Tuberculosis susceptibility and protection in children

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Children represent both a clinically important population susceptible to tuberculosis and a key group in whom to study intrinsic and vaccine-induced mechanisms of protection. After exposure to *Mycobacterium tuberculosis*, children aged under 5 years are at high risk of progressing first to tuberculosis infection, then to tuberculosis disease and possibly disseminated forms of tuberculosis, with accompanying high risks of morbidity and mortality. Children aged 5–10 years are somewhat protected, until risk increases again in adolescence. Furthermore, neonatal BCG programmes show the clearest proven benefit of vaccination against tuberculosis. Case-control comparisons from key cohorts, which recruited more than 15 000 children and adolescents in total, have identified that the ratio of monocytes to lymphocytes, activated CD4 T cell count, and a blood RNA signature could be correlates of risk for developing tuberculosis. Further studies of protected and susceptible populations are necessary to guide development of novel tuberculosis vaccines that could facilitate the achievement of WHO's goal to eliminate deaths from tuberculosis in childhood.

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Introduction

Traditionally, the field of paediatric tuberculosis has been neglected, although recent years have seen a welcome increase in policy focus including the goal of zero childhood tuberculosis deaths.[1] To achieve this ambition, substantial progress now needs to occur, given that more than 1 million children developed active tuberculosis in 2016 with 250 000 children dying of the disease.[2] This number represents 10% of the total global burden of incident tuberculosis and 15% of associated total mortality.[2] Children can equally be affected by resistant strains of *Mycobacterium tuberculosis*, with an estimated 25 000 children developing multidrug-resistant (MDR) tuberculosis and 1200 developing extensively drug-resistant (XDR) tuberculosis in 2014 alone.[3] There are numerous social, epidemiological, immunological, diagnostic, and therapeutic differences between childhood and adult tuberculosis,[4] hence paediatric tuberculosis requires specific considerations in clinical, public health, and research aspects.

Children and adolescents represent clinically important populations with increased susceptibility to tuberculosis, and key groups in whom to study mechanisms of protection. The precise definition of paediatric tuberculosis is debated: WHO reports tuberculosis data for those younger than 15 years, the UN Convention on the Rights of the Child defines a child as someone younger than 18 years, and adolescence is increasingly considered to last until the age of 24 years.[2,5,6] In this Review, we use the term paediatric to broadly refer to those younger than 18 years, while acknowledging that the burden of disease in adolescents is therefore underestimated.

Children aged under 5 years have the highest risk of progressing to disease after infection, with infection defined as mycobacterial sensitisation shown by a positive tuberculin skin test (TST) or interferon-γ release assay (IGRA). They are also at the highest risk of disseminated forms of tuberculosis such as miliary tuberculosis and tuberculous meningitis.[7] Young children are also the most likely to die, with tuberculosis mortality rates from the pre-treatment era of nearly 50% in those younger than 5 years, substantially higher than in older children.[8]

By contrast, young school-aged children (5–10 years old) seem to be protected against tuberculosis, before a second peak in incidence during adolescence and into adulthood (figure 1).[12] In fact, many children control *M tuberculosis* without intervention. Studies from the pre-chemotherapy era show that most children survive tuberculosis disease without treatment,[8] pathological appearances on pulmonary radiographs frequently clear spontaneously,[9] and *M tuberculosis* can be cultured from recently infected children who are asymptomatic and do not proceed to become unwell.[13] Contemporary data confirm that some children with culture-confirmed MDR tuberculosis remain well and symptom free in the absence of treatment.[14] Therefore, the interactions between human host and the mycobacterial pathogen are increasingly recognised to develop along a spectrum rather than falling within clearly delineated categories.[15–18]

This diversity in human responses to *M tuberculosis* exposure raises the question of whether a protective immune response can be promoted by a new vaccine. The development of a protective vaccine by 2025 is a cornerstone of the WHO End TB Strategy.[19] Children are the only group for whom there is strong evidence of inducible protection though vaccination.[20–22] BCG, a live, attenuated vaccine, was first given to humans in 1921 and has been administered to more people than any other vaccine in history. Infants (younger than 12 months) were the target population for the first phase 2 randomised placebo-controlled clinical trial[23] of a new tuberculosis vaccine, the modified vaccinia virus Ankara expressing antigen 85A (MVA85A). Unfortunately, the MVA85A vaccine showed no additional efficacy against tuberculosis disease or infection beyond that of BCG in South African infants. These results, which did not confirm previous animal models and human immunogenicity data, prompted reappraisal of future strategies in the field of tuberculosis vaccine development.[24–26]

The investigation of human correlates of protection against *M tuberculosis* remains a major research priority.[19,24]

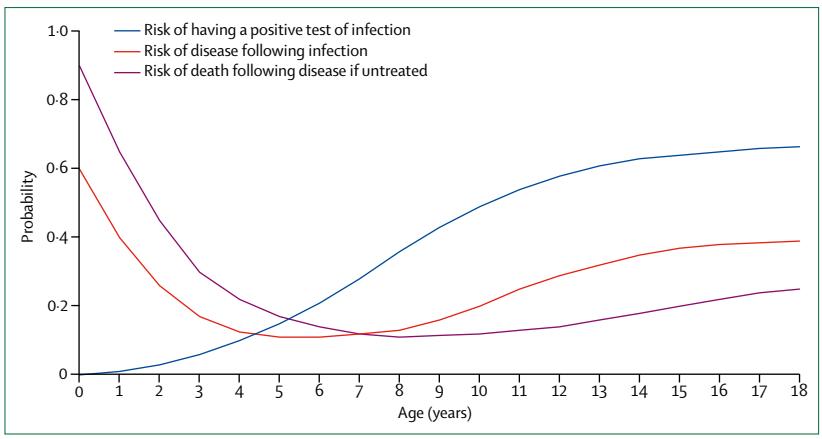

Figure 1: **Schematic diagram of the effect of age on risks associated with *Mycobacterium tuberculosis* in high burden settings**
Graph shows the probability of infection, disease, or death.[8,9,10,11]

