Conflicting results in biomedical research: factors to be considered and implications of these studies

Maurice R. Odiere, Ph.D* and Pauline N. Mwinzi, Ph.D

Maurice R. Odiere, Ph.D  Kenya Medical Research Institute, Centre for Global Health Research, P.O. Box 1578 Kisumu, 40100 Kenya. Tel (Lab): +254 57 2022902 Ext. 495, Fax: +254 57 2022981. Email: Modiere@ke.cdc.gov
Pauline N. Mwinzi, Ph.D  Kenya Medical Research Institute, Centre for Global Health Research, P.O. Box 1578 Kisumu, 40100 Kenya. Tel (Lab): +254 57 2022902 Ext. 495, Fax: +254 57 2022981. Email: Pmwinzi@ke.cdc.gov

* Corresponding author:

Keywords: biomedical research, conflicting results, science

Introduction

Science encompasses a system of acquiring and organizing knowledge based on the scientific method or research, which seeks to explain the complexities of nature in a replicable way. “The body of science is constituted by the collective judgement of scientists, in so far as there is substantial agreement. The fact that there are very large areas of agreement, in spite of the individualistic, antiauthoritarian nature of science, is partial evidence for the validity of scientific methods. However, there are cases where universal agreement has been attained for an untruth, though this has more often been the case with sweeping generalizations than with the basic observations” [1].

The role of biomedical research in modern society cannot be overemphasized. Biomedical research is crucial as a source of new knowledge; a means to identify problems and solutions; and may also serve as a means to implement, monitor and evaluate public health interventions. Biomedical science research aims at advancing human welfare, knowledge and understanding of the numerous health problems afflicting local populations; with the ultimate goal of alleviating human suffering through diseases. A lot of biomedical/health research takes place around the world, focusing on a broad range of subjects. Interestingly, some of the research on the same areas has more than often produced conflicting results. Studies (ranging from clinical, behavioral to laboratory) that have produced conflicting results are the focus of this review, which addresses some of these studies, some factors to be taken into consideration, and the implications of these conflicting results.

1) Malaria-Helminth co-infections

Research in parasite ecology has often focused on single parasites in individual hosts. However, we know that under natural conditions, one host may be infected by more than one parasite - as is the case with Plasmodium and most helminthes that occur in the same endemic areas. Because of this, the immunological networks activated as a result of these infections are multifactorial and complex to study [2]. It is now widely acknowledged that the concomitance of various parasitic infections in human populations can induce modifications of the specific immune response to each pathogen and therefore induce changes in their clinical expression. In general, immunity against malaria parasitemia is largely dependent on the type 1 immune response, while immunity to helminths is largely driven by the type 2 responses. There is evidence that interactions occur between these co-infections, although it is unclear whether this effect is beneficial or harmful to the host. The type 1-dependent control of malaria parasitemia might be impaired by the type 2 milieu of pre-existing helminth infection. Alternatively, immunomodulatory effects of helminths might affect the likelihood of malarial immunopathology [3]. In addition, immunological responses to malaria parasites may affect the balance between pro- and anti-inflammatory cytokines (TNF-α, IL-6 and IL-10) on the one hand and T helper (Th) type 1 and 2 cytokines on the other. Infection with helminths has a profound effect on the immune system resulting in polarization towards Th2, characterized by high levels of cytokines such as IL-4, IL-5, IL-13 and high serum levels of immunoglobulin E (IgE) [2].
Several studies on the effect of malaria-helminth co-infections, however, have produced conflicting results. The differences have been with respect to findings and conclusions about the strength and direction of any interaction between the parasites. Some studies reported that infection with helminths increases the susceptibility to malaria infection. Both Schistosoma mansoni and Schistosoma haematobium infections have been shown to increase the incidence of malarial fevers [4, 5]. An association of helminth infection with an increased risk for malaria incidence has also been confirmed in Thailand [6]. In Zaire, a study on mothers and children described a positive association between infection with Ascaris and the occurrence of P. falciparum [7]. Helminth infections not only slow down the development of antimalarial immunity [8], but also increase incidence of P. falciparum malaria [5, 6].

