Vaccine xxx (xxxx) xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Do vaccines increase or decrease susceptibility to diseases other than those they protect against?

Alberto Rubio-Casillas [a,b], Cesar Manuel Rodriguez-Quintero [b], Elrashdy M. Redwan [c,d], Munishwar Nath Gupta [e,1], Vladimir N. Uversky [f,*], Mikolaj Raszek [g]

[a] Autlan Regional Hospital, Health Secretariat, Autlan 48900, Jalisco, Mexico
[b] Biology Laboratory, Autlan Regional Preparatory School, University of Guadalajara, Autlan 48900, Jalisco, Mexico
[c] Biological Science Department, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia
[d] Therapeutic and Protective Proteins Laboratory, Protein Research Department, Genetic Engineering and Biotechnology Research Institute, City for Scientific Research and Technology Applications, New Borg EL-Arab, Alexandria 21934, Egypt
[e] Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology, Hauz Khas, New Delhi 110016, India
[f] Department of Molecular Medicine and USF Health Byrd Alzheimer's Research Institute, Morsani College of Medicine, University of South Florida, Tampa, FL 33612, USA
[g] Merogenomics (Genomic Sequencing Consulting), Edmonton, AB T5J 3R8, Canada

ARTICLE INFO

Keywords:
Non-specific effects of vaccines
Tolerance
Immune training
Excess deaths
IgG4 antibodies

ABSTRACT

Contrary to the long-held belief that the effects of vaccines are specific for the disease they were created; compelling evidence has demonstrated that vaccines can exert positive or deleterious non-specific effects (NSEs). In this review, we compiled research reports from the last 40 years, which were found based on the PubMed search for the epidemiological and immunological studies on the non-specific effects (NSEs) of the most common human vaccines. Analysis of information showed that live vaccines induce positive NSEs, whereas non-live vaccines induce several negative NSEs, including increased female mortality associated with enhanced susceptibility to other infectious diseases, especially in developing countries. These negative NSEs are determined by the vaccination sequence, the antigen concentration in vaccines, the type of vaccine used (live vs. non-live), and also by repeated vaccination. We do not recommend stopping using non-live vaccines, as they have demonstrated to protect against their target disease, so the suggestion is that their detrimental NSEs can be minimized simply by changing the current vaccination sequence. High IgG4 antibody levels generated in response to repeated inoculation with mRNA COVID-19 vaccines could be associated with a higher mortality rate from unrelated diseases and infections by suppressing the immune system. Since most COVID-19 vaccinated countries are reporting high percentages of excess mortality not directly attributable to deaths from such disease, the NSEs of mRNA vaccines on overall mortality should be studied in depth.

1. Introduction

Human vaccines were created to protect against infectious diseases such as measles, smallpox, polio, and tuberculosis. Edward Jenner (1749–1823) is generally referred to as father of vaccination technology, as his smallpox vaccine heralded the era of vaccination as a major preventive therapeutic strategy, which eventually culminated in the eradication of smallpox [1,2]. It is often not highlighted that Jenner's small pox vaccine and all those who followed fitted within the framework of the "magic bullet" concept in chemotherapy given later by Paul Ehrlich (1854–1915) [3]. Jenner's first use of cowpox virus clearly indicated that vaccines can result in collateral advantages in diseases other than those which results from the pathogen, which is used for the design of the vaccine. The functioning of the biological world has classically been viewed and interpreted in terms of the biological specificity at the cellular and molecular levels [4]. In the context of enzymes and antibodies, the specificity concept changed to accommodate "cross-reactivity". Recognition of this trait quite early has resulted in our not

* Corresponding author.
E-mail addresses: alberto.rubio@sems.udg.mx (A. Rubio-Casillas), cesar.rodriguezq@academicos.udg.mx (C.M. Rodriguez-Quintero), lradwan@kau.edu.sa (E.M. Redwan), mn7gupta@gmail.com (M.N. Gupta), vuversky@usf.edu (V.N. Uversky), info@merogenomics.ca (M. Raszek).
[1] Current address: 508/block 3, Kirti Apts., Mayur Vihar Phase 1 Extension, New Delhi 110091, India.

https://doi.org/10.1016/j.vaccine.2023.12.060
Received 29 August 2023; Received in revised form 16 November 2023; Accepted 20 December 2023

especially concerning ourselves with immune response to cowpox virus protecting against smallpox virus.

The "lock-and-key" hypothesis has been the anchor of the one structure – one biological function paradigm, which has had overarching impact on our views on the way biological specificity operates in both *in vivo* and *in vitro* worlds of biological systems. Over the time, all this has turned out to be over-simplification. For example, proteins can be highly non-specific as seen in the phenomena of protein promiscuity and moonlighting. These phenomena have been exploited in drug designs and have led to the concept of drug repurposing [5–8]. Hence, it is not surprising that even vaccines turn out to be non-specific in the sense of influencing immune responses of the diseases for which they were not designed. Just to be unambiguous, these non-specific effects are not based upon cross-reactivity of antibodies etc. They are seen in diseases which are, unlike cowpox and smallpox, quite unrelated.

In more recent times, these non-specific effects have assumed great importance with wider perspectives. This overview is about the lack of specificity observed with many vaccines which is generally described as ''Non-specific effects'' (NSEs) of vaccines. We also discuss that while looking at NSEs, we get drawn into the hugely controversial and debatable issue of using live attenuated viruses vs. killed (inactivated) viruses as vaccines. This debate has not still ended and continues to impact policy decisions in many countries in the world. This debate started seriously in the case of Polio and now has got enmeshed with the discussion on NSEs of vaccines. NSEs of vaccines have also raised few other questions, which merit a closer attention. We hope that an updated information and a critical look at NSEs in this review would be helpful in future vaccination programs.

2. Methodology

Research reports from the last 40 years were compiled. PubMed was searched for the epidemiological and immunological studies on the non-specific effects (NSEs) of the most common human vaccines. To this end, we used the "non-specific effects of child vaccines" and "non-specific effects of vaccination" search terms to find corresponding articles. These searches generated 345 results, from which most review articles and research studies not related to the human vaccines were excluded. Resulting set of 196 paper was analyzed and the results of this analysis are included to this review.

3. Impact of NSEs on overall benefits of vaccination

Anecdotal data from the past indicates that the smallpox vaccine decreased the probability of developing several illnesses [9]. As early as in 1931, Albert Calmette, co-inventor of the Bacillus Calmette-Guérin (BCG) vaccine noticed that "The general mortality of 8,075 vaccinated children exposed to tuberculous infection, aged from one month to one year, controlled by 114 dispensaries, has been 4.6 %, whereas in non-vaccinated children of the same age, living under similar conditions, it is at least 16 %, and often exceeds 25 %." Based on these observations, he wondered: "does the harboring of BCG, followed by its digestion and elimination, confer on the organism a special aptitude to resist those other infections which are so frequent in young children?" [10]. The Russian virologist Voroshilova carried out extensive studies in the 1960s and 1970s on live enteroviruses, which included the oral polio vaccine, and discovered that they dramatically decreased the probability of contracting influenza [11].

In the 1980s, Danish scientists performed systematic research into the effects of routinely administered infant immunizations on overall health, the conclusion was that the majority of vaccines had an impact on overall mortality that was greater than what was caused by the disease they were intended to prevent. The phrase "non-specific effects" of vaccines was used to describe such outcomes [12]. In simple terms, vaccination may have an impact on illnesses that it is not intended to protect [12].

The standard procedure for evaluating novel vaccines has been disease-specific: does it produce clinically effective protection against the targeted infection and generate protective antibodies or cellular immunity? This belief about vaccinations is fading away, as more epidemiological [13–15] and immunological [16–18] investigations identified either positive or negative non-specific effects (NSEs) of vaccines (also known as heterologous or un-targeted effects). The mortality rate for children has significantly dropped since 1990. The number of children under the age of five who died globally has reduced from 12.8 million in 1990 to 5 million in 2021. Globally, the mortality in kids less than five years old has fallen by 59 %, from 93 fatalities per 1000 live births in 1990 to just 38 in 2021 [19].