To date, our understanding of protection in children has been derived from three main research approaches. One such approach has been the use of case-control studies, nested within paediatric and adolescent interventional trials or cohorts. Commonly, patients who developed tuberculosis disease, are compared with those who remained well. Such studies have enrolled more than 15 000 children and young people between them, almost entirely from South Africa, and have used various laboratory approaches in the search for correlates of risk and protection (table).[16,23,27–39] A second approach has been active contact tracing, assessment, and follow up of individuals exposed to *M tuberculosis*, for example, through household contact studies or outbreak investigations.[10,40–49] A third approach has been identification of genetic defects in children with severe forms of mycobacterial disease. By examining the immunological pathways involved, critical aspects of the human immune response necessary to contain *M tuberculosis* can be identified.[50–52] In this Review, we first summarise key components of the paediatric immune response to *M tuberculosis* (figure 2) before focusing on the understanding of risk and protective factors when children encounter *M tuberculosis* (figure 3). For clarity, we have structured our discussion around the concepts of exposure, infection, pulmonary disease, severe disease, and death, although we acknowledge that this structure represents a simplification of the clinical spectrum.

Key components of the paediatric immunological response to *M tuberculosis*

There are several possible outcomes when a child inhales *M tuberculosis*; there can be changes in status over time as well as heterogeneity of outcomes within an individual's lungs.[18] These outcomes are: elimination of *M tuberculosis* by the innate immune system; asymptomatic control of the mycobacteria accompanied by a cell-mediated immune memory response (tuberculosis infection) with or without persistence of viable organisms; direct progression to pulmonary disease (previously known as primary tuberculosis) or delayed development of pulmonary disease (often described as reactivation); and extension beyond the lung and lymphatic system causing severe disseminated disease. The immune mechanisms involved in regulating the fine balance between host control and disease are poorly defined and a correlate of protective immunity remains elusive. It is often assumed that the differences in susceptibility to *M tuberculosis* between children and adults are attributable to age-related differences in the immune response, but few studies have assessed this.

Various innate and adaptive cell types and immune mediators are involved in the host response to *M tuberculosis* (figure 2). Antigen-presenting cells, including macrophages and dendritic cells, are key to the initial response. Young children have fewer circulating dendritic cells than adults, with reduced functional capacity, macrophage phagocytosis, and recruitment.[54] Other components of the innate response to *M tuberculosis* are also different in neonates and early infancy compared with older children and adults. These differences include collectin concentrations and the complement pathway.[55,56] Maturation of toll-like receptors in the first year of life might also be relevant.[57–59] Neutrophils are the most commonly infected phagocyte in human tuberculosis,[60] and drive a type I interferon-inducible transcript signature in adult whole blood that correlates with clinical severity, suggesting that neutrophils might be involved in disease pathogenesis.[15] The importance of CD4-positive T cells and interferon γ is evident through the significantly increased risk of tuberculosis in infants with HIV infection, and severe mycobacterial disease in those with defects in the interferon-γ pathway.[51,52,61] Although T-helper-17 cells (producing cytokines interleukin 17 and interleukin 22) reportedly play a role in adult tuberculosis, paediatric studies have not shown their contribution to protection or susceptibility.[38,62–64] The potential immunoprotective role of non-conventional T cells, which link the innate and adaptive immune responses, such as γδ T cells and natural killer cells, has also been investigated.[65,66] These cell types expand in response to mycobacterial infection and show effector T cell functions such as interferon γ production and granulysin release in children. Children with tuberculosis have higher levels of regulatory T cells than healthy controls, even after 6 months of tuberculosis treatment.[62]

Risk of exposure to *M tuberculosis*

The relationship between paediatric tuberculosis and poverty is overwhelming (figure 4) and confounds other risk factors.[70,71] The influence of poverty extends across the whole spectrum of paediatric tuberculosis and is associated with increased risk of being exposed to tuberculosis, of becoming infected, of developing disease, and of poor outcomes.[72]

The risk of exposure to *M tuberculosis* is a combination of epidemiological, environmental, sociocultural, and

	Number of children with tuberculosis included in the analysis*	Methods used to identify correlates of risk/protection	Potential correlates of risk	Potential correlates of protection
Double-blind randomised placebo-controlled phase 2b trial of MVA85A vaccine boost of BCG[23] (n=2797 infants† in South Africa)				
Analysis 1	71	Ex vivo interferon-γ ELISpot assays: Ag85A IgG; BCG mycobacterial growth inhibition assay; flow cytometry[27,28]	Percentage of HLADR+CD4+ T cells[27]	BCG ELISpot;[27] percentage of CD4-positive T cells at baseline;[27] D28 post-boost vaccination Ag85A-IgG[27]
Analysis 2	28	Quantitative QFT[29]	QFT conversion at interferon-γ values higher than 4·00 IU/mL	None
Primary isoniazid prophylaxis against tuberculosis in HIV-exposed children[30] (n=1336 infants† in South Africa and Botswana)				
Analysis	187	Full blood count monocyte to lymphocyte ratio	Increased monocyte to lymphocyte ratio[31]	None
Adolescent Cohort Study [16,32-34,36] (n=6363 adolescents aged 12–18 years in South Africa)				
Analysis 1	30	Flow cytometry	Percentage of HLADR+CD4+ T cells[27]	None
Analysis 2	46	RNA sequencing data of unstimulated whole blood	16-gene signature[16]	None
Analysis 3	96	Serial QFT	Persistently positive QFT; converting from negative to positive QFT; converting from positive to negative QFT[36]	None
Randomised trial to compare the incidence of tuberculosis over 2 years in infants vaccinated at birth with intradermal BCG or with percutaneous BCG[37] (n=5724 infants in South Africa)				
Analysis 1	29	BCG stimulation: flow cytometry;[28] 29-plex supernatant cytokine/chemokine;[39] lymphocyte proliferation assay;[39] cytotoxic marker assay;[39] quantification of myeloid and lymphoid cell populations[29]	None	None
Analysis 2	46	Microarray RNA gene expression analysis of BCG-stimulated PBMCs[39]	None	None

MVA85A=modified vaccinia Ankara virus expressing antigen 85A. PBMCs=peripheral blood mononuclear cells. ELISpot=enzyme-linked immunospot. qRT-PCR=quantitative real-time polymerase chain reaction. QFT=QuantiFERON-TB-Gold In-Tube. *Given the availability of samples, different numbers of cases were included in different analyses, despite being derived from the same cohort.†Younger than 12 months.