In contrast to studies described above, some recent studies have reported a protective effect of helminth infection on malaria. In Senegal, S. haematobium was shown to have had a protective effect on infection by decreasing P. falciparum densities [9]. However, this was only the case for light infections of S. haematobium (1–9 eggs/10 mL of urine), since no significant association with malaria densities was found for higher infection intensities. In this same study, no association between intestinal helminth infections (mainly A. lumbricoides) and malaria densities was observed, indicating that not all species of helminths might have the capacity to modify the course of malarial infections. In a recent study that compared S. haematobium-infected children with helminth-free children in Mali, infected children demonstrated a delayed time to first symptoms of clinical malaria, fewer malaria episodes and lower parasite densities, although only in the younger age group (4-8 years of age) [10]. Again, the age-associated protective effect on malaria seemed stronger in the children with light S. haematobium infections (< 50 eggs/10 mL of urine) than in children with higher egg loads, although this was only significant for the time to first malaria infection. Infection by A. lumbricoides has been shown to protect subjects in Thailand from cerebral malaria [11]. In another study [12], it was found that pre-existing Ascaris infection could increase tolerance of the host to different Plasmodium species, thus facilitating their coexistence. Elsewhere, a significant increase in malaria was reported in children treated for helminth infections compared with a group of children who received a placebo [13].

2) Inhibition or exacerbation of allergic and autoimmune diseases by infection

Studies on the impact of infections on the inhibition or exacerbation of allergic and autoimmune diseases have also produced conflicting results. Data from some of these studies suggests that infections or the exposure to non-pathogenic bacteria or helminths protect individuals from developing some autoimmune and atopic disorders. Most of these findings support the ‘hygiene hypothesis’, which attributes the rise in autoimmune and atopic disorders to a lack of infections that normally keep the immune system balanced by inducing immunoregulation [14].

Autoimmune infections

It has been suggested that infections within the first year(s) of life decrease the risk of developing type 1 diabetes (T1D), inflammatory bowel disease (IBD) or multiple sclerosis (MS) [15-17]. Exposure to a wide variety of infectious agents, including viruses [15], mycobacteria [18], Salmonella [19] and helminths [20], protects the diabetes-susceptible non-obese diabetic (NOD) mice from spontaneously developing T1D [18]. A protective role for pathogens in murine models of IBD has been suggested [21]. It has also been established that the incidence and severity of experimental autoimmune encephalomyelitis (EAE), can be slightly reduced by a pre-established infection with parasites or mycobacteria [22, 23]. A protective role for pathogens in rodent models of arthritis has also been suggested [24].

However, other studies have shown that infections do not always protect from the development of autoimmunity. This is evidenced in some autoimmune diseases which occur shortly after infectious diseases. Examples include; post-infectious encephalitis disseminata and rheumatic fever [15, 25]; lyme arthritis and spondylarthropathies that develop after bacterial infections [26]; congenital rubella infection and T1D [20], protects the diabetes-susceptible non-obese diabetic (NOD) mice from spontaneously developing T1D [18] and exacerbation of MS is 2-3 times more likely to occur during, or shortly after, common respiratory, gastrointestinal or urological infections [15, 25].

Allergies and atopy

The development of asthma and allergy, is thought to be favored by the absence of infections [27, 28]. Bacterial infection, (in particular with Mycobacterium tuberculosis and Mycobacterium bovis) [14] and Chlamydia trachomatis, Listeria monocytogenes or lactic acid bacteria [29-32] is thought to be one factor associated with protection from allergic disorders.
Since these infections induce strong Th1 responses and because IFN-γ has inhibitory effects on Th2 responses, it has been speculated that infections with mycobacteria might protect humans from developing allergies [14]. Indeed, it has been reported that a positive tuberculin test result was associated with a decreased risk of atopy and asthma in Japanese school children [32]. The effects of BCG vaccination and its protection against allergic disorders have been documented [32-34]. However, other studies have reported that some types of bacteria are associated with increased allergic responses. Chlamydia pneumoniae and Mycoplasma pneumoniae can exacerbate asthma [35, 36] or allergen-induced bronchial hyper-reactivity [37] and Staphylococcus aureus can trigger the exacerbation of atopic dermatitis [14]. In addition, allergic inflammation in the airways is enhanced by infections with Bordetella pertussis [38].