A portion of this reduction can be attributed to a decrease in pediatric ailments that can be prevented, many of which are frequently credited to the success of vaccinations [20]. Vaccines are developed to defend against particular pathogens [21], but they can also have additional effects because live vaccines can boost immune function and reduce mortality in ways that go beyond what can be elucidated by just halting a specific infection [16,22–24]. Because of these positive NSEs, live vaccinations might have had a greater impact on the drop in childhood mortality than is typically thought [25]. Numerous studies suggest a pattern in which live vaccines (like the smallpox vaccine, the Bacillus Calmette-Guérin (BCG) vaccine, the measles vaccine (MV), and the oral polio vaccine (OPV) enhance immunity to vaccine-unrelated infections, primarily sepsis and pneumonia, and thereby decrease global mortality far beyond what was anticipated from preventing the disease they were designed for [26].

The fact that these live vaccines protect against both target and non-target illnesses confers a greater beneficial effect. In opposition, non-live vaccines, such as "diphtheria-tetanus-pertussis (DTP) vaccine, the pentavalent vaccine for DTP, hepatitis B virus (HBV), and Haemophilus influenzae type b, the inactivated polio vaccine, single hepatitis B virus (HBV) vaccine, the RTS,S/AS01 Malaria vaccine, and the H1N1 influenza vaccine" enhance vulnerability to diseases not related with the vaccine, particularly in females. So, non-live vaccinations may protect against the target disease but have unfavorable effects by making people more susceptible to infections that are not the target [26]. Current vaccination guidelines are solely focused on the disease-specific results of vaccines, which frequently depend on assessments of the potential for preventing targeted illnesses. Nevertheless, it should be also investigated if vaccines change susceptibility to other illnesses [25].

4. Live and killed pathogens and other vaccine designs

Broadly, the various designs of vaccines, which are in use, are as follows [27,28]. An important class is where live but attenuated organisms have been used. Viruses of measles, mumps and rubella (together to form MMR vaccine), yellow fever, varicella-zooster, and Sabin's polio vaccine are examples of this. The classical Bacille Calmette-Guérin (BCG) vaccine for tuberculosis also uses a live nonvirulent strain of *Mycobacterium bovis*. The organisms, when used as a vaccine in such cases, do proliferate and provoke both innate and adoptive immune responses. Hence, these vaccines are not advisable for using with immunocompromised persons. There is also a very small danger of virus (by reversion to the virulent form) actually causing the disease. Such outcomes are accepted with the philosophy of risks outweighing the benefits. As we discuss later, the benefits seem to extend beyond the expected one of the protection against the specific disease caused by that particular pathogen in such vaccines.

Inactivated or killed viruses of rabies, polio (Salk's vaccine), and influenza vaccine are examples of the other kind. This review largely focusses on these two kinds, in which the vaccines act as complex antigens. Inactivated toxins (toxoids) are also used as antigens in vaccines for diphtheria, tetanus, and new cholera vaccines. Vaccines for meningococcus, pneumonococcus, haemophilus, and new vaccine for typhoid use capsular polysaccharides as the antigens. Hepatitis B vaccine is

based upon surface antigen. The last 3 kinds, based upon subcellular fragments as the antigens (rather than the whole organisms) are often called subunit vaccines. Diphtheria-Tetanus-Pertussis (DTP) vaccine consists of toxoids of diphtheria and tetanus bacterial toxins combined with either nonvirulent pertussis bacteria (DTwP) or antigens from tetanus bacteria (DTaP). Another combined vaccine is the pentavalent vaccine for protection against five killer diseases – diphtheria, pertussis, tetanus, hepatitis B, and Hemophilus influenza type B (Hib) bacteria.

In addition, there are conjugate vaccines such as one for meningitis where the polysaccharide antigens are coupled with the diphtheria toxoid as a carrier to ensure better immune (T-cell) response. There are quite a few other experimental designs exemplified by DNA vaccines. Vector vaccines suddenly occupied a center stage when accelerated approval for mRNA vaccines was given during COVID-19.

5. Live vaccines induce beneficial NSEs

An interesting pattern appeared in which the effects of live attenuated vaccines and non-live vaccines differed. The live attenuated vaccines have generally positive non-specific benefits that are noticeable when they are the most recent immunization [26]. For instance, African children who were injected with live vaccines had much lower all-cause mortality than children who did not, and this disparity cannot be explained by variations in mortality resulting from the infection that the vaccine is intended to protect [26].

Since infectious diseases are the primary reason why people die in such environments, vaccines may reduce the susceptibility to independent infections or their seriousness, and in cases in which studies have been able to categorize by cause of death, investigations have demonstrated a specific protective action against infectious diseases [29,30]. For four live vaccines, all-cause mortality that is lower than anticipated has been consistently found [26], and randomized studies have mainly confirmed the positive non-specific outcomes of the BCG vaccine [29,31], the measles vaccine [32,33], and the oral polio vaccine [34].

5.1. The oral polio vaccine

Salk's vaccine was developed first and licensed in USA in 1955. It was an injectable preparation of inactivated polio virus. In 1960, Sabin's oral vaccine, which was an attenuated preparation of the virus, was also licensed and soon became to be used more widely in most of the countries. Unlike Salk's vaccine, it provided intestinal immunity as well [35–37]. Ideally, attenuation should stop the possibility of reversion to the virulent form but rare cases of vaccine-assisted polio (due to possible reversion or immunocompromised candidates) and vaccine-derived (due to recombination with other enteric viruses) have been reported [37].

It was reported that the oral polio vaccine (OPV) specifically decreased diarrhea morbidity and mortality in Latin America when it was first given in the 1960s [38], and investigations in the Soviet Union demonstrated that the OPV had a prophylactic impact on respiratory infections [11,39]. Additionally, some investigations from Bangladesh, Burkina Faso, Ghana, and Guinea-Bissau have discovered that OPV was linked to significantly lower rates of child mortality [40–43]. Campaigns administering only OPV dramatically decreased the probability of passing away from respiratory illnesses by 62 % post-natally (1–35 months) according to a recent study conducted in Bangladesh [44].

An investigation from Denmark reported a similar advantageous result. Investigators looked at the rate of admissions for infectious diseases among kids who received the live attenuated OPV as their latest vaccination versus infants who received the inactivated diphtheria-tetanus-pertussis-polio-Haemophilus influenzae type b vaccine (DTaP-IPV-Hib) or the live measles-mumps-rubella vaccine (MMR). Similar to the MMR vaccine, having the OPV vaccine as the latest immunization was linked to reduced admissions for lower respiratory infections [45]. In Finland and Bangladesh, randomized control trials (RCTs) contrasting the OPV with the inactivated polio vaccine (IPV) discovered that the OPV safeguarded against upper respiratory infections and diarrhea [46,47].

When BCG, which is frequently administered along with the OPV, or the MV was the last vaccination administered, these protective effects have been noticeably beneficial [13,14,48]. The combined injection of the OPV and the diphtheria-tetanus-pertussis (DTP) vaccinations, however, has not been linked to a lower mortality rate in multiple studies from West Africa [13–15,48,49]. Because DTP and OPV vaccines are generally given jointly, it was challenging to discriminate between their individual effects [50].

The researchers used the sporadic scarcity of the DTP vaccine in Bissau city to evaluate the distinct influence of OPV on mortality at the pediatric hospital because the DTP vaccine had been inconsistently provided in Guinea-Bissau. The case fatality ratio (CFR) was 0.29 (95 % confidence interval (CI) 0.11–0.77) for 719 infants under the age of five whose vaccination cards were evaluated when admitted and who had not received a measles vaccination yet. Only receiving the OPV vaccine was linked to a case fatality rate of 6 %, as opposed to 15 % for kids who had the DTP and OPV vaccines together [50].