***Table*: Summary of key paediatric case-control studies, their parent cohorts, the methodologies employed to explore correlates of protection, and major findings**

behavioural factors that reflect how children, adolescents, and adults interact within societies. The probability of exposure relates to background prevalence of tuberculosis, although this factor can be heterogeneous within countries and cities.[12] Age-related and culture-related factors such as sleeping practices, care-giving, play, religious practices, and school will influence how and where children interact with adults who might have tuberculosis and how much risk these interactions carry.[73] Similarly, population density, household composition, crowding, transport systems, and ventilation, both at home and in health-care facilities, all contribute to the risk of exposure.[74,75] Adults living with HIV have an increased risk of tuberculosis,[76] and therefore children in the same households have an increased risk of exposure, in addition to the direct risks associated with vertical transmission of HIV and subsequent suppression of protective cellular immune responses. Social factors, such as household alcohol consumption, are known to enhance the risk of exposure, as adults who drink more than 40 g of alcohol per day or have an alcohol use disorder are more likely to develop tuberculosis than those who drink no or small amounts of alcohol, and are also less likely to seek care or have successful treatment outcomes.[77]

Risk of infection with *M tuberculosis*

After exposure, the risk that a child will develop *M tuberculosis* infection is influenced by the infectiousness of the source case, the duration and intensity of the interaction, the infectivity of the organism and the immune responses of the child.[73] Source cases are more infectious if they have a high bacterial load, which is clinically reflected by sputum smear-positivity.[42] Extensive pulmonary disease in the source case, defined as affecting multiple zones on a chest radiograph, is associated with increased risk of TST positivity in contacts, independent of mycobacterial load.[10,42] The duration of cough increases the risk of transmission: the longer the source case has been coughing, the more likely children are to be infected.[10] If the source case is a first-degree relative then child contacts are more likely to become infected than if the source case is a more distant relative,[10] and the physical proximity of sleeping arrangements in households influences transmission, with close sleeping proximity associated with increased

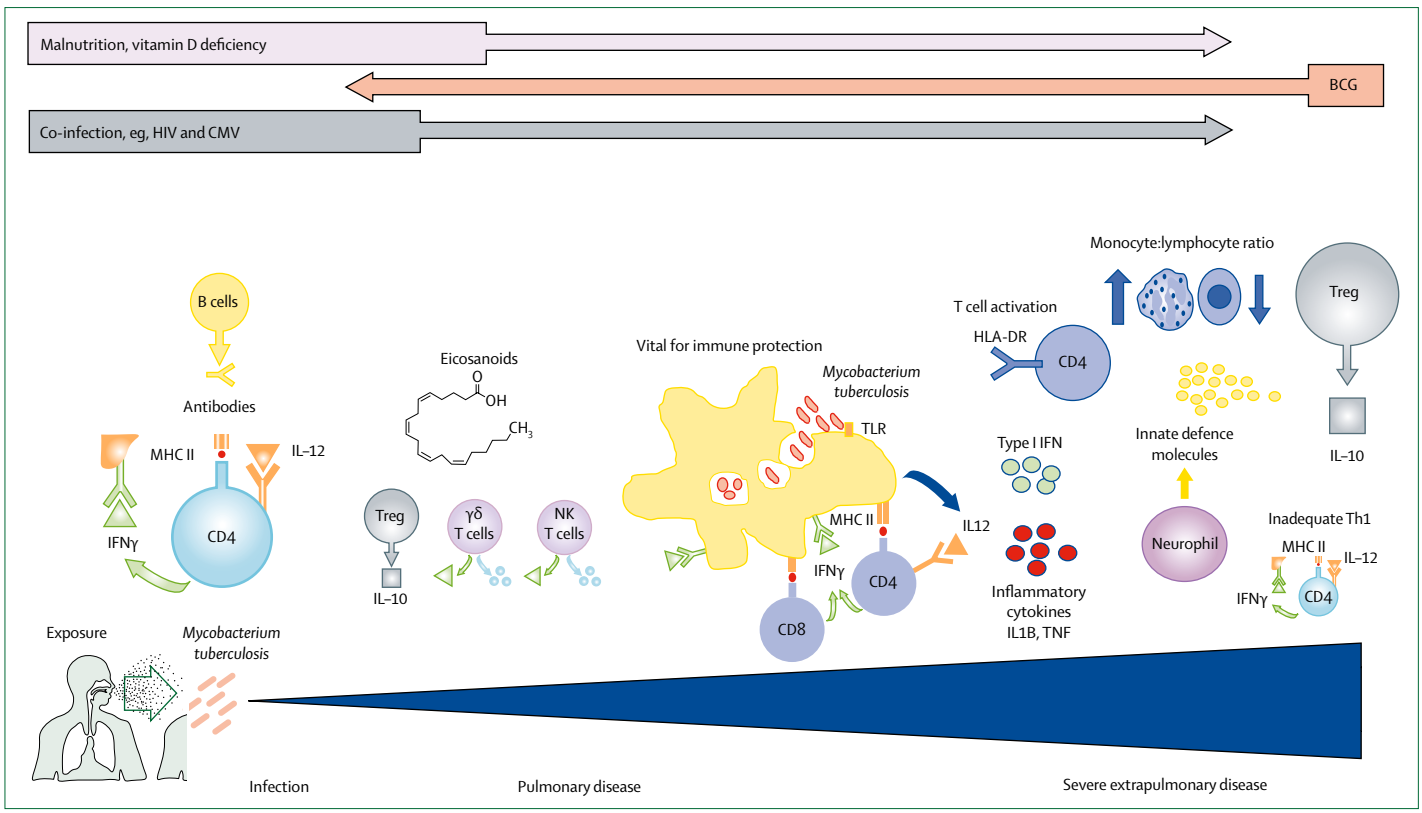

Figure 2: **Summary of key immunological factors involved in paediatric response to *Mycobacterium tuberculosis*, with hypothesised associations with risk and protection**
Adapted from Jones et al.[53] APC=antigen-presenting cell. CMV=cytomegalovirus. IFN=interferon. IL=interleukin. MHC=major histocompatibility complex. NK=natural killer. TLR=toll-like receptor. TNF=tumour necrosis factor.Treg=regulatory T cell.