Evidence exists that suggests that infections with viruses, e.g. hepatitis A protect against atopy development, and these anti-allergic effects of influenza A virus infection are associated with Th1 responses [39]. However it has also been shown that Th1 responses do not necessarily protect from allergy but can sometimes even exacerbate allergic responses [25]. For instance, respiratory viruses, such as rhinovirus (RSV), influenza A and metapneumovirus, can exacerbate the symptoms of asthma in humans or can directly induce wheezing [27]. In addition, RSV infections in the first year of life might be a risk factor for the development of childhood asthma and can directly induce airway eosinophilia and hyper-reactivity [27]. Low grade RSV infection, however, has been shown to protect mice from allergen-induced inflammation in the airways [40], suggesting that the severity and frequency of an RSV infection might also be important in determining what effect the infection will have on the development of asthma [14]. In allergen-exposed mice, Influenza A virus induces increased airway responsiveness [27, 39]. However, depending on the time point of infection, influenza A could also protect from allergen induced airway eosinophilia [39].

Controversy continues as to whether the Th2 response to helminths is protective or acts to dampen potentially damaging inflammatory responses against abundant and well-established tissue parasites [41]. Nevertheless, helminthic infections have been associated with a lower incidence of atopic diseases [28]. Infections with schistosomes or hookworms are associated with a reduced atopic phenotype [27, 28].

Retrospective [27] and interventional studies [42] have shown that anti-helminthic chemotherapy results in increased levels of skin-test reactivity against common allergens. Mice infected with Strongyloides stercoralis [43] or Nippostrongylus brasiliensis [39] have been found to express suppressed pulmonary allergic responses. Helminths are known to induce strong Th2-type responses in contrast to most other types of infections [28]. It is therefore plausible that helminths promote allergic disorders by generally enhancing Th2-type inflammation [14]. Indeed, it has been established that infections with helminths can directly induce an asthma-like phenotype in mice and rats [27] or can lead to the breakdown of oral tolerance against allergen [44]. Frequent occurrence of allergic symptoms has been reported in children seropositive for Toxocara or Ascaris species than in seronegative children and anti-helminthic treatment ameliorates asthma [27, 28]. Chronic helminth infections or the exposure to helminth-derived products have also been linked to urticaria [45].

Scientific evidence exists that clearly shows that infections have multiple and seemingly opposing effects on both autoimmune and allergic diseases (reviewed in [14]). Numerous types of infections have been shown to have an impact on atopy but not on autoimmunity and visa versa. Helminths and mycobacteria seem to be particularly good at protection and the timepoint (infections before the onset of atopy or autoimmunity have the greatest impact), age at infection, route, localization and dose of the infection all have a role [14].

3) Breast-feeding and intelligence in children

There has been a raging debate within the scientific cycles as to whether breast-feeding promotes intelligence in children. The duration of breastfeeding has been shown to affect the intelligence quotient (IQ) [46]. The study shows that babies who are breast-fed for at least six months grow to be more intelligent than their peers who are breast-fed for less time. This is supported by another study that involved a meta-analysis of 20 articles and showed that breast-fed babies’ IQ is higher than that of formula-fed babies [47]. This study adjusted for factors that may influence intellect, including the mother’s age and intelligence, birth order, race, birth weight, gestational age and socioeconomic status, and found that breastfeeding may raise a child’s IQ by more than five. Results of an 18-year study of 1,000 New Zealand children concluded that children who were breast-fed achieved...
consistently higher IQ scores, higher grades, higher classroom performance ratings and better high school achievements than non-breastfed children [48]. It has also been reported that children who were breast-fed performed better in tests of intellectual competence than those who were not [49]. Studies on neurological and cognitive outcomes in breastfed children found that early visual acuity and cognitive function of these children is greater than in non-breastfed children [49, 50].

However, several other studies dispute the view that breast-feeding promotes intelligence. A recent study found a positive impact for breastfeeding on intelligence only when other potential contributors such as the mother’s IQ and the parents’ educational and economic status were not taken into consideration [51]. Their study included 5,475 children and mothers in the U.S. who participated in an ongoing youth development survey. This study found no significant difference in intelligence among the breastfed and non-breast-fed siblings when they considered the variables above. In their study, the mother’s IQ was by far the most important variable, accounting for 70-75% of the difference [between children who were and were not breast-fed]. The position held by The US Department of Health and Human Services Office on Women’s Health [52] is that the observation that cognitive function of breast-fed children is greater than in non-breastfed children has not been conclusively proven. This position by US DHHS is supported by results from other studies [53, 54]. Although the majority of studies concluded that breastfeeding promotes intelligence, the evidence from higher quality studies is less persuasive [55]. These authors further argue that no convincing evidence exists regarding the comparative effects of breastfeeding and artificial feeding on intelligence.