The outcomes of administering live attenuated vaccinations simultaneously were studied in two RCTs. One compared BCG and OPV to BCG alone. Until participants received campaign OPV, BCG, and OPV immunizations were linked with a 32 % (range 0–55) decrease in newborn mortality [34]. In contrast to receiving only the MV, the co-administration of OPV and the MV decreased diarrhea morbidity in the other RCT [47,51]. In Bangladesh and Finland, RCTs comparing OPV and inactivated polio vaccine (IPV) discovered that OPV was linked to a decreased incidence of otitis media and diarrhea [46,47]. These findings cast doubt on the concept that OPV only prevents polio infection and raise questions about the decision to substitute the OPV with the IPV [47]. These multiple observations, although most based upon epidemiological studies, indicated that at least in the case of polio vaccine, the oral one was a better choice. The results with respect to the other vaccines also indicate that, in general, live vaccines are superior to the vaccines based upon inactivated viruses.

5.2. The Bacillus Calmette–Guérin (BCG) vaccine

Tuberculosis (TB) vaccine, also known as BCG, is a live attenuated vaccine and it is one of the most often used today, having been administered more than 4 billion times worldwide, plus an extra 100 million neonatal BCG immunizations annually [52]. Unexpectedly, epidemiological research soon after the BCG vaccine's debut in the 1920s showed that it decreased child mortality regardless of its impact on tuberculosis (reviewed in [53,54].

Children who received the BCG vaccine experienced a 50 % decrease in overall mortality, according to various observational surveys conducted in West Africa. The result was too significant to be attributed to tuberculosis immunity alone [14,55,56]. Comparable effects were found in other nations, and lately, they have been confirmed by randomized controlled trials (RCTs) and a *meta*-analysis of three RCTs [29,57,58]. The induction of protection against unrelated pathogens seems to be the cause of the BCG-induced decrease in infant mortality [52].

According to some investigations [59–61], the BCG vaccine has positive NSEs on child survival, which are more pronounced in females [62]. The campaign to prevent tuberculosis (TB) was the initial primary endpoint of BCG studies conducted in the US and the UK in the 1940s and 1950s, and a reanalysis of those trials revealed that BCG immunization was linked to a 25 % decrease in non-TB and non-accident mortality [53,54]. A further mechanism through which BCG immunization can safeguard babies from sepsis was just uncovered. The BCG vaccine stimulates the growth factor granulocyte colony-stimulating factor (G-CSF) a few hours after its injection, which consequently triggers a mechanism termed "emergency granulopoiesis", that boosts the creation

of neutrophils prepared to fight pathogens [63]. The characteristics of this rapid reaction to the BCG vaccine provide solid proof for the epidemiological evidence that the BCG vaccine can safeguard infants after a few days after its administration [30,64].

5.3. The measles vaccine (MV)

There is a growing consensus that the measles vaccine (MV) causes advantageous NSEs. According to studies on the introduction of MV in Africa, mortality decreased by 50 %, which was greater than the predicted prophylaxis of ± 10 % of measles-related deaths [65,66]. Later observational research and randomized controlled trials (RCTs) confirmed these results (reviewed in [24,67]). A "booster" dosage may also enhance the favorable NSEs for several live-attenuated vaccines [67–71]. In 1963, an RCT in West Africa showed that 27 in the group of control children who were immunized with the Diphtheria-Tetanus-Pertussis (DPT) vaccine had three deaths reported, whereas, in the cohort of 26 young children who had received the MV, there were no deaths when monitored for 18 months [72].

The Kasongo Project in Zaire, which was completed in 1981, produced the first prospective cohort study. The research showed that measles immunization led to a 48 % relative decrease in global infant mortality between 7 and 35 months of age. The total mortality rate was found to have decreased by 3.0 % according to the same study, yet the total decrease was greater in young children [73]. More significant death reductions have been attributed to the implementation of the measles vaccine than those accounted for by preventing measles infection and its prolonged repercussions in numerous countries, including Guinea-Bissau [13], Bangladesh [74], Senegal [75,76], and Haiti [77]. What is the support for the outstanding argument that the Schwarz vaccine's normal doses decrease mortality from diseases different than measles? First off, while measles per se accounts for 10 % of infant deaths worldwide, the vaccine lowers mortality in underdeveloped nations by at least 30 % [74]. Second, compared to unvaccinated children, immunized children without measles had a much lower mortality rate [13,78]. Girls had a higher decline in non-measles mortality than boys did [74,76].

The Bandim Health Project kept records of all kids from the studied region who were admitted to the pediatric section of the major hospital in Bissau, Guinea-Bissau, between 1990 and 1996. 2079 hospitalized kids from the Bandim study area, ranging in age from 1.5 to 17 months, were included in the research. The case fatality ratio registered for measles-immunized infants versus un-immunized infants was 0.51 (0.27–0.98), with females benefiting significantly more than boys (test of interaction, $p = 0.050$). Children with pneumonia and suspected malaria responded most favorably to the measles vaccine (MR = 0.28 (0.07–0.91) and MR = 0.40 (0.13–1.18), respectively). In summary, measles vaccination was linked to a decreased risk of death from non-measles infections, with a higher positive effect for girls than boys [79]. The favorable outcome of vaccination protocols indicates that measles can be eradicated [80]. It has been advised to continue administering the MV to children in low-income nations even after the illness is eradicated because there is compelling evidence that it offers protection against death from diseases other than measles [81].

5.4. The smallpox vaccine

Following the discovery of the last case in Somalia in 1977, smallpox was declared extinct in 1980 [82]. Older People in Guinea-Bissau's city and rural areas were examined for smallpox scars to determine whether immunization against smallpox continued to have health advantages after the disease had been eradicated. Results were significantly above what was anticipated [83,84]. A 40 % (95 %CI: 13–59 %) decrease in mortality for persons over 25 years of age was found in the urban trial when smallpox scars were compared to no scars over the following 4 years [83]. The mortality decrease in the rural study was 78 % (39–92 %) [84]. Scientists examined the vaccination history described on the school vaccination certificates of youngsters in Copenhagen to look into the effects of halting the BCG and smallpox vaccinations in Denmark. They centered on the 47,622 kids born between 1965 and 1976, the cohort that underwent the simultaneous discontinuation of the BCG and smallpox immunizations [85].

Being immunized with both smallpox and BCG vaccines was linked to a 46 % (19–64 %) lower mortality rate from natural causes between the start of school and the age of 45, after controlling for socioeconomic status, year of birth, and sex. Receiving smallpox and/or BCG was linked to a 34 % (4–54 %) decreased chance of contracting HIV-1 in a different study that used data from Guinea-Bissau and Denmark [86]. Thus, in both high- and low-income countries, the smallpox vaccine was linked to positive NSEs that persisted for decades after the smallpox extinction. No study has documented harmful NSEs following smallpox immunization. Therefore, it is likely that discontinuing vaccines against measles, polio, and smallpox had a detrimental impact on survival [87].

6. Non-live vaccines induce negative NSEs

As opposed to live vaccines, non-live vaccines, while protecting against the disease for which they were designed, in some circumstances may also enhance the risk of other diseases, especially in females [88]. For instance, in low-income environments, girls who received the non-live diphtheria-tetanus-pertussis (DTP) vaccine died at a rate that was 1.5–2 times greater than girls who did not receive the vaccine, and a comparable enhanced risk above that of male recipients of the DTP vaccine [88]. The DTP vaccine [26], the H1N1 influenza vaccine [43], the pentavalent vaccine [89], the DTP plus hepatitis B and Haemophilus influenzae type B vaccines [90], the hepatitis B vaccine [90], the inactivated polio vaccine [91], and the RTS,S malaria vaccine [92] are six non-live vaccines for which this pattern has been identified.

