LEADING ARTICLE

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Development of an Asthma Vaccine
Research into BCG

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Abstract

Asthma is an atopic disorder characterised by the activation and recruitment of eosinophils to the lung resulting in chronic swelling and inflammation of the airways. Allergic disorders such as atopic asthma and dermatitis have been increasingly prevalent in developed countries, and the inverse correlation between exposure to major diseases such as tuberculosis and atopy prevalence has been reported. Intranasal administration of *Mycobacterium bovis*-Bacillus Calmette-Guerin (BCG) has been demonstrated to suppress airway eosinophilia in a model of atopic asthma. This immunomodulation is attributed to the ability of interferon (IFN)-γ produced by BCG-specific T_H1 lymphocytes to inhibit the development of lung T_H2 responses such as airway eosinophilia. The mechanism of IFNγ–induced inhibition is yet to be defined, but could involve activation of macrophages, direct suppression of developing T_H2 lymphocytes, or altered dendritic cell activation and antigen presentation. Mycobacteria such as BCG and certain mycobacterial fractions are strong inducers of a T_H1 immune response. The effectiveness of BCG in inhibiting atopic airway eosinophilia suggests its potential as a useful therapeutic agent in the treatment of atopic asthma.

Asthma is an atopic disorder characterised by the activation and recruitment of eosinophils to the lung resulting in chronic swelling and inflammation of the airways. T lymphocytes of the immune system are a key instigator of much of the pathology seen in atopic asthma.[1,2] In particular, the type 2 T helper (T_H2) lymphocyte through its production of the cytokines interleukin (IL)-4 and IL-5 programmes the timing and characteristics of atopic airway disease including mast cell sensitisation and degranulation, eosinophil and lymphocyte recruitment and activation in the airways,[3,4] and mucus secretion.[5] Current theory holds that the T_H2 lymphocyte subset is principally designed to regulate or control infection by metazoan parasites which migrate through the tissues often involving the lung and gut.[6,7]

The other major CD4+ T helper lymphocyte subset is comprised of T_H1 lymphocytes which produce IL-2, interferon (IFN)-γ and tumour necrosis factor (TNF)-β, cytokines which attract and activate macrophages and induce the production of complement fixing antibodies by B lymphocytes.[8,9] The T_H1 lymphocyte subset is thought to be more directly involved in neutralising viral infections and intracellular pathogens such as mycobacteria. One of the intriguing features of the T_H subsets is that they appear able to reciprocally balance each others' activity.[10,11] Such observations have raised the possibility that either the T_H1 or T_H2 cytokines or the agents which selectively induce T_H1 or T_H2 lymphocyte populations could be used to modulate or regulate the activity of opposite T_H1 or T_H2 immune responses.

1. Intranasal Administration of BCG Vaccine Can Inhibit Atopic Airway Disease

Prior intranasal administration of *Mycobacterium bovis*-Bacillus Calmette-Guerin (BCG) into the airways of mice is able to block the development of allergen (ovalbumin)-induced airway eosinophilia.[12] The inhibition of eosinophilia correlated with a greatly reduced IL-5 production by T_H2 lymphocytes in the lymph nodes draining the lung. BCG was found to exert its inhibitory effects through the action of IFNγ as BCG infection failed to inhibit atopic airway inflammation in IFNγ receptor deficient mice. The conclusion of this study was that IFNγ produced by BCG-specific T_H1 lymphocytes was able to inhibit the development of local T_H2 immune responses in the lung leading to suppression of allergen-induced airway inflammation. Importantly, the BCG-induced suppression was confined to the atopic immune response in the airways as serum levels of allergen-specific antibody and blood eosinophilia were unaffected. In related experiments, other workers have demonstrated that either administration of IFNγ directly into the airways of allergen-challenged mice or injection of IL-12 will also suppress atopic airway inflammation.[13-16] This data has been made all the more compelling by the many recent reports speculating that in the developed world lack of frequent exposure to infections by organisms such as mycobacteria has lead to an increased risk of developing atopy.[17-20]

2. *Mycobacterium bovis* Contains Many Immunostimulatory Molecules

Mycobacteria are well recognised as powerful inducers of T_H1 type immune responses, known to stimulate the development of IFNγ–producing T_H1 lymphocytes, and cause extensive recruitment and activation of macrophages.[21,22] The ability of mycobacteria to induce the T_H1 immune response has been attributed to diverse features of the mycobacteria including its cell wall constituents,[23] the proteins it secretes,[24] the intracellular environment of the macrophage in which it sequesters, and even the unmethylated copies of DNA it releases.[25] However, it is generally accepted that the unique characteristic of mycobacteria, both in terms of their biology and interaction with the host immune system, is the chemical structure of their cell wall.[23] It is this cell wall which gives the mycobacteria bacilli its gram positive and acid fast nature, making it resistant to acids and alkalis and to dehydration, and contributing to its prolonged survival within sequestering macrophages. The essence of the mycobacterial cell wall is the mycolyl arabinogalactan-peptidoglycan complex (mAPG) and the associated lipoarabinomannan (LAM). The mAPG constitutes the underlying core of the mycobacterial cell wall, whereas LAM has been shown to stimulate many of the cellular inflammatory events associated with mycobacterial infection, including macrophage activation and production of the cytokines IL-12, TNFα and IL-6.[26-30]

Another important antigenic preparation of mycobacteria is purified protein derivative or, as it is most commonly known, PPD.[24] PPD represents the conglomerate of proteins which are extracted from the supernatant of heat-killed *M. tuberculosis* or *M. bovis* cultures. Depending on the classification system used, between 7 to 50 different protein antigens have been recognised in this crude preparation, with most of them being able to stimulate either T cells or B cell antibody responses.

