Collaborative studies in mucosal immunology in Goroka

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SUMMARY

A collaborative program between the Papua New Guinea (PNG) Institute of Medical Research and the Hunter Mucosal Group has completed studies relevant to protection of the airways against bacterial infection. Specifically, these studies addressed the mucosal capacity to produce local immunoglobulins and the capacity of the airways to respond to an oral vaccine containing inactivated nontypeable *Haemophilus influenzae* (NTHi). The mucosal IgA response to NTHi antigens was blunted in both children and adults in PNG compared with that found in Australian children and adults, whose airways are colonized only intermittently. Despite this, when oral NTHi is given to Papua New Guinean adults with chronic airways disease, it is followed by a significant (50%) reduction in incidence of acute bronchitic episodes, and a 3-log reduction in density of colonization, which persisted about 10 months. The implications of these key findings are discussed with respect to both mechanism and wider control of pathology emanating from abnormal airways colonization in a PNG environment.

Introduction

The Hunter Mucosal Immunology Group has had two major objectives: first, to understand the mechanisms whereby host-parasite relationships at mucosal sites determine health and disease outcomes; and second, to develop therapeutic strategies that shift the balance of these relationships towards protection against disease. One particular focus has been the airways. When our collaborative work with the Papua New Guinea Institute of Medical Research (PNGIMR) began, the dogma regarding airways immunity was as follows:

i Protection of the gas exchange apparatus (eg, from *Streptococcus pneumoniae*) was a systemic IgG antibody response.

ii Protection against chronic parenchymal infection (eg, tuberculosis) was mediated by a systemic T lymphocyte response.

iii Protection against endobronchial infection (eg, nontypeable *Haemophilus influenzae*) (NTHi)) was mediated by a local IgA antibody response (1).

Two collaborative studies in the PNG highlands provided important information, taking forward our understanding of mucosal immunology in relation to both the PNG and western environments.

The Studies


The clinical pattern of airways disease in PNG reflects environmental conditions from birth, with smoking an additional threat in many adults. Children are colonized soon after birth by both NTHi and *S. pneumoniae*, with all children colonized by 3 months (3). The commonest cause of death in the first year of life is bacterial pneumonia (4,5), usually caused by the biotype and/or serotype of the dominant colonizing bacterial
species. In adults aged ≥30 years living in the highlands, 16%-25% of deaths are due to chronic lung disease, with a further 18% caused by acute lower respiratory tract infections (4,5). NTHi and S. pneumoniae can be grown, respectively, from 90%-100% and 30%-40% of subjects with chronic lung disease (6).

This study found that the local salivary IgA antibody response to colonizing NTHi in the PNG highlands is blunted, with downregulation most apparent in adults. This suppression of antibody response in the mucosal compartment was selective, as a vigorous serum IgG and IgA response to NTHi antigens was seen in serum samples of adult Papua New Guineans.


This was a clinical study examining the effect of an oral NTHi vaccine in adults with chronic airways disease. An oral vaccine comprising inactivated NTHi bacteria had been developed to reduce morbidity from endobronchial infection and was also used to assess mechanisms of control of colonization of damaged airways in chronic obstructive pulmonary disease (COPD). In Australia it had been shown to reduce the incidence of acute exacerbations, with an associated reduction in frequency of isolates of NTHi from sputum, without an increase in specific IgA antibody in airways secretions (8). The oral vaccine was given monthly for 3 consecutive months, each cycle with 6x10^{11} orally administered NTHi. The PNG study was in adults with chronic airways disease (70%-80% with a history of smoking). The study followed recognition that acute inflammatory episodes in the lower airways at all ages were a major health problem. A major reason for the study was as a precursor to studies in the first year of life, where early universal colonization with NTHi (and S. pneumoniae) was a determinant of lower respiratory tract infections and middle ear infections. A successful study in Papua New Guinean adults would extend proof of concept to include NTHi isolates in PNG, providing a framework to assess the vaccine’s value in early life. A double-blind study was conducted in PNG in which 30 subjects received active tablets and 32 subjects placebo tablets monthly for 3 consecutive months, and they were all followed up for 12 months. The major outcomes were:

i Episodes of acute bronchitis were reduced by 50% (p <0.05) over the 12-month period.

ii Colonization density of NTHi was reduced by 3 logs for about 10 months (p <0.05). S. pneumoniae carriage density was also reduced over a similar period.

iii No reduction in pneumonia was observed.