Whenever a certain vaccine is the most recent, these non-specific side effects are more evident. Little research has been done on how long non-specific effects last if no further vaccines are administered because the majority of research has been conducted on children who were frequently vaccinated. Nevertheless, non-specific effects appear to persist for at least six months [26,93] and occasionally for years [85,94]. The non-specific outcomes of vaccines were first discovered in low-income nations with high infectious disease mortality, but non-specific outcomes were reported in some investigations from high-income nations that assessed the risk of hospitalizations for infectious diseases that are not specifically targeted [95,96], confirming that vaccines can influence the likelihood of contracting unrelated infections [97]. Studies from Europe and the USA demonstrated that the live MV and the BCG vaccine reduced hospital admission for unrelated diseases in high-income nations, whereas the non-live DTP vaccine increased it [95,96].

6.1. The DTP vaccine

The diphtheria-tetanus-pertussis (DTP) vaccine has a high level of effectiveness in lowering morbidity and death from diphtheria, tetanus, and pertussis. However, observational studies have discovered that, despite this specific protective benefit, a link between whole-cell DTP immunization and elevated total mortality when DTP is the last vaccine applied [91,98–100]. Girls are more likely to be affected by this effect, which may be linked to higher risks of contracting unrelated diseases [88,89,101,102]. Girls' cytokine reactions to irrelevant stimuli are reduced in West African newborns who received the whole-cell DTP vaccine [103]. Despite protecting against particular diseases, the implementation of the diphtheria-tetanus-pertussis vaccination (DTP) in the 1980s was linked to greater total mortality [104–106].

Later investigations comparing the mortality of DTP-vaccinated infants with that of DTP-unvaccinated children corroborated deleterious NSEs, particularly in girls [14,91,99,107,108]. According to the WHO review of NSEs, there is insufficient evidence to confirm or

disprove whether DTP-related NSEs are beneficial or harmful [24,109]. Nevertheless, the WHO report included trials with severe survival bias; whenever the *meta*-analysis was limited to investigations with continuous follow-up and vaccination status validation, children who received the DTP immunization had mortality rates that were twice as high as those of infants who did not receive the DTP vaccine [100].

The World Health Organization (WHO) advises administering BCG followed by DTP, and potential negative effects have been reported in Senegal [60], Guinea-Bissau [99], Gambia [110], and Malawi [111]. In contrast, kids who were immunized with BCG and DTP at the same time as their first vaccinations had a lower death rate than kids who began the immunization plan with BCG-only and then DTP according to further research from Senegal [60], Bangladesh [112], and India [113]. According to some investigations, administering BCG and DTP simultaneously lowers mortality compared to the immunization plan suggested by the WHO, which administers BCG first and then DTP/Penta [111].

Children in Bangladesh who were injected with the DTP1 vaccine (first dose) after receiving BCG had a mortality rate that was two times greater than those who were immunized with DTP1 and BCG at the same time. In comparison to children who initiated their immunization plan with BCG first or DTP1 first, infants in rural India who were injected with BCG and DTP1 at the same time had a fourfold decreased mortality rate until 9 months of age [112]. Similar to this, a Senegalese study discovered that children who received BCG and DTP1 at the same time had lower mortality compared with those who received BCG or DTP1 first [60].

Combining data from these three studies, the Strategic Advisory group of Experts on Immunization (SAGE) evaluation predicted that giving BCG and DTP at the same time may result in mortality that is 48 % (20–66 %) lower than giving BCG first and then DTP [114]. While other studies revealed few deaths, they were consistent with the notion that giving BCG and Penta at the same time rather than BCG then Penta would lower mortality in infants between the ages of 6 weeks and 9 months. It was discovered in that study that if the Penta vaccine was the most recent vaccine administered, it was linked to higher mortality for girls than for males [115].

Ten countries, including Bangladesh, have recently shown higher than predicted rates of under-5-year-old female mortality [116,117], which they attribute to differing treatment of girls and boys [118–120]. The sex-differential under-5-year mortality, however, may have at least two causes: sex-differential therapy and immunizations. It was found that while BCG was the most recent vaccine delivered, girls died at lower rates than boys, but when Penta was the most recent vaccine applied, girls' mortality was greater than in boys. The idea that some additional female deaths are caused by Penta vaccination could be supported by the reversal of the female-male mortality rate ratio (F/M MRR) from BCG as the most recently applied vaccine to Penta as the most recently applied vaccine [115].

The existing data demonstrate that, in low-income countries, the DTP vaccine could cause the death of more infants from other illnesses than it prevents from diphtheria, tetanus, or pertussis. Although a vaccine defends kids from the disease it is intended to prevent, it may also make them more vulnerable to unrelated infections [104]. This study showed that in comparison to children who had not gotten the DPT vaccine, mortality was five times higher among kids who had received the vaccine. Unexpectedly, the same study discovered that children who were immunized only with DPT and no OPV had a mortality rate that was ten times greater [105].

6.2. The influenza vaccine

An H1N1 influenza outbreak started in Mexico and the USA in March and April of 2009. On June 11, 2009, the virus spread in many countries and the WHO proclaimed it as a pandemic, because, according to that organization, it reached Phase 6, the highest level [121]. Vaccine research was quickly started [122], and a vaccine was ready in August 2009 [121]. Researchers in Guinea-Bissau examined the hypothesis that children who received the H1N1 vaccine would consult with doctors more frequently than children who did not receive the vaccine, despite having immunity to the H1N1 influenza. Although Guinea-Bissau did not perform any influenza monitoring, data from Senegal, a nearby nation, showed that H1N1 influenza was present in the area in October 2010 [123].

If H1N1 influenza was prevalent, vaccinated kids (who are less susceptible to H1N1 influenza) would be predicted to have a smaller number of influenza consultations with physicians. Children who received the H1N1 vaccine should have experienced a greater drop in consultation rates if the vaccine had no NSEs. Since the opposite happened, researchers concluded from those results that the non-live H1N1 vaccine, along with other non-live vaccines, could render children more susceptible to other infectious diseases [124]. This is also applicable to Hepatitis B [90], inactivated polio [91], and DTP vaccines [100], all of which have resulted in increased overall mortality rates for females notwithstanding conferring immunity to the intended illnesses [124]. Importantly, the study found no evidence that a possibly detrimental outcome of H1N1 was greatest for females, contrary to what research for other non-live vaccines demonstrated [124].

The impact of H1N1 influenza vaccines on overall mortality has not been thoroughly studied. One of them recently discovered that children monitored in Guinea-Bissau's randomized vaccine trials had higher age-adjusted mortality rates following the H1N1 immunization campaign [68]. In Kenya, hospital employees who had received the trivalent inactivated influenza vaccine (including the H1N1 strain) had higher rates of self-reported respiratory symptoms and absence from work than hospital personnel who had not received the vaccine [125], and children in a small randomized controlled trial in Hong Kong who had received the vaccine suffered a higher incidence of respiratory illnesses other than influenza [126].

The biological process underlying NSEs is unresolved. Though mechanisms for heterologous immunity have been identified in both the innate and adaptive immune systems, according to an increasing number of studies [16,17,127]. It is interesting to note that the live BCG vaccine and the non-live trivalent seasonal influenza vaccine have recently been contrasted for their impact on the innate immune system. According to the research, the trivalent influenza vaccine causes negative NSEs, which is in contrast to the overall immunostimulatory effect that was seen after BCG immunization. Influenza vaccination was linked with reduced IFN-gamma and IL-1beta production in response to heterologous pathogen stimulation, or "innate tolerance," which may be a sign of negative NSEs [128]. More investigation into the mechanisms of non-live vaccine NSEs is required [124].

