Nonspecific effects of neonatal and infant vaccination: public-health, immunological and conceptual challenges

Peter Aaby, Tobias R Kollmann & Christine Stabell Benn

Vaccines can have nonspecific effects through their modulation of responses to infections not specifically targeted by the vaccine. However, lack of knowledge about the underlying immunological mechanisms and molecular cause-and-effect relationships prevent use of this potentially powerful early-life intervention to its greatest benefit. The World Health Organization has identified investigations into the molecular basis of nonspecific vaccine effects as a research priority.

Globally, programs for the immunization of newborns and infants target serious infectious threats, from polio and tuberculosis to diphtheria, tetanus, pertussis, invasive *Haemophilus influenzae* type b (Hib), hepatitis B virus (HBV), pneumococcus, measles, mumps, rubella and rotavirus. The vaccines against these diseases and pathogens have had a substantial role in diminishing mortality and morbidity in early life and rightfully represent major triumphs of preventive medicine and public health[1].

Such common childhood vaccines were originally introduced in high-income countries on the basis of studies documenting clinical protection against the targeted diseases or following documentation of immune responses that correlated with protection[2]. However, studies did not examine whether vaccines had effects in addition to the targeted, disease-specific prevention. The Expanded Programme of Immunization followed the same strategy when the program was launched in low-income countries in the 1970s[2]. For example, the decision to recommend administration of the measles vaccine (MV) to children at 9 months of age was based on studies in Kenya of post-vaccination measles-specific seroconversion rates for children between the age of 5 months and 12 months (**Fig. 1**). The expected effect on child survival was extrapolated from those seroconversion rates; however, no randomized controlled trials (RCTs) examined the age at which vaccination against measles had the best overall effect on child survival[2].

Peter Aaby and Christine Stabell Benn are with Bandim Health Project, Indepth Network, Bissau, Guinea-Bissau; OPEN, Institute of Clinical Research, University of Southern Denmark/Odense University Hospital, Denmark; and Research Centre for Vitamins and Vaccines, Bandim Health Project, Statens Serum Institut, Copenhagen, Denmark. Tobias R. Kollmann is with the Division of Infectious Disease, Department of Pediatrics, University of British Columbia, Child & Family Research Institute, Vancouver, Canada.
e-mail: p.aaby@bandim.org

Nonspecific vaccine effects

The concept of 'nonspecific vaccine effects'—i.e., that vaccines have effects on morbidity and mortality that are not explained by the prevention of the targeted diseases—was first recognized in a series of RCTs in the late 1980s[3]. These RCTs tested whether a high-titer MV (HTMV) could be given at 4–6 months of age and could be as effective against measles infection as the standard MV given at 9 months of age. Early administration of the HTMV prevented measles infection just as effectively as did the standard MV given at 9 months of age. However, trials in Guinea-Bissau, Senegal, Sudan and Haiti found early administration of the HTMV to be associated with twofold higher overall mortality among females (there was no difference in mortality for males)[3]. This first observation that vaccines could protect against the target disease but at the same time affect mortality after infection with other pathogens, in a sex-differential manner, led to several further studies showing that other vaccines might also have such nonspecific effects. Nonspecific vaccine effects have now been documented for almost all vaccines in use (the most pertinent findings are summarized in **Box 1**). In brief, live vaccines are associated with beneficial nonspecific effects[2,4–6], whereas inactivated vaccines are associated with deleterious effects[7], and both positive and negative effects are greatest for females[3–5,7]. The vaccine most recently administered determines the outcome, and thus the sequence and combination of vaccines becomes very important. An illustrative example is the HTMV; during subsequent analyses it became clear that it was not the live HTMV itself that was deleterious for females but the fact that the HTMV was given so early that most children received the DTP (diphtheria-tetanus-pertussis) vaccine, the IPV (inactivated polio vaccine) or the DTP-IPV afterward, and this was the real cause of the increased female mortality[3].