risk of transmission.[10,78] The influence of solid fuel smoke and cigarette smoke is complex, but children are more likely to become infected if someone in the household smokes cigarettes or if solid fuels are used for cooking or heating.[79]

Whether certain mycobacterial strains are more infectious than others is unclear, with conflicting findings regarding an increased infectiousness and virulence of the Beijing strain, compared with other strains.[43,80] A large Indian study showed that isoniazid-resistant strains were associated with higher rates of infection but similar rates of disease compared with isoniazid-susceptible strains,[81] and a study from Peru suggested that contacts of people with MDR-tuberculosis cases were less likely to develop tuberculosis than contacts of people with drug-susceptible tuberculosis.[49]

Most contact studies show that children have an increased likelihood of a positive TST with increasing age (figure 1).[10,11,82] Given that these tests reflect infection at any point in their lives, these results are not surprising, since older children are more likely to have been exposed to people with infectious cases of tuberculosis in the community in addition to the identified household source case. BCG vaccination and exposure to non-tuberculous mycobacteria are also known to influence TST results. Whether uninfected older children are more likely than uninfected younger children to develop a positive TST after known exposure is unclear. Outbreak investigations in low prevalence settings suggest that perhaps young children are most vulnerable, but this finding could be explained by increased intensity of exposure.[45]

Co-infections also have a role.[83] Even when vertical transmission is interrupted, infants who are HIV-exposed but uninfected have transient altered responses to BCG vaccination in vitro early in life and are at increased risk of tuberculosis infection.[84–88] The effect of parasite co-infection on risk of tuberculosis infection in exposed children remains unclear.[73,83,89] Helminth infections, in particular *Ascaris lumbricoides* and hookworm, induce a T-helper-type-2-associated immune response, characterised by the presence of cytokines including interleukin 4, interleukin 5, interleukin 9, interleukin 10, and interleukin 13.[89] A randomised clinical trial showed no effect of deworming on either TST or IGRA in children[90] and helminth infections have been shown to increase[91] or reduce[92] the risk of TST positivity after exposure to an infectious source case.

Protection against infection by *M tuberculosis*

Understanding why some children who are exposed to *M tuberculosis* show no signs of infection is crucial in

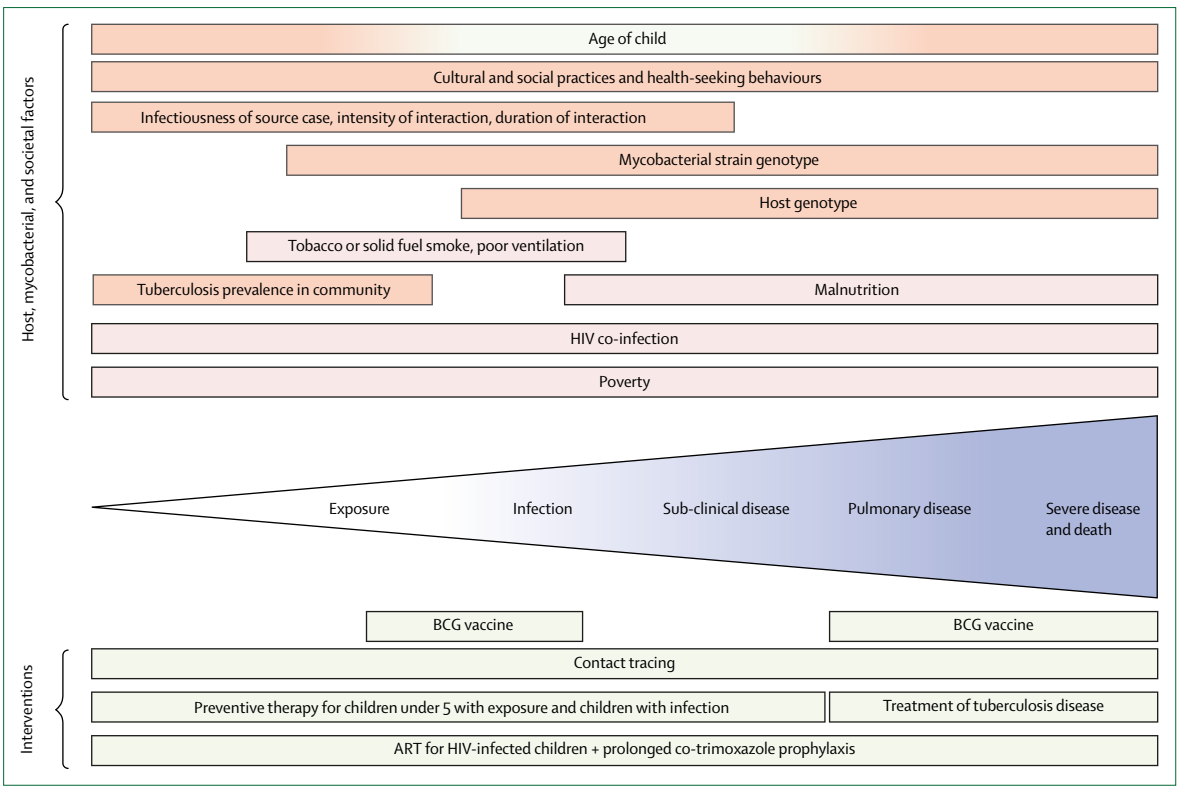

Figure 3: **Schematic representation of risk factors and protective interventions in paediatric interactions with *Mycobacterium tuberculosis***
Red background denotes risk factors, green background denotes protective factors, and orange denotes factors that can be either protective or increase risk. ART=antiretroviral therapy.