6.3. The malaria vaccine

According to calculations, there were 247 million clinical cases of Malaria and 619,000 fatalities from it in 2021 [129]. 10 % of all pediatric fatalities in Africa occur in children under the age of five due to malaria. In phase III clinical studies with young children, the RTS,S/AS01 malaria vaccine was found to be 18 to 36 % effective in preventing clinical malaria [130]. The vaccination may be somewhat effective against the disease, but it has little effect on total mortality [92]. The non-live RTS,S/AS01 malaria vaccine increased by two-fold the all-cause mortality in African girls in a randomized study. Additionally, there was a propensity for RTS,S to be connected to a lower risk of malaria mortality in boys (malaria mortality ratio, 1.07 [0.52 to 2.18]), but not in girls (malaria mortality ratio, 1.90 [0.82 to 4.37]) [92].

Contrary to popular belief, RTS,S was not related to a decrease in malaria deaths, but rather to a case fatality ratio that was twice as high in children who contracted severe malaria [131]. According to the WHO, the increased death rate among girls "could be due to chance" and was "largely due to the low female mortality in the control arm", despite a P value of 0.0006 for girls and a mortality rate of 2.4 % in girls versus

1.8 % in boys following RTS,S vaccination (risk ratio, 1.33 [1.02 to 1.74]). The WHO may be right to speculate that this discovery was the result of randomness, but these data point to a call for precaution and more investigation. Before RTS,S is included in normal immunization regimens, it must be established whether RTS,S/AS01 enhances mortality in girls, and plausible pathways involved should be also investigated [92]. Findings from numerous trials of non-live vaccines, such as DTP and the inactivated polio vaccine (IPV), demonstrate that females are more adversely affected by these non-live vaccines than boys are [15,91]. The higher female mortality following RTS,S/AS01 vaccination should therefore not be considered an unexpected discovery that was randomly produced [92].

6.4. The hepatitis B vaccine

Research performed in high mortality areas has demonstrated that the DTP, Influenza, and Malaria vaccines were linked with enhanced mortality of girls in comparison to boys. Hence, scientists looked into how sex-specific variations in mortality in Guinea-Bissau were also related to the hepatitis B vaccine (HBV). A sub-cohort of 876 kids received HBV at 7½, 9, and 10½ months of age as a part of a measles vaccination randomized study. The researchers wanted to determine if this cohort's death rate and female-male mortality ratio changed from the previous and subsequent birth cohorts recruited in the same study [90].

The mortality rate (MR) for children aged 7½-12 and 1½–7½ months in groups that did not get the HBV was 0.97, while the MR for the group that was injected with the HBV at 7½ months was 1.62. Children who got the HBV vaccine between the ages of 7½ and 12 months who were participating in the measles immunization experiment had higher mortality than both the prior and following groups who were not immunized with the HBV vaccine (MR = 1.81; the effect was especially substantial for girls (MR = 2.27). The female-male MR between 9 and 24 months of age was 2.20 in the cohort that was injected with the HBV and MV, as opposed to 0.96 among trial subjects who were only given the MV. Longer monitoring showed no change in these trends. In conclusion, although HBV vaccination protected children against this disease, it induced higher mortality in girls, as reported for the other non-live vaccines [90].

7. The effect of vaccination sequence on the mortality rate

According to the present vaccination model, the order and combination of the vaccines do not really matter; for instance, it is of little significance if DTP is administered before MV, MV is administered before DTP, or DTP and MV are administered simultaneously in terms of pertussis or measles immunity [26]. Nonetheless, studies on DTP, inactivated polio, and hepatitis B vaccines, indicate that non-live vaccines injected after live attenuated vaccines impair the positive nonspecific effects of the live attenuated vaccines [26]. DTP given after MV, for example, is linked to twice as much mortality as when MV is given before DTP [26,114,132].

Observational investigations [15,42,89,99,107,132,133] and RCTs [59,108] showed that the sequence of immunizations was crucial since NSEs were most significantly correlated with the most recent vaccination. Inactivated vaccines given after medium- or high-titer MV have been contrasted with standard-titer MV following an inactivated vaccine in RCTs. According to a *meta*-analysis of studies, being immunized with an inactivated vaccine after a live MV was linked with a mortality rate ratio (MRR) of 1.38 (95 % CI 1.05 to 1.83) compared to being immunized with a live MV after an inactivated vaccine, with the unfavorable effect being especially high in females [57]. When DTP was given after MV, more females died, according to other research [59,91].

The number of injections of the DTP vaccine increased the relative female-male mortality rate ratio (F/M MRR) by 50 % (95 % CI: 10–106 %), however, the ratio decreased thrice after receiving the MV. When DTP was administered following MV, it considerably spiked once more [132]. When Tdap (the use of capital letters in this acronym denotes the presence of full-strength antigen concentration for Tetanus bacteria in the vaccine. The lowercase "d" and "p" indicate that a fewer concentration of diphtheria and pertussis antigen is used in this vaccination. The "a" in Tdap stands for "acellular," which denotes that the component of whooping cough only consists of some of the bacteria rather than the entire bacteria) and BCG are administered concurrently, their nonspecific immunological effects interact, indicating that the sequence in which vaccines are administered is essential for the result of nonspecific immune consequences.

This is consistent with epidemiological investigations that show the BCG vaccine, given concurrently with or after DTP, is linked with decreased mortality, as opposed to receiving DTP after BCG (as per the recommended schedule by the WHO), implying that the most recent vaccination given is of the utmost importance in determining the positive or negative nonspecific effects [22,24,112]. This reinforces the hypothesis that giving live-attenuated vaccines to young children as their last immunization can elicit protective, non-specific benefits [134].

Compared to in-sequence immunizations, out-of-sequence vaccinations were linked to greater mortality. It was discovered that DTP not followed by MV was associated with increased mortality and that children who received their immunizations out of order had a higher mortality rate compared to those who received them in order. As a result, the current evaluation criteria, which place a strong emphasis on DTP3 coverage, may not maximize the effect of the vaccination scheme on children's health. Such findings suggest that more priority should be given to enhancing the MV coverage and receiving DTPs and MV in the suggested order [25].

To sum up, (a) there is an interplay between the gender-specific responses of the immune system and the sequence, in which vaccines are administered; (b) there is a viral interference and/or synergy involved when multiple vaccinations are administered. It may not be practical to gather such data but it would be useful to find out the effect of time-wise spacing of the administration of different vaccines. What would complicate matters is that different vaccinations have different immunological memories; (c) replacing a live vaccine with a killed vaccine either merely abolishes the positive NSEs or leads to negative NSEs. More on this later (the viral interference is discussed in this review elsewhere).

While we still do not have complete understanding of gender-specific responses by the immune system, it is by now clear that these differences are complex and operate in diverse ways. Klein and Flanagan (2016) have reviewed the differences in the gender-specific immune responses [135]. They note that adult females in general have higher innate and adoptive immune responses as well as are more susceptible to inflammatory and autoimmune diseases. Females, for example have higher $CD4^+$ and higher $CD4^+$ /$CD8^+$ ratios during immune responses as compared to males. They further report that age, reproductive status, nutritional status, microbiome compositions all affect these differences in gender-specific responses which are regulated by sex-specific genes and hormones [135].

Ortona *et al.* (2019) have summarized 19 publications in a special issue devoted to different aspects of gender-specific differences in immune responses [136]. Unlike Klein and Flanagan (2016) who covered several species [135], this entire set of articles (including both reviews and original research) focusses on humans [136]. The take home message, though, is similar – we need to understand the cross-talk between the endocrine (sex chromosomes and sex hormones) and immune systems better [136]. More recently, Sciarra *et al.* (2023) have specifically pointed out that "Androgens and progestogens mainly promote immunosuppressive or immunomodulatory effects, whereas estrogens enhance humoral immunity both in men and in women" [137]. Furthermore, they report that neutrophils, monocytes, mast cells and immature B and T cells express androgen receptor. On the other hand,

eosinophils, dendritic cells, NK cells and mature B and T cells express the receptor for this male hormone [137]. These kind of details would help us in understanding the complex correlation between gender, age and responses to vaccinations.