Those findings have been confirmed in many different low-income countries in Africa and Asia[2,7]. Notably, a large population-based cohort study of Danish children

Figure 1 WHO-recommended vaccination schedule used in most low-income countries since commencement of the Expanded Programme of Immunization in the 1970s. Booster doses of the DTP vaccine and OPV at 18 months are administered in some countries but not in others. The DTP vaccine has been replaced by the pentavalent DTP+HBV+Hib vaccine (consisting of the DTP vaccine, the vaccine against HBV and the vaccine against invasive Hib) in most low-income countries. The yellow fever vaccine is administered together with the MV in some countries. The rotavirus vaccine and pneumococcus vaccine have been introduced in some countries to be administered at the same time as the DTP vaccine and OPV. A second dose of the MV in the second year of life is beginning to be introduced in countries with a high coverage for the first dose of this vaccine.

has emphasized that nonspecific vaccine effects are not restricted to only resource-poor regions of the world[8].

In April 2014, the Strategic Advisory Group of Experts for the immunization program of the World Health Organization (WHO) recognized the importance of nonspecific effects of vaccines and recommended further research into this issue[9]. This recommendation grew out of an independent and extensive epidemiological review of the bacillus Calmette-Guérin (BCG) vaccine, DTP vaccine and MV and their effect on overall mortality in children under 5 years of age[10]. Specifically, this review concluded that the BCG vaccine and MV reduce mortality by ~50%, a beneficial effect not explained by the prevention of tuberculosis or measles alone. On the other hand, despite the undoubtedly beneficial effect of reducing the incidence of diphtheria, pertussis and tetanus, most studies of the DTP vaccine have found it to be associated with a nonspecific increase in mortality[10].

In several ways, the public health approach has been built on the perspective that 'health' is the absence of specific diseases; the vaccine community and industry are therefore developing and testing vaccines to generate individual and herd immunity targeting specific pathogens[1]. The recognition of vaccine-mediated nonspecific effects challenges this narrowly focused view. Here we will synthesize from the existing data a cohesive vision outlining how to overcome the considerable challenges now faced in terms of vaccine-mediated nonspecific effects at the public-health, immunological and broader conceptual level.

Box 1 Nonspecific vaccine effects: key findings

- All live vaccines studied so far (BCG, MV, the smallpox vaccine and OPV) reduce mortality more than can be explained by prevention of the targeted infection(s)[4–6].
- Inactivated vaccines such as the DTP vaccine, the vaccine against HBV and the inactivated polio vaccine may have nonspecific deleterious effects, particularly for girls[7].
- Effects often differ by sex; girls seem to receive not only stronger beneficial effects from live vaccines[4,5] but also stronger deleterious effects from inactivated vaccines[3,7].
- The most recent vaccination appears to be most important in determining the nonspecific outcome. For example, when children transit from receiving the BCG vaccine at birth to receiving the DTP vaccine at 6 weeks of age to receiving the MV around 9 months of age (**Fig. 1**), the female-versus-male mortality rates change, with females suffering lower mortality than males in the time window following administration of the BCG vaccine, higher mortality than males in the window following vaccination against DTP and again lower mortality than males following administration of the MV. This pattern is illustrated in the sex- and age-related changes in mortality in studies of children from Guinea-Bissau and The Gambia (**Fig. 2**). This indicates that the sequence or combination of vaccinations is an essential driver for the nonspecific effect on child survival[3,7].
- The success of current immunization practices in reducing the threat posed by the vaccine-targeted pathogens ('herd immunity') at the same time leads to a relative increase in the importance of nonspecific effects. For example, in an RCT comparing two doses of the MV at 4.5 and 9 months of age versus the standard dose of the MV at 9 months, the intervention reduced mortality by 30%, only 4% of which could be explained by prevention of measles[4].
- Vaccines interact with other interventions or conditions that modulate the immune system; for example, supplementation with vitamin A may amplify the deleterious and beneficial effects of vaccines[28], and other micronutrients may have similar effects[29].