4) Energetic costs of an immune response

Existing evidence for the direct mechanisms, particularly energetic costs, underlying indirect costs of immune activity in non-human vertebrates is currently equivocal. Unfortunately, a majority of studies on energetic costs of immune responses have involved the use of non-replicating or non-living antigens, and it remains to be investigated if other types of immune responses, to relevant living pathogens, are energetically more costly. Different authors [56-58] have argued that by using non-replicating antigens instead of an experimental infection with a parasite, it is easy to assess the energetic costs associated with an immune response alone, irrespective of the costs that would be imparted by parasite proliferation and subsequent tissue damage and repair. However, we know that this is far from what happens in natural populations in the field, and non-replicating antigen-induced immune response can only be a crude surrogate for a parasite-induced immune response. Therefore, there is a critical need for use of live replicating antigens, which will induce a more ecologically relevant antigenic challenge. Nevertheless, several studies have tried to quantify the energetic costs of an immune response, using resting metabolic rates (RMR) or basal metabolic rates (BMR) as an index of energy expenditure. Most of these studies have found significant increases in the RMR/BMR associated with an immune response. The table below summarizes some of these studies.

Table 1. Summary of some studies on energetic costs (percentage increase in resting or basal metabolic rate as compared to controls) of an immune response to various antigenic challenges.

<table>
<thead>
<tr>
<th>Species</th>
<th>Immune challenge</th>
<th>Increase in RMR/BMR</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Sepsis</td>
<td>30%</td>
<td>[59]</td>
</tr>
<tr>
<td></td>
<td>Sepsis</td>
<td>30%</td>
<td>[60]</td>
</tr>
<tr>
<td></td>
<td>Sepsis and injury</td>
<td>57%</td>
<td>[61]</td>
</tr>
<tr>
<td></td>
<td>Sepsis</td>
<td>49%</td>
<td>[62]</td>
</tr>
<tr>
<td></td>
<td>Typhoid vaccination</td>
<td>16%</td>
<td>[63]</td>
</tr>
<tr>
<td></td>
<td>Sickle cell disease</td>
<td>15%</td>
<td>[64]</td>
</tr>
<tr>
<td>Laboratory rat</td>
<td>IL-1 infusion</td>
<td>18%</td>
<td>[65]</td>
</tr>
<tr>
<td></td>
<td>Inflammation</td>
<td>28%</td>
<td>[66]</td>
</tr>
<tr>
<td>Laboratory mouse</td>
<td>KLH challenge</td>
<td>30%</td>
<td>[56]</td>
</tr>
<tr>
<td></td>
<td>Heiligmosoides bakeri</td>
<td>9% &amp; 14%</td>
<td>[67, 68]</td>
</tr>
<tr>
<td></td>
<td>Heiligmosoides bakeri</td>
<td>0%</td>
<td>[69]</td>
</tr>
<tr>
<td></td>
<td>Heiligmosoides bakeri</td>
<td>0%</td>
<td>[70]</td>
</tr>
<tr>
<td>Sheep</td>
<td>Endotoxin</td>
<td>28%</td>
<td>[71]</td>
</tr>
<tr>
<td></td>
<td>Endotoxin</td>
<td>10-49%</td>
<td>[72]</td>
</tr>
<tr>
<td>Blue tits</td>
<td>SRBCs</td>
<td>9% (BMR)</td>
<td>[73]</td>
</tr>
<tr>
<td></td>
<td>Diphtheria–tetanus vaccine</td>
<td>0% (BMR)</td>
<td>[74]</td>
</tr>
<tr>
<td>Chicken</td>
<td>SRBCs</td>
<td>0%</td>
<td>[75]</td>
</tr>
</tbody>
</table>

NOTE: 0 % indicates no significant increase.
SRBCs – Sheep red blood cells; KLH – Keyhole limpet hemocyanin.
It is interesting to note that some of the studies in Table 1 above [69, 70, 74, 75] found no significant difference in metabolic rates between the experimental and control groups. Whereas these four studies are not conflicting sensu stricto, the fact that they observed no significant change in RMR/BMR deserves mention here.