Significance

There are two outcomes of these studies – specific information related to the therapeutic value of oral NTHi therapy in PNG, and a contribution to a broader understanding of mucosal immunity in humans and the mechanism of protection following oral immunotherapy with NTHi. The major outcome relevant to PNG is that oral immunotherapy is an apparently safe way to downregulate bronchial inflammation in adults with chronic bronchitis and COPD. NTHi isolates cross-react (perhaps due to highly conserved outer membrane antigens) with the isolate used effectively in Australian trials and thus a single vaccine is relevant to different geographical areas. By extension it is likely that a reduction in morbidity in infants with respect to lower airways infection could occur. Studies to control abnormal colonization in the upper airways – targeting otitis media and its sequelae (an original aim of the PNG trial) – are appropriate, but require an infant formulation. The clear demonstration that the effector mechanism (phagocytosis) is non-specific with significant impact on both NTHi and S. pneumoniae in sputum reinforces the potential for NTHi immunotherapy in PNG populations, where a range of pathogens occur. The key requirement for a successful clinical outcome is sensitisation to NTHi.

The results of the two PNG studies had a profound effect on understanding the mechanism of action and subsequent development of NTHi immunotherapy. First, the positive clinical results confirmed the
findings of the initial Newcastle study (8), broadening the geographical value of immunotherapy. Second, clinical benefit could be demonstrated in the most difficult microbiological climate – the natural history in PNG is a lifelong pattern, often involving high-density polybacterial colonization. Third, downregulation of IgA antibody responses in airways secretions was consistent with the absence of a local IgA antibody response following oral NTHi immunotherapy. Further studies in rodents showed that only thoracic duct T cells from immune animals could transfer immunity and antigen-specific T cells were detected following oral immunization with NTHi in subjects with COPD (9). Recently, the critical role of gut-derived Th17 cells in respiratory tract immunity has been demonstrated, and we have detected IL-17 in bronchial washings following oral immunization (unpublished observations). Fourth, the most significant observation in understanding the sequence of events following oral immunization with NTHi – a reduced antigen mass within bronchi – was the 3-log reduction in colonization density of NTHi and the significant reduction in heavy growth of *S. pneumoniae*. This observation became a basic tenet of the hypothesis subsequently developed, that acute exacerbations in patients with COPD represent a hypersensitivity response involving Th17 cells to colonizing bacteria. Reduction in the antigen load after oral NTHi ‘buffers’ against both bacterial- and viral-initiated episodes (ie, the resident bacterial population is a common denominator for most acute episodes, irrespective of initiating organism). Co-infection of mice with NTHi and influenza virus is followed by an increase in the titre of both microbes; prior oral immunization with NTHi abrogates these changes (10). These observations challenged the existing idea that NTHi descended the airways following intercurrent virus infection. Fifth, the duration of protection was best defined by the PNG study, where both quantitative NTHi and *S. pneumoniae* data in the active group merged with placebo at about 10 months. Thus annual re-immunization is now recommended. Sixth, an increase in adult serum IgG antibody in PNG is consistent with recent data obtained in Newcastle and the USA, where a specific IgG increase in serum has been linked to unprotected exposure to NTHi in those with damaged airways (10). In the Australian study, this increase in IgG antibody is seen as a surrogate parameter of mucosal protection (11,12).

**Conclusion**

Review of the impact of studies in mucosal immunology highlight the value of collaborative research between PNG and western countries, with obvious benefits to both the societies involved, in progressing understanding and in defining future objectives. Studies with oral NTHi immunotherapy have continued – a recent Phase 2 study in Australia confirms protection, with a 90% reduction in admission into hospital in those with most severe disease (13). That is being followed in 2011 with a multisite study across Australia in 340 subjects, using a commercial-quality highly characterized product (HI-164OV). Work with oral NTHi immunotherapy has substantially added to a current recognition of the importance of bacteria in driving acute (and possibly chronic) manifestations of COPD. It is time to return to PNG populations, in particular to explore the value of oral NTHi in infants with respect to lower and upper airways infections. Furthermore, earlier studies in Goroka suggesting that infants are infected with NTHi and *S. pneumoniae* from the adults with COPD who share their living space (R Grimley, unpublished manuscript) can now be tested using oral NTHi immunotherapy. The work done in Goroka has had a substantive influence on changing our understanding of airways protection, with a primary non-antibody role of T cells in controlling endobronchial colonization, and on the value of quantitative bacteriology in understanding these mechanisms where local antibody secretion has been suppressed.

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