Nonspecific effect of the BCG vaccine

All live vaccines studied so far have been shown to reduce mortality more than can be explained by prevention of the targeted infection(s) (**Box 1**). However, we will focus our discussion of nonspecific effects on neonatal immunization with the BCG vaccine because evidence from RCTs as well as studies of possible molecular mechanisms has advanced furthest for the BCG vaccine.

The WHO recommends immunization with the BCG vaccine at birth in areas of the world with a high incidence of tuberculosis. However, immunization with this vaccine has effects on health beyond tuberculosis. Several studies have suggested that such vaccination may reduce atopy, particularly when given early in life[11]. Furthermore, in multiple observational studies, immunization of neonates with the BCG vaccine has been shown to provide beneficial effects on overall mortality. For example, children with a scar or a positive skin test resulting from the BCG vaccine are less likely to develop sepsis and exhibit an overall reduction in child mortality of around 50% (refs. 12–14) (**Table 1**). Such observations encouraged researchers to examine the beneficial nonspecific effects of the BCG vaccine on overall health in RCTs. Since immunization with the BCG vaccine is recommended to be given at birth in countries with a high incidence of tuberculosis, it would have been unethical to randomize children into 'BCG' or 'no-BCG' groups. However, many low-income countries delay immunization with the BCG vaccine for low-birth-weight infants; this offered the opportunity to directly test the effect of the BCG vaccine on overall mortality (**Table 2**). In the first two RCTs, receipt of the BCG vaccine plus the oral polio vaccine (OPV) at birth was associated with a 21% reduction in all-cause mortality (95% confidence interval (CI), –2% to 39%) compared with receipt of the OPV only ('delayed BCG'), over the entire first year of life; mortality was reduced by 48% (CI, 18–67%) in comparisons focused only on the first month (neonatal period) and by 58% (CI 8–81%) in comparisons focused on only the first few days following immunization with the BCG vaccine[6]. Lower mortality rates from sepsis as well as respiratory infections among children immunized with the BCG vaccine were also noted. These results have since been replicated in a third trial (S.

Table 1 Mortality after immunization with the BCG vaccine, with and without scarring

Study	Subject with a scar	Mortality (deaths/total subjects)		MRR (scar present/no scar)	Adjustment
		Scar present	No scar		
Bissau, 1996–1998 (ref. 12); reading at 6 months; follow-up, 12 months	92% (1,676/1,813)	102/1,676	19/137	0.47 (0.29–0.70)	Age at BCG; height for age; weight for age
Bissau 1998–1999 (ref. 13); reading at ages 3–60 months; follow-up, 12 months	68% (794/1,167)	14/794	13/373	0.45 (0.21–0.96)	Sex; ethnic group
Bissau 2000–2002 (ref. 14); reading at 2 or 6 months; follow-up to 12 months of age	84% (1,321/1,572)	49/1,321	17/251	0.44 (0.23–0.81)	Low birth weight; season; BCG strain; supervision; age at immunization with BCG vaccine; sex; ethnic group, place of vaccination; place of residence; electricity in house; maternal schooling; marital status of parents

Mortality among children immunized with the BCG vaccine, with or without a scar resulting from the BCG vaccine, adjusted for various parameters (far right) and presented as an all-cause mortality rate ratio (MRR), with 95% confidence interval in parentheses.

Biering-Sørensen, P.A. and C.S.B., unpublished data).