5) Antibody responses in mice infected with Heligmosomoides bakeri (Nematoda)
A recent study [76], found a clear difference in anti-adult IgG1 levels between two strains of mice (SWR and CBA) infected with H. bakeri. This was in tandem with earlier reports that adult worms induce a polyclonal B cell stimulation, with CBA mice, having the greater number of adult worms, showing the larger response [77]. They acknowledged that this finding contrasted with results of earlier studies utilizing single pulse infections where the responder mice strains such as SWR, SJL and NIH were shown to have faster and more intense adult worm specific IgG1 responses [78, 79]. In addition, evidence exists that IgG1 antibodies to adult worms, in repeatedly infected mice, are host protective [80]. It was not clear why CBA were unable to control worms under the trickle infection protocol used in their study.

6) Are BALB/c mice weak or strong responders to Heligmosomoides bakeri (Nematoda) infection?
There is close agreement between different researchers as to which mouse strains are weak responders to H. bakeri infection, with the exception of BALB/c. Behnke and others [100] found the performance of BALB/c to range from weak (< 20% protection) to strong (almost 100% protection), and therefore designated this strain as an intermediate responder. Some studies using BALB/c have found them to be inferior responders relative to NIH mice [81] and LAF1/J mice [82]. However, other studies [83, 84] have found BALB/c mice to be among the strong responder strains. In addition, BALB/c have been reported to be much more resistant than C57BL/6 to challenge infections [85, 86].

Factors to be taken into consideration to address conflicting results and implications of such studies Several factors need to be addressed in dealing with conflicting studies. The epidemiological settings and methodology used in some of the studies must be evaluated if any standard comparisons are to be made. In malaria-helminth co-infections, there is need to study each type of helminth infection separately, since each might have different effects on the course of malaria [2]. The intensity of a helminth infection must be reported, since the intensity might be an important determinant for the outcome of immune responses to the malaria parasite [4, 9, 10]. The analysis of the immunological profiles at the level of the cellular immune system will shed more light on the mechanisms that are involved, and murine co-infections will help to dissect the molecular immunological pathways involved in controlled models [2].

There is need to control for confounding factors or effect modifiers in scientific research. For instance, the traditional approach among researchers towards controlling for confounding and modification in parasitological surveys is either through matched case control designs or by stratifying on common confounders such as age and sex [87]. Location of residence is among significant confounders in malaria-helminth co-infections. Simply observing an association between two species of parasite (or their associated morbidities) in the absence of controlling for location cannot be interpreted as evidence of biological interaction [87]. Controlling for confounding factors can help reduce heterogeneity in several parameters that frequently remains unexplained, and will ultimately lead to correct inferences.

Chronic helminth infections induce strong type 2 and regulatory immune responses, and are known to influence immune activity to other antigens such as allergens and vaccines [2]. Many malaria vaccines have been shown to target parasite antigens that have the capacity to induce specific antibodies capable of inhibiting parasite growth. In the ideal sense, vaccines should also stimulate an efficient CD4+ Th1 response [2]. Infections with helminths have been shown to affect immune responses to tetanus vaccines and to BCG [88, 89]. It has been suggested that helminth infections may also affect the induction of efficient Th1 type of immune response to a potential malaria vaccine, and the presence of helminth infections in a population should be considered as a confounding factor for the assessment of the efficacy of malaria vaccines [2]. These authors further argue that a better answer to the question of whether helminth infections affect course of malarial infection and disease will come from longitudinal studies with a placebo-controlled anti-helminth treatment design.

Since helminth infections are prevalent throughout tropical regions where malaria parasites are transmitted, their co-infections will frequently be observed. It is clear that malaria-helminth co-infection
pose a significant challenge with real world consequences. Arguing from the point that helminth co-infection ameliorates cerebral malaria [11], mass anti-helminthics will be disastrous and of no benefit where malaria is endemic. On the other hand, the additional burden of helminth infection may increase the severity of malaria infection [5]. This stresses the importance for clear, accurate and consistent results on malaria-helminth co-infections. Scientists must carefully evaluate the impact that conflicting results on malaria-helminth co-infections might have on the implementation and efficacy of control interventions such as malaria vaccines. The interaction between malaria parasites and helminths is complex on its own, and this is worsened when there is no consensus among studies in this area.