understanding an effective early human immune response to *M tuberculosis*. This knowledge could definitively inform the design of vaccines able to induce protection against infection.[93,94] In the absence of a gold standard, protection against infection is considered to be absence of a positive TST or IGRA despite documented exposure or living in high prevalence regions. A Colombian household contact study showed that approximately 65% of the variability in TST results is attributable to genetic contributions.[95] Specifically, a genome-wide linkage study of 128 families, including 350 children, in Cape Town, South Africa investigated 6000 single-nucleotide polymorphisms and identified a major locus for TST positivity (TST=0 mm *vs* >0 mm) at chromosome region 11p14, and a locus for quantitative TST reaction at chromosome region 5p15.[96] The 11p14 result was replicated in a prospective household contact study of 97 families in Paris, an area of low tuberculosis prevalence,[97] and the locus overlaps a region involved in production of tumour necrosis factor (TNF) α production.[98] Investigation of the persistently TST negative phenotype in both children and adults in Uganda suggests that persistent TST-negativity is not attributable to clinical or epidemiological characteristics alone, but also has genetic contributions and differential interferon γ responses.[46,47,99]

In addition to genetic contributions, modifiable factors such as vaccination or co-infection also play a role. The improved specificity of IGRAs to identify infection has enabled detection of a vaccine-inducible protective effect, with BCG having an estimated 19% effectiveness (95% CI 8 to 29) to protect against infection.[21,94] To date, this protection has proven difficult to improve on, with the MVA85A phase 2b vaccine trial in South Africa showing no additional efficacy against *M tuberculosis* infection (–3·8%, 95% CI –28·1 to –15·9). However, the study was not powered for this endpoint.[23] Some findings suggest that protozoal infections might protect against tuberculosis infection.[91,100]

Risk of pulmonary tuberculosis disease

Data from the pre-chemotherapy era showed clearly that age is one of the most important factors in predicting which children will progress to disease. In the absence of any preventive therapy, infected infants have a 50% risk of progression to disease; however, this risk reduces between 5 and 10 years of age, before rising again as children enter adolescence (figure 1).[9,101–103] The pattern of increased susceptibility with young age is supported by contemporary data, with progression rates for untreated patients with IGRA-confirmed infection of 43% for children under 5 years, significantly higher than the

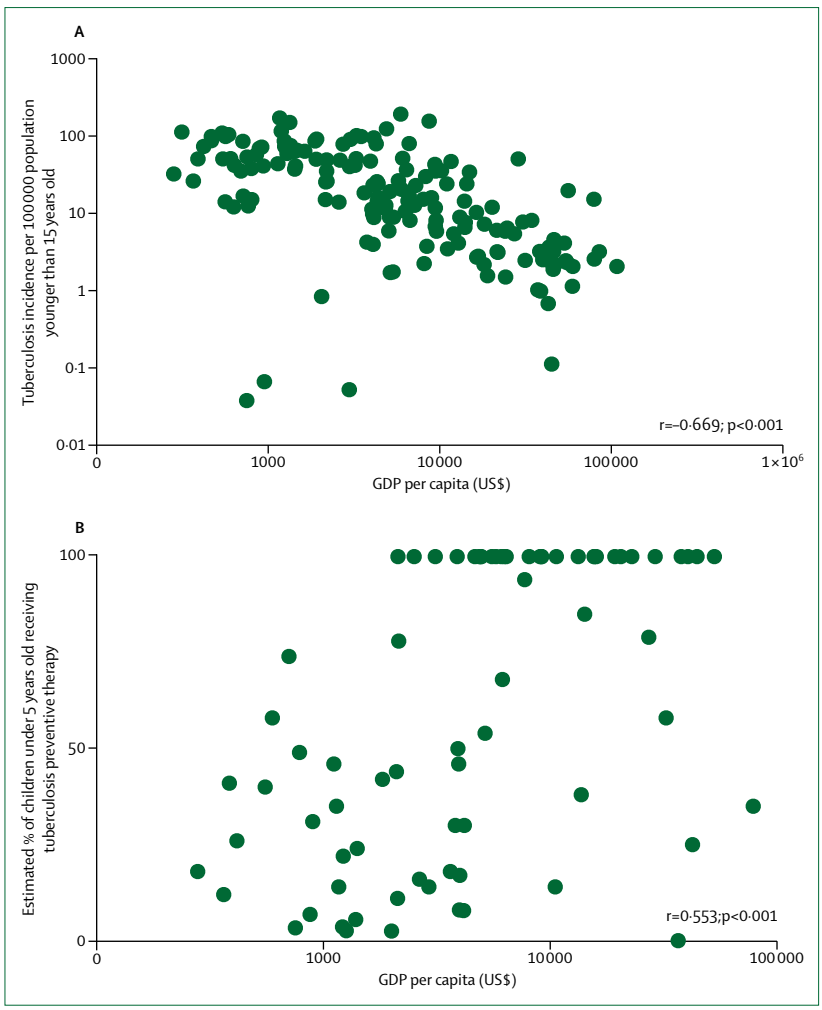

Figure 4: **Association between gross domestic product per capita and (A) paediatric tuberculosis incidence in 168 countries with available data and (B) provision of tuberculosis preventive therapy to eligible children under 5 years old who are household contacts of tuberculosis cases**

Relatively few countries (n=75) report data on provision of tuberculosis preventive therapy to eligible children under 5 years old, and 33 of those countries report perfect provision (100%). Data sources: 2015 GDP data, World Bank;[67] 2015 population under 15 years, United Nations, Population Division, Department of Economic and Social Affairs;[68] 2015 tuberculosis cases under 15 years old and estimated proportion of children under 5 years who received tuberculosis preventive therapy, are household contacts of tuberculosis cases, and who are eligible for tuberculosis preventive therapy, WHO.[69]

equivalent rate of 10.3% for adults.[104] A Dutch study of contacts of patients with tuberculosis again showed a significant age-related effect, with a 5-year risk of prevalent and incident tuberculosis of 33·3% in contacts less than 5 years compared to 6·7% in those older than 15 years.[103]

Addressing HIV co-infection is essential in this context—infants with HIV are 24 times more likely to develop tuberculosis than infants without HIV,[61] with falling CD4 counts increasing risk.[105,106] Antiretroviral therapy significantly reduces the risk, with most of the effect achieved within the first year of treatment.[107,108] However, the risk of tuberculosis remains consistently higher than that of the general population.[107] Restoration of CD4 cell count is not associated with substantial increases in production of interferon γ in response to mycobacteria.[109] Children who are malnourished or who have other forms of immune deficiency also have a heightened vulnerability.[110,111] However, it remains difficult to establish whether the tuberculosis leads to malnutrition or vice versa.