The finding that the beneficial effect of immunization with the BCG vaccine 'declined' from 48% after 1 month to 21% after 12 months of follow-up was confusing at first and distracted from the overall strong confirmatory result of a beneficial nonspecific effect of such vaccination on neonatal mortality. However, the reason for the 'decline' in beneficial nonspecific effects soon became clear and in fact provided further proof of the potent nonspecific effects of such vaccination: nearly all infants eventually received the BCG vaccine; i.e., these trials compared the BCG and no-BCG groups only up to the point at which the no-BCG group received the BCG vaccine, usually in the second month of life. The nonspecific reduction in mortality following administration of the BCG vaccine became less pronounced as time progressed and as more children in the 'delayed-BCG' group received their BCG vaccine. Furthermore, the infants in the 'delayed-BCG' group who received the BCG vaccine after 6 weeks of age would receive the DTP vaccine and the OPV at the same time, whereas the other group was given the BCG vaccine first and then received only the DTP vaccine and the OPV after 6 weeks of age. Several studies in Senegal, Guinea-Bissau, India and Bangladesh have shown that the deleterious effect of the DTP vaccine is diminished if it is administered together with the BCG vaccine rather than being given in the recommended schedule of immunization with the BCG vaccine first, followed by vaccination against DTP ~6–14 weeks later[10,15]. The trial of low-birth-weight children confirmed that the 'delayed-BCG' arm that received the BCG vaccine with the DTP vaccine had a relative benefit from the age of vaccination against DTP onward, which contributed to a reduction in the overall difference in mortality between the groups[15]. In short, immunization with the BCG vaccine leads to a profound reduction in neonatal and infant mortality and may offset otherwise deleterious effects of certain other vaccines.

Mechanisms of nonspecific effects

The review by the WHO in 2014 concluded that nonspecific immunological effects of vaccines are plausible and common, but that their biological effects are not fully understood[9]. This places a high premium on understanding the mechanisms by which vaccines affect overall mortality beyond reducing infection with the targeted pathogen. We will continue to use the example of the BCG vaccine to delineate possible mechanisms and strictly focus on BCG vaccine–induced reduction of infectious causes of death.

Host defense against infection can broadly be categorized into anatomical and physiological barriers, innate immunity, and adaptive immunity[16]. BCG vaccine–induced alterations to anatomical and physiological barriers early in life has not been investigated, to our knowledge. Immunization with the BCG vaccine is known to induce production of potent antimicrobial peptides in epithelial cells[17] and can activate mechanisms of adaptive immunity as well as innate immunity in the bladder mucosa that nonspecifically target cancer cells[18]. Enhanced mucosal and epithelial barrier function following immunization with the BCG vaccine thus might contribute to the nonspecific protection against infection, but this theoretical possibility will have to await experimental investigation.

There is strong support for the notion that immunization with the BCG vaccine early in life nonspecifically alters subsequent adaptive immune responses. For example, immunization with the BCG vaccine alters the immune responses to standard childhood vaccines given months later[19]. In particular, immunization with the BCG vaccine biases subsequent T cell responses toward an adaptive immune response dominated by type 1 helper T cells (T_H1 cells), which produce IFN-γ, and the T_H17 subset of helper T cells, which produce interleukin 17 and interleukin 22 (ref. 20). Following immunization with the BCG vaccine, a T_H17 bias would be predicted to result in an increase in protection against pathogens at mucosal sites that require strong neutrophil responses for protection, while a T_H1 bias would be predicted to result in increased protection against intracellular pathogens. This pattern has been confirmed in animal models, in which immunization with the BCG vaccine confers partial protection against unrelated pathogens such as *Babesia*, *Plasmodium*, *Toxoplasma*, trypanosomes and vaccinia virus[21,22]; each of these pathogens is known to be controlled at least partially by mechanisms mediated by a T_H1 and/or T_H17 immune response.

Table 2 Mortality after immunization with the BCG vaccine

Follow-up period	MRR	MRR	Combined MRR
	Small RCT	Large RCT	
3 days	0.17 (0.02–1.35)	0.49 (0.21–1.15)	0.42 (0.19–0.92)
4 weeks	0.18 (0.06–1.37)	0.55 (0.34–0.89)	0.52 (0.33–0.82)
12 months	0.41 (0.14–1.18)	0.83 (0.63–1.08)	0.79 (0.61–1.02)

Mortality after immunization with the BCG vaccine at birth, assessed after 3 days, 4 weeks or 12 months of follow-up (far left column), compared with the mortality of control children who received delayed BCG vaccine and presented as an all-cause mortality rate ratio as in **Table 1**. Data are a summary of two RCTs assessing low-birth-weight children in Guinea-Bissau[6].