3. Suppression of T_H2 Lymphocyte Immune Responses by Interferonγ

Several studies have reported the suppressive effects of IFNγ on T_H2 lymphocyte development, but very few have demonstrated or speculated a clear mechanism of action.[10,13,31,32] This is largely because in *in vitro* studies the T_H2 immune response (principally IL-4 activity) appears to dominate and suppress T_H1 development.[33] In contrast, *in vivo* studies have reported clear immune suppressive effects of IFNγ on T_H2 immune responses.[14-16,34] There are several possible explanations for this apparent contradiction. The T_H2 lymphocyte which develops *in vivo* may be more sensitive to the ac-

tion of IFNγ than the *in vitro* T_H2 lymphocyte. The development of T_H2 lymphocytes may be indirectly influenced through the action of IFNγ on the antigen presenting dendritic cell or macrophage. We have previously reported that T_H2 immune responses in the airways are exquisitely dependent on costimulatory signals provided by the molecules CD80 and CD86.[35,36] It is possible that IFNγ is able to suppress T_H2 lymphocyte activation through its ability to modulate CD80 upregulation on dendritic cells. The caveat to this would have to be that antigen presentation to the T_H1 lymphocytes would not be affected by IFNγ, possibly because under the circumstances of mycobacterial infection macrophages can present the antigen and this macrophage presentation ability is enhanced by exposure to IFNγ.

Mycobacteria are not the only microbial agents that can provoke a T_H1 lymphocyte response in a mammalian host. Infection with a virus such as influenza,[37,38] protozoan parasites such as *Leishmania*[39] and *Toxoplasma*,[7] or gram negative bacteria such as *Brucella abortus*[40] have all been demonstrated to induce quite profound T_H1 immune responses characterised by the production of IFNγ. However, each of these infectious agents does vary with respect to specific cytokine profiles and additional effector arms of the immune response they induce. For example, influenza also induces a strong cytotoxic CD8+ T cell response and high levels of immunoglobulin (Ig)G2a antibody production.[37,38] Paradoxically, many studies concerning influenza infection have associated it with exacerbation of many of the symptoms of asthma in both children[41-43] and adults.[44,45]

4. Future Directions

The effectiveness of BCG in inhibiting atopic airway eosinophilia suggests its potential as a useful therapeutic agent in the treatment of atopic asthma. However, important questions remain to be addressed relevant to its clinical application. One important aspect of vaccine development is to determine whether *M. bovis* BCG needs to be viable to induce immune suppression of airway inflammation. Possible advantages of infection with viable organisms is that the quantity of the bacterial load is increased, or that viable bacteria immediately sequester to the intracellular compartment of the macrophage modulating its subsequent function, or that some rare molecules are only produced in sufficient quantities by viable bacteria. Recently it has been reported that concurrent administration of allergen and heat-killed *Listeria monocytogenes* modified primary and secondary antigen-specific immune responses, enhancing systemic T_H1 cytokine response and attenuating antigen-specific IgE production.[46] Although this study did not address the specific efficacy of this treatment to modify asthma-like responses in the lungs, it does suggest that treatment with nonviable BCG, or possibly mycobacterial extracts such as LAM, may be able to induce suppression of atopic airway inflammation.

Research conducted in our laboratory has demonstrated that a single dose of BCG administered subcutaneously or intraperitoneally versus directly into the airways is less effective at inducing the local T_H1 effects associated with inhibiting airway eosinophilia.[12] However, others have reported that BCG administered intravenously was able to suppress antigen-induced airway eosinophilia and reduce airway hyperresponsiveness.[47] More recently, intraperitoneally administered *M. tuberculosis*-containing adjuvant was also reported to suppress airway eosinophilia following antigen exposure.[48] Further investigation is needed to determine the most effective route of delivery for potential mycobacterial therapeutics.

5. Conclusion

Our recent finding that BCG vaccine can be used to inhibit airway eosinophilia has the potential to open up several new therapeutic strategies for treating or vaccinating against diseases such as atopic asthma. There are now several studies which describe the basic phenomenon of IFNγ–dependent T_H1 immune responses inhibiting T_H2 immune responses, but there is very little *in vivo* data available identifying the target cells for IFNγ (i.e., the

developing T_H2 lymphocyte or the local antigen presenting dendritic cell). Future research should attempt to elucidate the mechanism of action of the BCG-induced suppression of airway eosinophilia. Furthermore, determining which component(s) of the BCG is T_H2-immune suppressing and identifying the optimal route of vaccine delivery will prove very important for the future development of BCG as a therapy.

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