL-4. An additional OM-85-induced Treg pathway exists leading to increased IL-10 and thus further promoting Th1 activity. Therefore, immune

modulation with OM-85 promotes balance of the Th1/Th2 system in allergic conditions in which Th2 activity is elevated.

CONCLUSION

OM-85 activation of DC cells at induction sites within the GALT leads to migration of activated immune cells to the respiratory mucosa, activating both innate (pro-phagocytic and antiviral cytokines) and adaptive (immunoglobulins, B cell activation/maturation, and CD8⁺ cells) immune processes through the release of cytokines. In addition, OM-85 plays a rebalancing role in allergic conditions that feature immune dysfunction by promoting Th1 activity (increased IFN γ and IL-10) and reducing the upregulation of Th2 activity (reduced IL-4).

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