Other than age-related differences, the ability to distinguish children with high risk of progressing from infection to disease based on currently available clinical tests is poor. In a multi-site study in Europe of 5020 tuberculosis contacts, in which 495 (9·8%) contacts were aged less than 14 years, the positive predictive values for IGRAs were below 2%.[48] A systematic review and meta-analysis, in which eight out of 15 of the included trials involved paediatric participants, showed similar incidence rate ratios for tuberculosis disease progression for a TST response of greater than 10 mm induration (1·60, 95% CI 0·94–2·72) or a positive IGRA (2·11, 1·29–3·46).[112] Prospective follow up of a South African adolescent cohort study[33] showed that IGRA conversion from a baseline negative test result to a positive result had a positive predictive value for the development of tuberculosis disease within 2 years of 2·6%, representing an eight-times higher risk than those who did not convert. An increased relative risk remained even in cases where a positive IGRA reverted to negative.[36]

Whether the magnitude of TST reactions or interferon γ responses can further stratify risk is debatable.[29,104,112,113] A study in Hong Kong, where BCG vaccination rates in newborn babies are high, of nearly 20 000 children who were administered a TST aged 6–10 years, showed that those with a result greater than 15 mm had a 12-times increased risk of developing tuberculosis during adolescence compared with those with a result between 10 mm (the WHO threshold for a positive TST result) and 14 mm.[113] The odds of developing tuberculosis nearly doubled with every unit of interferon γ measured per millilitre in IGRAs in another study,[104] but did not significantly affect risk of tuberculosis in other populations.[48,112] An analysis of the infant cohort from the MVA85A vaccine trial showed that infants with conversion to a positive IGRA with conventional thresholds showed no greater risk of developing tuberculosis than those with a persistent negative IGRA. However, those who had a quantitative result ten times greater than the conventional threshold for positivity had significantly higher risk of developing tuberculosis than infants who were IGRA-negative, and those with a lower magnitude of IGRA positivity.[29]

Analyses of the MVA85A trial[23] and South African adolescent cohort study[34,35] have identified additional novel risk factors for developing disease (table).[16,29,33] A 16-gene, whole blood RNA signature identified in adolescents could predict progression from a positive IGRA to tuberculosis before developing symptoms or clinical diagnosis, with

increasing sensitivity the closer to the time of diagnosis (table).[16] Whether this signature reflects early subclinical disease or could be used to stratify treatment and follow-up for children infected with *M tuberculosis* remains to be established. In both studies' populations, activated CD4 cells expressing HLA-DR were associated with increased risk of tuberculosis (table).[27] This observation, alongside the recently recognised importance of type 1 interferons,[15] has prompted much discussion as to whether part of the susceptibility to tuberculosis is related to viral co-infections including cytomegalovirus. In the MVA85A cohort,[23] cytomegalovirus was identified as a co-factor of interest, with a statistical association with HLA-DR-positive CD8 cells, which in turn was associated with HLA-DR-positive CD4 cells. Cytomegalovirus responses were, however, not directly associated with HLA-DR-positive CD4 cells themselves, nor with risk of tuberculosis in this study.[27] In an unrelated study, CD27 effector memory phenotype of cytomegalovirus-specific interferon γ producing CD4 cells differed in HIV-uninfected adults with tuberculosis compared with controls, although no effect was detectable in children.[114] An increased monocyte-to-lymphocyte ratio has been identified as a further risk factor for progression from case-control comparisons within a cohort of infants exposed to HIV (table) and in a Madagascan household contact study.[31,115]

Genome-wide association studies to investigate genetic contributions to pulmonary tuberculosis susceptibility at a population level have primarily focused on adults. Several significant loci have been identified, but with relatively small effect sizes, challenges in replicating results in other populations, and uncertain functional importance.[116–118] Involvement of HLA class II alleles[119,120] and *SLC11A1* (formerly known as *NRAMP1*)[121] represent the most robust findings. Enzymes involved in inflammatory eicosanoid pathways might also have a role in paediatric susceptibility to tuberculosis.[122] By focusing on relatively early age of onset of pulmonary tuberculosis, several additional genetic loci have recently been identified (*FOXP1* and *AGMO*,[123] *TOX*,[124] and *STAT4*[125] with tuberculosis onset at age <25 years). Concentrations of vitamin D, in the context of genetic polymorphisms in receptors and the interaction with HIV, are additionally implicated in risk of disease.[126,127] Seasonal peaks in childhood tuberculosis have been described in different communities and probably reflect a mixture of environment-related exposure factors combined with seasonal variations in concentrations of vitamin D.[128]

Once again, the influence of helminth infection is unclear. In vitro studies show that pre-exposure or co-incident infection with filaria, hookworm, strongyloides, or schistosoma is associated with downregulated or suppressed T-helper-1 and T-helper-17 responses to mycobacterial antigens.[89] These immunological in vitro findings have not been replicated in patients with tuberculosis disease, however.

Protection against pulmonary tuberculosis disease

Existing, safe, and effective interventions to prevent children exposed to or infected with tuberculosis from progressing to disease already exist.[129,130] WHO makes a dual recommendation that household or close contacts of patients with tuberculosis are actively traced with particular focus on children,[131] and that children younger than 5 years who are found not to have tuberculosis disease should be given isoniazid daily for 6 months.[132] The decades of experience with isoniazid preventive therapy in children show an estimated risk reduction for developing tuberculosis of 59%[133] and a protective effect that can last at least 30 years.[134] In one study, the positive predictive value of a positive IGRA for progressing to disease was 17% in patients who did not receive preventive therapy, compared with 4% for the whole study population. A third of the patients were children.[135] Negative predictive values for developing tuberculosis for both IGRAs and TST are consistently high (>98%), reinforcing that many children exposed to tuberculosis are able to effectively control the pathogen.[48,104] The risk of developing isoniazid resistance through preventive therapy is extremely low,[136] with a theoretical indirect benefit to drug-resistant strains; surveillance for drug resistance is key to identifying isoniazid resistance.[137] Shorter regimens, including 3 months of isoniazid and rifampicin, and 12 weekly doses of isoniazid and rifapentine, are equally efficacious in children and young people, and might help improve treatment completion rates, which are known to be lower in adolescents aged 15–18 years than in children younger than 5 years.[138,139] Appropriately chosen preventive therapy regimens in paediatric contacts of patients with MDR-tuberculosis appear to be effective.[140] However, contact tracing and preventive therapy are not widely implemented in countries with high tuberculosis burden (figure 4B), which is an example of the need to strengthen health systems alongside advocacy and human rights approaches to implement such strategies with already proven success.[5,141]