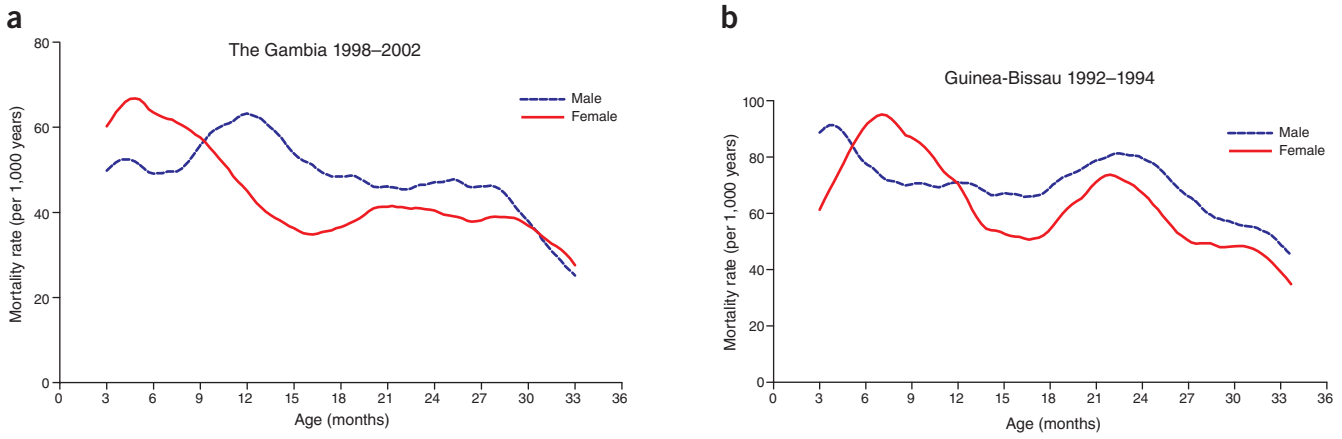

Figure 2 Effect of vaccination on sex- and age-specific mortality rates. (**a**) Sex- and age-specific mortality of children from Farafenni, The Gambia, who received the BCG vaccine plus the polio vaccine (OPV1) plus the vaccine against HBV (HBV1) at birth; the polio vaccine (OPV2) at 1 month; the DTP vaccine (DTP1) plus the polio vaccine (OPV3) plus the vaccine against Hib (Hib1) plus the vaccine against HBV (HBV2) at 2 months; the DTP vaccine (DTP2) plus the polio vaccine (OPV4) plus the vaccine against Hib (Hib2) at 3 months; the DTP vaccine (DTP3) plus the polio vaccine (OPV5) plus the vaccine against Hib (Hib3) plus the vaccine against HBV (HBV3) at 4 months; the MV and yellow fever vaccine at 9 months of age; and booster doses of the DTP vaccine and OPV at around 16–18 months of age; all were assessed between birth and 3 years of age, from 1998 to 2002, with a 'bandwidth' (time in the program) of 4 months. The female mortality rate is greater than that of boys in the age groups in which inactivated vaccines (against DTP, invasive Hib and HBV) are the most recent vaccination. (**b**) Sex- and age-specific mortality of children from Bissau, Guinea-Bissau, who received the BCG vaccine plus the OPV at birth; the DTP vaccine plus the OPV at 6, 10 and 14 weeks of age; the MV at 9 months of age; and booster doses of the DTP vaccine plus the OPV at 18 months; all were assessed between birth and 3 years of age, 1992–1994. The female mortality rate is greater than that of boys in the age groups for which DTP is the most recent vaccination.