The gender specific differences in efficacy of vaccinations are also dependent upon parameters, such as number of booster doses (if any) and if the vaccine was live one or not. There is interesting and intriguing data with regard to DPT vaccine. The third injection of DTP (DTP3) is utilized as an indicator of the percentage of one-year-old children who survive after receiving three doses of the DPT vaccine and is commonly employed to evaluate the efficacy of the immunization system [138–140]. Children who had DTP3 as their most recent vaccination had a 66 % (32–109 %) greater female mortality rate than male mortality rate. Shifting from DTP1 to DTP3, the female-male MRR increased by 50 % (10–15 %). The female-male MRR decreased three times after standard MV vaccination compared to DTP; the reduction in the F/M MRR from DTP to MV was always lower for females than for men. The F/M MRR raised again if the DTP vaccine was administered after the MV [132].

For DTP and MV, opposite-sex-differential outcomes have been described in several prior research, including the SAGE review [24,99]. Girls' mortality was lower than boys' mortality in the first eight weeks of life whenever BCG prevails; after 3–4 months of age, when DTP was likely to be the prevailing vaccine, girls' mortality exceeded boys' mortality, according to research that compared girl and boy mortality rates by monthly age cohorts. Female mortality drops below male mortality after 9 months when MV predominates [22,110,113,141,142]. It is important to note that this pattern is not present when vaccination percentages are low or when vaccinations are administered at various times. For instance, in Niakhar, Senegal, where DTP coverage was poor and MV practically absent in the late 1990s, infant death rates were not greater for females than for males [60].

Two prior investigations revealed that the adverse outcome of DTP-vaccinated versus DTP-unvaccinated infants was exacerbated by further DTP doses [143,144]. The same phenomenon was discovered in other investigations of DTP [90] and pentavalent DTP-containing vaccines [141,142]. Boosting with live vaccines improves the favorable NSEs, according to prior work [70]. In opposition, since most non-live vaccines induce negative effects with boosting, this is essential because the WHO intends to administer more non-live vaccines in the 2nd year of life, e.g. booster DTP and booster RTS,S malaria vaccine.

It is unclear why the mortality is enhanced with each additional immunization [132]. When compared to one dose (DTP1), the Female/Male Mortality Rate Ratio considerably increased after three doses of the DPT vaccine (DTP3), demonstrating that repeated inoculation with non-live DTP vaccines further exacerbates the harmful NSEs for girls [132].

8. The influence of vaccine antigen concentration on the measles mortality rate

Significantly favorable NSEs have been linked to four live vaccines. An early intriguing observation was that the high antigen concentration (with more than $10^{4.7}$ plaque-forming units) in the high titer measles vaccine (HTMV), which is also a live vaccine, induced detrimental NSEs. In addition, the standard measles vaccine (MV), which had 10^3 to 10^4 plaque-forming units, induced more significant beneficial NSEs for females, whereas HTMV was linked to higher female mortality [12]. The MV is often administered between 12 and 15 months of age in high-income countries since seroconversion rates are greater at that age than in younger kids. The MV is often administered between 6 and 9 months of age, although many children in developing nations succumb to the disease before 12 months old [77].

Due to its significantly greater seroconversion rates than the regular doses of the Schwarz vaccine administered at 6 months, the World Health Organization recommended large doses of the Edmonston-Zagreb vaccine in 1990. This advice was then withdrawn after it was demonstrated that girls who received the high-titer Edmonston-Zagreb vaccine had a greater mortality rate than those who received the regular Schwarz vaccine. The girls did not contract more measles, and they did not die at a faster rate than children who were not inoculated, proving that the higher mortality was not the result of vaccine malfunctioning. The rationale appears to be that the high-titer Edmonston-Zagreb vaccine was not protective against death from illnesses other than measles (a consequence that was more pronounced in girls than boys [77].

The high-titer measles vaccine (HTMV) was discontinued by the WHO in 1992 because it was linked to a rise in female mortality [145]. The WHO advised HTMV to start at 6 months of age in 1989. When administered at this early age, HTMV proved protective against infection with measles in both boys and girls [146,147]. Nevertheless, HTMV compared to standard-titer MV at 9 months of age was linked with twice as much increased female mortality when children in Guinea-Bissau and Senegal were followed up to age 5 [15]. For boys, HTMV had no impact. The WHO withdrew the HTMV when a comparable pattern was discovered in Haiti [145].

It had not been clarified how an efficient live vaccine may be connected to increased female mortality. Finding an answer that matched all of the available facts took ten years. The non-live DTP vaccine had been linked in several studies to greater female mortality [13,91]. The majority of kids received doses of DTP or inactivated polio vaccine (IPV) after receiving HTMV, which was administered around 4–5 months of age. It was investigated if the non-live DTP and IPV vaccines injected after HTMV contributed to female mortality [15]. Female mortality was greater in Sudan, Senegal, and Guinea-Bissau if DTP/IPV was given after HTMV but not in the control group of children who were immunized with the MV at 9–10 months and were prone to acquire a non-live vaccine after MV [148]. Female mortality did not increase, when kids were not vaccinated with the DTP/IPV after HTMV [15]. Therefore, rather than HTMV specifically, the sequence of a non-live vaccine after early MV explained the enhanced female mortality. Numerous investigations conducted after the HTMV incident revealed that DTP and other non-live vaccines have been linked to greater mortality rates in girls compared to boys [15,88,91,99,148].

Presumably, the antigen concentration in the vaccine also has an important influence on the immune response not yet recognized. This concept has been excellently summarized with the following maxim "A little bit of vaccine does you good—but a lot of vaccine is not so good" [81].

A recent article describes our current understanding of the way antigen concentration in vaccines affects their outcome [149]. The vaccine dose affects both humoral immunity as well as cell-mediated responses. Higher antigen concentration supports maturation of B-cells leading to higher production of antibodies but lowers production of memory B-cells. Higher dose also adversely affects affinity maturation which leads to high avidity antibodies when an infection persists. The effects of vaccine doses on T-cells are more complex and operate via T cell receptor (TCR) stimulation. The antigen concentration not only affects the ratio of Th1/Th2; it also influences the "induction of Tregs, Th-17, T-follicular helper" etc. [149]. It is critical to remember in this context that unlike live vaccines, those with inactivated pathogens are reported to have no cell-mediated immune responses. So, vaccine dose is also an important factor in understanding the effect on the sequences and choices in the vaccination programs.

9. Immunological mechanisms for non-specific effects (NSEs) of vaccines

We had compared the live and inactivated viruses for the design of the oral vaccines in case of Polio virus. The key lessons from that historical case have mostly turned out to be general in nature. In this section, we amplify the mechanistic insights into the limitations of the non-live virus-based vaccines. The main attraction of these vaccines is their better safety. Hence efforts continue to overcome their limitations and

disadvantages. We will also briefly outline these strategies so that a balanced picture of the debate between the two vaccine designs emerges. The way of how an attenuated live vaccine acts is illustrated in Fig. 1 with the example of the live attenuated influenza vaccine [LAIV]. The vaccine is administered intranasally and results in a long-lasting immune response which includes innate immunity as well as adoptive immunity of both humoral and cell-mediated kinds [150].

The killed/inactivated viruses do not enter host cells and replicate. This results in them not being able to use class 1 MHC pathway for antigen presentation. The cell-mediated response and memory cells production is thus not significant. However, use of adjuvants is known to induce dendritic cells to act as antigen presenting cells (APCs) even for killed viruses and even improve T-cell responses [151]. Another approach which has helped in some cases is the use of conjugated vaccines [152].