The evidence behind administering primary isoniazid prophylaxis without a known tuberculosis contact in children infected with HIV is less clear.[30,132,133,142,143] In a study before the rollout of antiretroviral therapy in sub-Saharan Africa, isoniazid decreased both all-cause mortality and confirmed or probable tuberculosis, leading to the study being stopped early.[142,143] However, more recently, 96 weeks of isoniazid primary prophylaxis showed no effect in HIV-exposed children, whether they were HIV-infected or not.[30] Although it acknowledges the low quality of the evidence, WHO recommends 6 months of isoniazid for children over 1 year old living with HIV in areas with high tuberculosis prevalence in the absence of symptoms of tuberculosis or a known tuberculosis contact.[132] A trial comparing the continuation or cessation of co-trimoxazole in children living with HIV and

receiving antiretroviral therapy beyond 96 weeks showed a three-times reduction in tuberculosis incidence among those continuing, compared with those stopping.[107] Whether this result represents a direct effect of co-trimoxazole on *M tuberculosis* or an effect on co-infections that might in turn affect the ability to contain *M tuberculosis* is unclear and merits further evaluation.

There is likely to be a vaccine-inducible protective effect against pulmonary tuberculosis disease in children.[22,130,144,145] Neonatal BCG vaccination, as recommended by WHO in countries with high tuberculosis prevalence, appears to provide 60% protection against pulmonary tuberculosis in childhood (95% CI 42–71), but offers much less protection against adult forms of pulmonary disease that contribute to transmission.[22] A transient cessation in universal BCG vaccination between 1991 and 1996 in Greenland offered a well-controlled opportunity outside of a randomised trial to show a vaccine effectiveness of 50% against tuberculosis.[144] A Peruvian contact tracing study showed a 65% decrease in prevalent and incident tuberculosis in BCG-vaccinated children under the age of 10 years, compared with their unvaccinated peers.[130]

The findings from case-control analyses that have investigated correlates of protection have been inconsistent to date. In the MVA85A cohort, in which all infants were BCG-vaccinated, BCG-specific T cells secreting interferon γ correlated with reduced risk of tuberculosis.[27] However, neither flow cytometric intracellular cytokine profiling, nor gene expression analysis of BCG-stimulated cells, yielded significant correlations between cases and controls from a cohort of unrelated South African infants (table).[37,38,39] The many potential explanations for the different results between these studies include the number of cases identified, number of controls per case, type of matching between cases and controls, case definitions, difference in age of participants, choice of laboratory methodology, and statistical design. Data from the MVA85A cohort suggested that IgG against the Ag85A protein 28 days after intervention might also correlate with protection.[27] Overall, immunological correlates of protection remain elusive. Other potential correlates of protection include foetal growth, since higher birthweight is associated with lower risk of tuberculosis.[111,146]

Risk of severe tuberculosis disease and death

Mycobacterial and host factors influence the risk of disease progression to disseminated disease or death.[147–149] The Beijing strain of *M tuberculosis* has been shown in some studies to be associated with disseminated disease in adults.[80] However, this pattern has not been shown convincingly in children.[149–151] Young age, MDR-tuberculosis, HIV infection, malnutrition, extrapulmonary tuberculosis, and a TST result of less than 5 mm, have all been found to be associated with death from tuberculosis in children.[7,8,147,148,152] Immunological explanations for why some children succumb to severe tuberculosis, however, remain elusive.[153] One of the major contributions to the field of tuberculosis biology has been the description of genetic susceptibilities underlying rare and severe infections by normally avirulent mycobacteria in children.[50–52] The subsequent identification of inherited defects in the interferon γ receptors and associated signalling pathways provided key mechanistic insights into the important role of interferon γ in humans. A hallmark of the clinical presentation in patients with complete interferon γ receptor deficiency is impaired granuloma formation, in line with the importance of interferon γ for containment of mycobacteria. Mutations in *STAT1*, components of the interleukin 12 pathway, and *NEMO* are among those more recently identified that lead to the clinical constellation of Mendelian susceptibility to mycobacterial disease.[52] Increased susceptibility to mycobacteria in more generalised primary immunodeficiencies, such as severe combined immunodeficiency, chronic granulomatous disease, and *GATA2* deficiency, confirm the crucial part that T cells, neutrophils, and antigen-presenting cells, respectively, play in the response to mycobacteria in children.[52] Iatrogenic immunodeficiency, through treat-ment of chronic inflammatory disorders with anti-TNFα monoclonal antibody treatment, is known to predispose children to severe mycobacterial disease. However, it occurs rarely in the context of screening for and treatment of infection before commencing immunosuppressive therapy.[154]

Protection against severe tuberculosis disease

Evidence for the efficacy of BCG is strongest for prevention of severe disease. BCG protects young children from tuberculous meningitis and miliary tuberculosis, with an efficacy of 75–85%.[20,22] However, this value varies in different geographical regions, with a reported efficacy in the UK of up to 80%, versus 0–20% in low-income countries nearer the equator, where there is increased prevalence of helminth infections and exposure to environmental mycobacteria.[22,145] The immunogenicity of BCG vaccines is decreased by suppressed cellular immune responses to mycobacterial antigens and increased TGF-β production in individuals with helminth infections.[21,155]

WHO states that tuberculosis contact investigations "contribute to early identification of active tuberculosis, thus decreasing its severity".[131] 10% of children under age 5 years, assessed through contact tracing in low-income and middle-income countries, have been found to have prevalent tuberculosis,[41] with small numbers of severe cases identified in clinical trials with active follow up. Combined with the natural history of tuberculosis in children, it is firmly established that early case identification and treatment initiation through contact tracing prevents progression to severe disease.[9]

