BCG-specific and non-specific effects: different questions, similar challenges

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Interest in the non-specific effects of BCG and the possibility that BCG may provide protection against non-mycobacterial infectious disease has been growing [1]. Trials in low birth-weight infants from Guinea-Bissau [2-5] and a recent trial in Ugandan neonates [6] showing significant reductions in all-cause infectious disease morbidity and mortality following BCG vaccination at birth have led to excitement about the use of BCG for prevention of neonatal infections, a major contributor to infant and child mortality world-wide. The ability of BCG vaccination to provide protection against viral infections [7, 8] has also stimulated interest in its use for protection against COVID-19 [9], with multiple current randomized controlled trials assessing the use of BCG for SARS-CoV-2 prevention in health workers [10]. The results of the study by Messina and colleagues in this issue of *the Journal of Infectious Diseases* [11] may, therefore, be disappointing to some, although of interest to those trying to unravel what BCG vaccination does to the immune system.

The article reports the results of a randomized controlled trial comparing BCG vaccination within the first 10 days of life with no BCG vaccination, investigating whether BCG has non-specific effects on all-cause infections, primarily lower respiratory tract infections (LRTIs) [11]. This carefully performed study in 1272 Australian infants did not find a significant effect on LRTIs in the first year of life, with a risk difference for LRTI of -3% (95% CI -9.2 to 2.6) in BCG-vaccinated compared to control infants. Lack of supportive evidence for significant non-specific effects of BCG on childhood infections was also found in the Danish-Calmette study in Copenhagen [12] and in a trial in Indian neonatal units [13], but these findings contrast with the significant benefits shown in neonates in Guinea-Bissau and Uganda [2-6] and in elderly Dutch adults [8].

So what may be responsible for these differing findings? One possibility, suggested by the authors, is that the non-specific effects of BCG may be setting dependent, an observation familiar to those studying the TB-specific effects of BCG. The protective efficacy of BCG against TB is greater with distance from the equator [14]. In contrast, the majority of observational and trial data supporting

non-specific beneficial effects of BCG, particularly in neonates, have come from equatorial Africa. The potential masking or blocking effects of exposure to environmental mycobacteria, may be an explanation for the reduced TB-specific responses seen in tropical countries [15]. Such exposures may boost any non-specific beneficial effects of BCG given shortly after birth, enhancing protection against non-tuberculous infectious disease in these settings. More recently, studies have suggested that the immunological and clinical non-specific effects of BCG may be particularly strong in infants born to BCG-vaccinated mothers [12] [16]. Maternal priming through BCG vaccination, as well as through exposure to environmental mycobacteria and tuberculosis itself, is more likely to occur in high TB-incidence, resource-poor settings where the greatest non-specific beneficial effects of BCG have been shown. However, the MIS BAIR study did not find larger BCG effect sizes on reducing LRTI infections in infants born to BCG-vaccinated mothers, although a potential effect of maternal BCG alone on LRTI incidence was seen.

Underlying immunological differences in the populations studied may also account for the varying magnitudes of BCG-specific and non-specific effects seen [17, 18, 19, 6]. Both the MIS BAIR [11] and Danish-Calmette studies [12] were designed to investigate the potential impact of BCG at birth on atopy and wheeze, as well as its effects on infection outcomes. As a result, both study populations were highly enriched for participants with a family history of atopy/allergy (83% and >64% respectively), compared to the much lower rates of atopy documented in African populations [20]. Allergic infants have high innate immune responses at birth, decreasing over time with attenuated TLR responses and increased Th2-dominant allergen-specific responses. In contrast, non-allergic children show low innate responses at birth, with progressive increases in cytokine responses to virtually all TLR ligands and increased Th1 responses to allergens and mitogens over time [21]. The potential for BCG to have non-specific beneficial effects may, therefore, be affected by differing susceptibilities to allergy/atopy seen in different settings.