Possible explanations for the NSEs of vaccines are now beginning to emerge from immunological investigations of innate immune training [18,22]. As discussed earlier in this review, live vaccines, such as BCG and vaccinia, elicit epigenetic alterations that train the innate immune system and increase immunity to unrelated infections. In opposition, non-live vaccines may promote "tolerance" that increases susceptibility to unrelated illnesses [153].

Tolerance is a critically necessary feature of the immune system [27]. Broadly described as non-responsiveness to an antigen, it prevents the immune responses against "self" antigens of the animal's own tissues. At mucosal level, it prevents recognition of diverse kinds of antigenic molecules produced by digestion in the food. Central tolerance results from deletion of the self-reactive immature B cells and T cells in bone marrow and thymus, respectively. Some lymphocytes directed against self-antigens, which escape clonal deletion or when exposed to low affinity antigens, develop peripheral tolerance. Both high zone tolerance (tolerance developed by B cells when high concentration of antigen is present) and low zone tolerance (induced in T cells when sub-immunogenic concentration of an antigen is there) are known. Tolerance can also result from antigen sequestration; failure in antigen presentation by APCs, which can be due to the absence of co-stimulatory molecules on APCs; secretion of immunosuppression directly by regulatory T cells or secretion of cytokines, such as IL-10 and transforming growth factor beta (TGF-β) by immune cells. Mucosal or oral tolerance [encountered with vaccines administered through oral or nasal routes] develops via dominance of Th2 cells along with secretion of suppressive cytokines and Treg cells [27].

Most pathogens enter our bodies through mucosal surfaces of the respiratory, gastrointestinal and genitourinary tracts [154,155]. Injected vaccines do not produce immunity right at these "ports of entry". At the same time, mucosal vaccines (those producing mucosal immunity) are capable of producing systemic immunity as such [156]. The licensed mucosal vaccines are exemplified by oral cholera vaccine, intranasal influenza vaccine, and oral vaccines against rotavirus and typhoid [157]. It is to be noted that apart from cholera one, all other vaccines consist of live attenuated pathogens.

Tarancón *et al.* (2020) have described that MTBVAC, a genetically modified form of *Mycobacterium tuberculosis* [Mtb], used as a live attenuated vaccine induced immunity against *Streptococcus pneumoniae* in a murine model [158]. MTBVAC, at a pre-clinical trial phase was known to protect against Mtb better than BCG (see Fig. 2). Their results

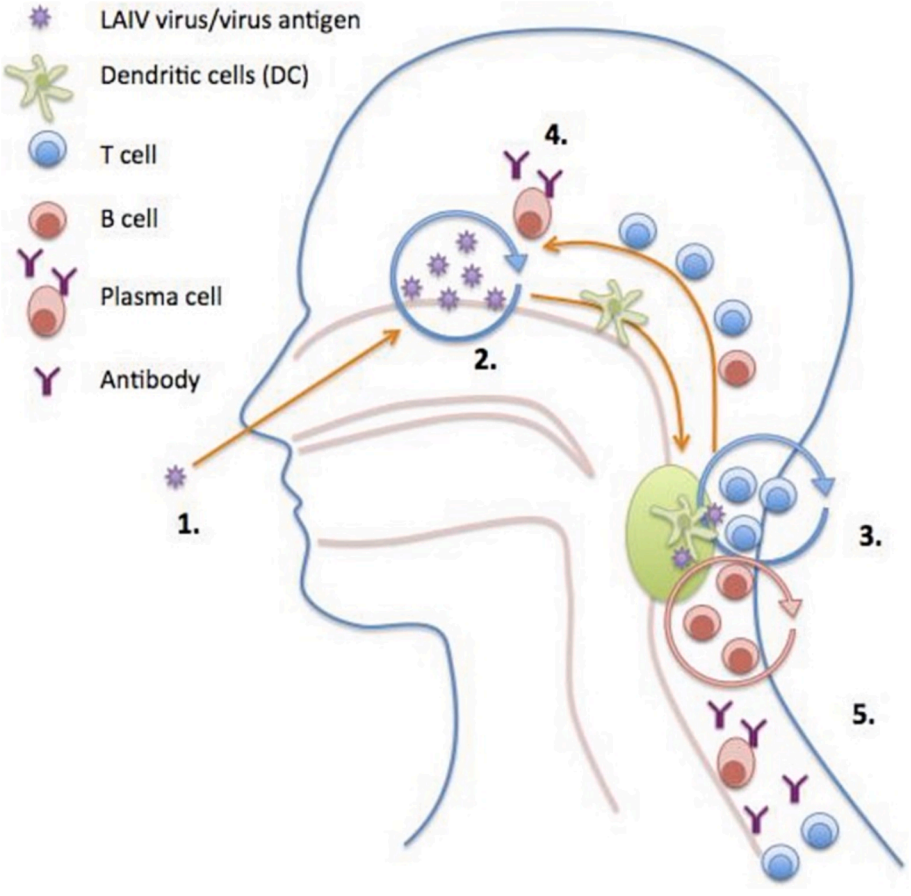

Fig. 1. Model of induction of immune responses after live attenuated influenza vaccination (LAIV). (1) Intranasal LAIV immunization; (2) Viral antigen is transported to the tonsils/adenoids by the Dendritic Cells (DCs); (3) Activation and proliferation of T and B cells in tonsils/adenoids with help from CD4 + T-cells. Affinity maturation of B cells; (4,5) Activated T and B cells home to site of infection and enter circulation. Plasma cells secrete antibody into the blood and at the mucosal surfaces. Reproduced from Sridhar et al. (2015) [150] under CC BY license.