However, the most likely mechanism by which the BCG vaccine reduces overall mortality is an alteration in innate immunity[23]. The fact that a nonspecific reduction in mortality and morbidity has been seen immediately following vaccination[6] already suggests that the BCG vaccine mediates protection via alteration of the innate immune system. In adults, immunization with the BCG vaccine leads to elevated production of the proinflammatory cytokines tumor-necrosis factor and interleukin 1β in response to non-BCG–related stimuli that is maintained for up to 3 months after vaccination. Furthermore, monocytes recovered 1 year after such vaccination still display increased expression of the coreceptor CD14, pattern-recognition receptors (for example, Toll-like receptor 4) and the receptor for mannose. The underlying molecular mechanisms that lead this sustained alteration in function of the innate immune system following immunization with the BCG vaccine appear to relate to changes in the epigenetic regulation of gene expression following innate stimulation[23]. It is possible that changes in innate immunity following such immunization differ according to age of the recipients; age-dependent differences between infants and adults have been noted in their innate immunity in general[24] and in their response to the BCG vaccine in particular[25].

Hence, there are several levels at which the BCG vaccine has been shown to alter host resistance to infection, from mucosal barrier function to adaptive and innate immunity. These data could guide future studies toward acquisition of the molecular knowledge necessary to explain mechanistically what has been observed at the population level. The current state of knowledge, however, provides only limited insight into how such changes could result in nonspecific protection and affect overall mortality. The call to action by the WHO highlights the importance of further investigations into the molecular cause-and-effect mechanisms of nonspecific protection.

Final thoughts

The concept of nonspecific vaccine effects challenges the narrowly focused notion of vaccines as disease-specific interventions. The good news is that the beneficial nonspecific effects provide potentially highly effective and yet relatively easy and affordable solutions to reduce morbidity and mortality in early life. For example, since live attenuated vaccines such as the BCG vaccine can have such pronounced beneficial effects on neonatal survival, other living but harmless microbes (for example, probiotics or *Candida*) administered under controlled conditions might do so as well. Hence, identification of the mechanisms underlying the beneficial effects of the BCG vaccine on childhood mortality will probably aid in identifying general avenues for reducing high neonatal mortality in low-income countries and possibly neonatal morbidity in high-income countries. Furthermore, even small changes to vaccine delivery policy might lead to an immediate and immense reduction in neonatal mortality. For example, to avoid wasting the vaccine, many providers open a new 20-dose vial of the BCG vaccine only on certain days. This can result in delay of vaccine administration; less than 50% of African children receive the BCG vaccine in the neonatal period[26]. Providing the BCG vaccine to all children at birth would probably save countless lives.

The bad news is that whether an intervention will have an overall beneficial effect just because it prevents the target infection or deficiency cannot be predicted. To address this, a paradigm shift is needed, from a focus on the pathogen to a focus on the host. The field of vaccinology has for too long too narrowly defined 'health' as the absence of a specific pathogen. Such a focus clearly is necessary during the initial discovery and development phases of vaccine production, but subsequent field testing of vaccines should be guided by the definition of health that the WHO has long embraced, that "health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity"[27]. The approach to evaluating vaccines should be amended to include in the assessment of vaccine effectiveness data on overall morbidity and mortality. Furthermore, since many studies have shown strong sex-difference effects of vaccines and micronutrients, it would also be wise to evaluate effects separately for females and males, not just for 'children', as is current practice. Finally, the data

summarized here for the BCG vaccine indicate that researchers and clinicians also need to be aware that subsequent interventions may modify or reverse the overall effect of a given vaccine. Such comprehensive vaccine evaluations, coupled with the powerful tools of modern molecular immunology, should help overcome the public-health, immunological and conceptual challenges that currently restrict the understanding of vaccines. Collectively, this could usher in an era of vaccine-mediated early-life immunological modulation that would bring benefit to millions of children all over the world.

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