Future directions

Despite many years of substantial research, through combinations of hypothesis-based and hypothesis-generating methodologies, with the evaluation of

unstimulated and mycobacteria-stimulated samples, and with more than 15 000 infants and adolescents enrolled into trials that enabled case-control comparisons, true correlates of protective paediatric immunity remain elusive (table). It is essentially unknown why BCG protects some children but not others. Differential responses to BCG vaccination identified through gene expression might have provided clues, although these findings also do not correlate with protection.[39] Although some correlates of risk have now been identified (eg, HLA-DR+CD4+ T-cells,[27] high monocyte to lymphocyte ratios,[31,115] and a 16 gene RNA signature[16]), the underlying biology still remains to be elucidated and these parameters are difficult to modulate through changes in vaccine design. There are at least 14 novel vaccines in clinical trials up to phase 2B,[156] but to date it has proven difficult to surpass the effectiveness of BCG,[23] which has been used in humans for nearly 100 years. Furthermore, despite failing to prevent cases of adolescent and adult tuberculosis that drive the ongoing epidemic, BCG induces strong interferon γ responses and not only protects children against infection, pulmonary disease, and disseminated tuberculosis, but also protects against leprosy, with an estimated efficacy of 26%,[157] and appears to have heterologous protective effects that are not related to the protection against tuberculosis.[158–160] Thus, even though BCG vaccination is clearly not halting the epidemic, it remains difficult to move beyond its use in infants when assessing novel vaccines. In areas where tuberculosis incidence is high enough to enable large studies with sufficient statistical power, infants exposed to HIV represent a specific subpopulation in which such vaccines can be trialled as a true alternative to BCG, given the known potential of BCG to cause dissemination in HIV-positive infants.[161] A variety of approaches, including combinations of novel vaccines with BCG in prime-boost strategies, post-exposure vaccination, and improved prevention of infection, are all also under consideration.[19,24,94] These endeavours are inevitably constrained by the scarcity of knowledge of what immune response an ideal tuberculosis vaccine should induce.

To make progress in protecting children against tuberculosis, parallel and multidisciplinary approaches are needed. Poverty remains the most important risk factor (figure 4) and therefore universal health coverage, social protection and justice, and poverty alleviation are all part of the solution.[5,70,72,162] Furthermore, there are already existing and efficacious interventions: contact tracing; preventive therapy for children under the age of 5 years; prevention, diagnosis and treatment of paediatric HIV; infection control precautions; and training of front line health workers to improve diagnosis.[5] Alongside implementation of approaches that are known to work, the research agenda needs to focus on unravelling the biology behind the glimmers of insight into innate, adaptive, and trained immunity, in both susceptible and protected populations, to enable rational vaccine development and make progress towards the goal of zero childhood deaths from tuberculosis (panel).[1,24]

Panel: Proposed areas of focus for future research

Studying populations at extremes of susceptibility and protection

Highly vulnerable with exposure to tuberculosis

- Children aged under 3 years
- Adolescents
- Immunocompromised people
- People with tuberculosis meningitis, miliary tuberculosis, or with high mortality

Relatively protected with exposure to tuberculosis

- Exposed uninfected children
- School-age and pre-adolescent children

Sociocultural and epidemiological factors

- Pre-school children—interactions between the child's health and that of the primary caregiver, role of nutrition and poverty
- Adolescence—pregnancy, sexual behaviours including risk of HIV infection, drugs, alcohol, tobacco, health-seeking behaviours, treatment concordance, poverty

Pathogen determinants of risk and protection

- Effect of mycobacterial strain on host immune response
- Effect of drug resistance on host immune response

Improved understanding of existing interventions

- Heterogeneity of responses to BCG
- Heterologous effects of BCG
- Re-evaluation of preventive therapy in HIV-infected children
- Optimal preventive therapy in those exposed to MDR-tuberculosis

Interpretation of host immune responses

- In vitro and in vivo experimental follow-up of correlates of risk and protection identified from clinical studies
- Biological exploration of gene expression data
- Ontogeny of the immune system through childhood into adulthood

Maximising yield from prospective studies

- Collaborative and co-ordinated approach
- Multiple study sites
- Combinations of hypothesis-testing and hypothesis-generating approaches
- Methodological developments to minimise required sample volumes
- Combinations of use of fresh and frozen samples
- Study of non-stimulated and mycobacterial-stimulated samples

Beyond blood

- Where ethically and clinically appropriate, further exploration of advanced radiological imaging in children exposed to tuberculosis
- Use of readily available non-sputum based samples—eg, urine, stool, saliva and nasopharyngeal aspirates
- Where ethically and clinically appropriate, use of samples from site of disease—eg, cerebrospinal fluid, lymph nodes, alveolar macrophages

Co-infection

- Exploration of immunological basis for increased risk of tuberculosis infection in HIV-exposed but uninfected children
- Exploration of influence of non-tuberculous mycobacteria, helminth, protozoal, cytomegalovirus, influenza, and microbiota interactions on paediatric response to mycobacteria

Search strategy and selection criteria

We searched PubMed for articles, using combinations of the search terms "tuberculosis", "risk", "susceptibility", "immune correlate", "epidemiology," "infection", "disease", "correlate", "preventive", "prophylactic", "protect*", "genome-wide association study", "genetics", and "immunology". Results were restricted to studies in people aged 0–18 years, without language restrictions, published any date before April 30, 2017. Articles resulting from these searches and relevant references cited in those articles were reviewed, giving preference for inclusion in this Review to the latest evidence from publications within the past 10 years.

Contributors

RB, JAS and BK initiated the review. RB, EW, JAS, and BK developed the scope of the manuscript. RB, EW, and JAS did the literature searches and prepared the first draft. BK critically reviewed the data and draft, and all authors subsequently modified the manuscript jointly. All authors approved the final submitted version of the manuscript.

Declaration of interests

BK holds a patent for a paediatric diagnostic biosignature. RB was a consultant for the Foundation for Innovative New Diagnostics, Geneva. All other authors declare no competing interests.

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