It is without doubt that the immune system benefits from regular encounters (priming) with some pathogens, which clearly has implications for interventions in autoimmunity and atopy. Available evidence indicates that under certain circumstances some infections can inhibit, induce or exacerbate allergic or autoimmune diseases (reviewed in [14]). The current, most popular, hypothesis on how infections inhibit the development of both types of disease, suggests that microbial molecules trigger certain programs, such as IL-10 production by dendritic cells (DCs), which thereupon instruct the development of regulatory T (Tr) cells [90]. Kamradt and colleagues [14] argue that although increasing evidence for this hypothesis is currently being published, it is far from proven and other mechanisms might contribute to infection-mediated protection. Data from these results needs careful interpretation because it can have far reaching ramifications regarding the development of novel therapeutic intervention strategies aimed at reducing autoimmune responses in humans by using pathogens or commensals. Several studies ranging from BCG vaccination and T1D [91], BCG and MS [92], probiotic bacteria and pouchitis in ulcerative colitis [93], Trichuris suis and ulcerative colitis [94], T. suis ova and Crohn’s disease [95], T. suis and IBD [96] provide us with an insight of how conflicting results in this area can play out in the real world.

The debate on whether breastfeeding promotes intelligence in children is an interesting one. There is a clear need to carry out evaluations on the impact of controlling for maternal intelligence and other confounding factors (e.g. socioeconomic status and maternal education) on this association. The importance of controlling for maternal IQ has been demonstrated by Der and colleagues’ who have shown that the association of breast feeding with cognitive performance in children drops from 4.7 to 0.5 points after adjusting for mother’s cognitive competence and other socio-environmental measures [51]. Other studies [53, 97] support Der and colleagues’ finding that maternal intellectual enrichment and genetic endowment, as measured by maternal IQ, are crucial potential confounders of the effect of breast feeding on cognitive competence in children. It remains to be determined what really accounts for breast milk’s brain-boosting power.

There is a general consensus that all physiological processes, including immune responses, consume energy [56, 74, 98, 99]; the only fundamental question being how much. The possible reasons given for the no significant change in RMR in some of the studies above (Table 1) include: differences in parasite cultures that produce different levels of infectivity and thereby elicit different degrees of immune response in mice that could differentially affect RMR [69]; very low sample sizes [74] and the effect of age [75]. Science could benefit through the empirical evaluation of the relationship between immune responses to various parasites and RMR/BMR, and between immune eliciting capacity and different parasite cultures in order to make concrete conclusions as to why there are no significant changes in some studies on energetic costs of immune responses.

The SWR and CBA mice share different MHC-linked background genes, with SWR expressing the H-2q haplotype and CBA the H-2K haplotype. Indeed, it has been established that the immune response phenotype in H. bakeri infection in mice is influenced by both background and major histocompatibility complex (MHC) genes [100]. This implies therefore, that thoughtful considerations should be made in comparisons between infections with H. bakeri using different mouse strains.

Whether BALB/c should be designated as a weak or strong responder to H. bakeri is still controversial. It is apparent that differences in the immunizing protocols that are employed in some of the studies are a likely cause of variation in the results [86, 100]. This therefore calls for control of the immunizing schedules for any meaningful comparisons and concrete conclusions to be made.
Conclusion

How can the scientific community benefit from the knowledge from conflicting results in research? Parasitic diseases, caused by a diverse spectrum of eukaryotic organisms, represent a major global health problem in terms of social and economic burden. Elimination of these diseases is probably not feasible in our lifetime. Scientists must therefore strive towards bridging the gap between some of these conflicting results. Researchers must explore concrete explanations or discussions for lack of consistency and reproducibility within their study settings. Conflicting results may not be a negative phenomenon after all, science is all about stimulation, creativity and critical thinking; and conflicting studies provide the foundation for reflections on new approaches/designs for prospective studies in research.

References


33. da Cunha SS, Cruz AA, Dourado I, Barreto ML, Ferreira LD, Rodrigues LC. Lower prevalence of reported asthma in adolescents with symptoms of rinitis that received neonatal BCG. *Allergy*. 2004;59:857-62.

34. Marks GB, Ng K, Zhou J, Toelle BG, Xuan W, Belousova EG, Britton WJ. The effect of neonatal BCG vaccination on atopy and asthma at age 7 to 14 years: an historical cohort study in a community with a very low prevalence of tuberculosis infection and a high prevalence of atopic disease. *Journal of Allergy and Clinical Immunology*. 2003;111:541-9.