In the MIS BAIR study, all the children had a birth weight of >1500gms, but only 45 of <2500gms, too few for a sub-group analysis. The observation in both the Guinea-Bissau and Ugandan studies [2-6] that the beneficial effects of BCG were most pronounced in low birth-weight infants (principally small for gestational age term infants) could also indicate that nutritional differences among settings may influence the varying non-specific responses seen. Infants born growth restricted have altered immunology compared to appropriately grown infants, with higher inflammatory activation and T-cell turnover, lower complement and IgG levels, higher NK-cells and IgM levels, reduced vaccine responses in childhood and increased risks of infectious mortality in the neonatal period and beyond [22]. The findings of significant non-specific effects of BCG in African studies may, therefore, be a function both of the higher numbers of growth restricted infants and the higher prevalence of the outcome measure of interest (infectious disease or death) in infants in these settings.

An interesting outlier in the observed differences between high vs. low-resource, high vs. low latitude settings is the trial conducted by Jayaraman et al, in Indian neonatal units [13]. This trial, in low birth-weight and preterm infants in a high-mortality, high TB-incidence setting relatively close to the equator, failed to find significant non-specific beneficial effects of BCG. These results may be partly explained by the varying effectiveness of different BCG strains, with BCG-Russia used in the Indian study, compared to BCG-Danish in moststudies reporting beneficial non-specific effects. Differences in induced immune responses by strain will again be familiar to those studying variations in the TB-specific, as well as non-specific, effects of BCG [23] [24], although it is worth noting that BCG-Danish was used in the MIS BAIR trial [11]. Perhaps as important, however, may have been a lack of specificity regarding the outcome of interest. Although the Indian study [13] was powered to show a 33% reduction in mortality rate, more than half of the study infants died from non-infectious causes for which BCG is not expected to have an effect. The MIS BAIR study [11] may also have been

limited by a lack of specificity in the measured primary outcome: LRTI. An episode of LRTI was based on parental-report of any episode of infant wheeze, rattle or rattly chest, an imprecise definition, particularly in a population enriched for family history of atopic disease and asthma. As the authors acknowledge, there was often discordance between physician-diagnosis and parental-reported symptoms. Optimally, all episodes of suspected infection in trials investigating the non-specific effects of BCG should be precisely identified by a blinded physician based on a combination of clinical findings with supportive diagnostic tests, rather than relying on proxy measures such as hospitalizations, deaths or parental-reported episodes. However, accurate identification of illness episode etiology not only remains a challenge in clinical practice but is expensive and time-consuming in research studies. The identification of immunological correlates of non-specific protection induced by BCG would, therefore, be of great benefit for future studies. Whereas trained innate immunity appears likely to play a role in the non-specific beneficial effects of BCG, no consistent signature of immune responses has yet been identified, and findings have again differed in different settings and in neonates compared to adults [25] [6].

Finally, the impact of other routine immunizations on the non-specific beneficial effects of BCG should be considered. The period of largest beneficial non-specific effects of BCG in the African trials [2-6] was prior to the receipt of DTP-containing vaccinations, with limited evidence for effects subsequently. The potential modifying effect of non-live vaccinations on the non-specific beneficial effects of BCG has been described in observational studies [26]. Although underpowered due to paucity of infectious outcome events during this early time-period in their settings, both the MIS BAIR [11] and Danish-Calmette studies [12] showed trends toward beneficial non-specific effects of BCG during the period prior to first DTP-containing vaccination. Interrogation of different vaccination schedules, with consideration of 'live-vaccine-last' regimens [27], is essential to ensure that maximum overall benefit to children may be achieved with these interventions.

Investigators of the non-specific effects of BCG have faced many of the same challenges that those investigating the TB-specific effects of BCG have faced before them. The findings of the MIS BAIR study [11] do not negate those studies showing significant non-specific benefits of BCG. Even if BCG vaccination can only offer significant protection to infants in the period shortly after birth in high-mortality settings, reductions in childhood mortality from ensuring the provision of BCG on the first day of life would still be great. The disparities in study findings illustrate that the ways in which the developing immune system, environment and infectious diseases interact are complex, and we are only beginning to understand the many ways in which they influence each other.

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