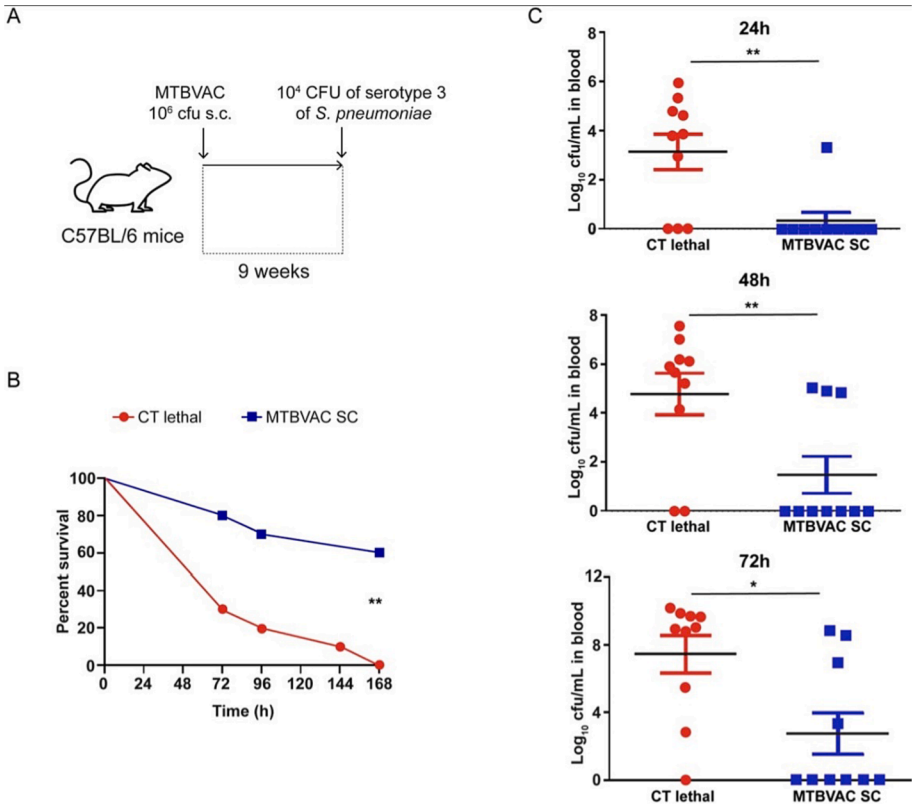

Fig. 2. Vaccination with MTBVAC protects against pneumococcal pneumonia. (A) Protocol of vaccination with BCG or MTBVAC and subsequent infection with *S. pneumoniae*. (B) Survival of mice subcutaneously (SC) vaccinated with 106 CFU of MTBVAC (blue squares) or placebo as lethal control (red circles) and infected by the intranasal route with 1 × 104 CFU of serotype 3 of *S. pneumoniae*. (C) Bacterial levels in blood at 24 h, 48 h and 72 h of the lethal control and MTBVAC vaccinated group. $p < 0.01$, Log-rank (Mantel-Cox test) for the survival experiment. $p < 0.05$ or $p < 0.01$ by two-tailed Student's *t* test; CT lethal: control lethal. Reproduced from Tarancon et al. (2020) [158] under creative common attribution license. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

showed that the trained immunity worked via induction of glycolysis and glutaminolysis and methylation of histones of the promoters of pro-inflammatory genes of monocytes. This is in line with the emerging picture of the interplay between cellular metabolism and epigenetic reprogramming during immunity induction [158].

From the beginning, the formation of memory B-cells and T-cells is believed to be the basis of vaccine action. Such memory cells are also formed during the actual infection, leading to frequently stated wisdom that natural infection is the best form of infection. In that sense, vaccines which are attenuated forms of live viruses come very close to the natural infection. The efforts to understand NSE have led workers to realize that memory is not limited to adoptive immunity mediated by the two lymphocytes [158,159]. Infection with a pathogen and even inflammation also leads to the memory cells associated with innate immunity—macrophages, natural killer (NK) cells, monocytes, and dendritic cells. These cells have been shown to undergo epigenetic reprogramming and "rewiring of cell metabolism" [159]. This memory of innate immune cells is part of what is now more frequently called "trained immunity". It is also now known that this trained immunity is not just relevant to vaccine action (or their NSE) but also involved in atherosclerosis, sepsis, and even cancer [158,159].

For immunosuppression, the transcription factor FOX P3 present on CD4 + cells turn out to be the master regulator. Among APCs, it is the dendritic cells, which play an important role in this context [160]. As manipulating tolerance is important for preventing tissue/organ graft rejection, strategies to over-ride tolerance continue to be developed [161]. Various antigen-specific tolerogenic vaccine designs have been developed some of which are at the human clinical trial level. This includes use of innovative adjuvants and nanoparticles in vaccine development platforms [160]. Pulendran *et al.* (2021) have provided a good overview of the updated information on role of adjuvants in specific vaccines [162].

For NSEs, it is hypothesized that both Th1 and Th2 immunity may be involved, as a live vaccine such as the MV induces Th1 and an inactivated vaccine like DTP induces a Th2 profile [47]. According to a study, after controlled human malaria infection (CHMI), a group of healthy participants who received the BCG vaccine exhibited increased NK cell and monocyte activation, which is correlated with decreased numbers of parasites in their blood. These results support the hypothesis that BCG vaccination could produce trained immunity with functional responses toward a different human pathogen *in vivo* [163]. Additionally, there is proof that BCG immunization lowers parasitemia in rodent malaria models [164–167], and in endemic locations, BCG immunization has been linked to lower malaria mortality [56].

Training of the innate immune system has been confirmed for live vaccines such as the BCG and smallpox vaccines [168], as well as the adenovirus-based COVID-19 vaccine [169], and could elucidate why they have favorable non-specific outcomes. On the other hand, various non-live vaccines (DTP vaccine [102], typhoid vaccine [170], and non-replicating smallpox vaccine [171] have been demonstrated to promote innate immune tolerance to unrelated pathogenic stimuli. The connection between non-live vaccines and greater vulnerability to other diseases may be explained by the enhanced innate tolerance toward other pathogens [96].

The DTP vaccine may enhance susceptibility to infections in females because it has been demonstrated to inhibit innate immunity and promote T cell exhaustion in girls but not in boys. Reduced post-vaccination pro-inflammatory responses to Toll-like receptor 4 (TLR4) stimulation in

DTP-vaccinated children suggest compromised innate immunity against gram-negative bacteria, while responses to TLR2, TLR5, and TLR7/8 were unaltered. This notion is further supported by the reduced levels of mostly type 1 interferon genes in DTP-vaccinated girls but not in boys. This is because the STAT1 pathway, which relies extensively on type 1 interferons, is an important pathway for "trained immunity" leading to altered innate memory [102].

The Tdap vaccine caused modifications in some immune cell subsets in peripheral blood, which likely caused the short-term activation of monocyte-derived cytokine responses and the short-term repression of T-cell responsiveness. Tdap suppressed nonspecific T-cell responsiveness three months after immunization and suppressed innate immunological reactions to unrelated pathogens. Ex vivo cytokine responses produced from re-stimulated peripheral blood mononuclear cells (PBMCs) after vaccination with a live vaccine (e.g., BCG) as opposed to a non-live vaccine (e.g., Tdap) may differ. In an *in vitro* model of trained immunity, it was discovered that a live smallpox vaccine produced trained immunity while the non-replicating Vaccinia Ankara promoted tolerance [171].

In conclusion, heterologous innate and T-cell-derived cytokine synthesis is affected in a nonspecific manner by the BCG and Tdap vaccines, and these outcomes overlap with one another. These findings are in line with the hypothesis that vaccination with an inactivated vaccine, such as DTP [102], suppresses heterologous immunity, while vaccination with a live-attenuated vaccine, such as BCG [17,42,172], enhances trained immunity. Up to months after immunization, BCG enhances cytokine reactions to unrelated pathogens [17,128,173]. BCG caused monocyte-derived cytokine responses to be both temporarily and permanently potentiated. Immuno-tolerance to unrelated pathogens was brought on by Tdap and was only partially reversed by BCG vaccination given concurrently or afterward. The BCG vaccine reversed the immunosuppressive effects of Tdap vaccination when administered together or after Tdap [153]. In conjunction with epidemiological evidence demonstrating contrary outcomes of live and inactivated vaccines on total morbidity and mortality [56,82,98,111], the results emphasize the relevance of improving our knowledge of the biological processes underlying these effects to achieve the best health results by improving currently vaccination programs [53].

It is noteworthy that comparison between live attenuated and inactivated pathogens as a component of vaccine design continues and "'additional observations" are surely welcome [174]. More recently, Gong *et al.* (2022) have listed the positive NSEs of BCG vaccination and limited insights, which we have about the mechanisms by which these NSE may operate [175]. For example, the positive non-specific effect of BCG vaccination on yellow fever virus involved monocytes and was associated with higher secretion of IL-1 beta. BCG vaccination was also reported to induce a functional antibody against HINI virus and release of interferon gamma (IFN-γ). It also helped children with warts caused by infection with HPV; the effect was found to be associated with activation of CD4 lymphocytes and increased release of IL-1, IL-2 and tumor necrosis factor alpha (TNF-α). Work with animal models have further shown that BCG vaccination shows promises of prophylactic effects and/or induction of resistance against HSV-1, HSV-2, Japanese encephalitis virus, *Klebsiella pneumonia*, and *Salmonella enteritidis* [175].

The other epidemiological observations, such as the sequence of multiple vaccinations, age of vaccinations, and antigen concentrations must be examined in the light of the mechanistic insights, which we have discussed at other places as well in this review. More important, before making any policy decisions, experts must factor in the emerging insights into NSEs of vaccines.

10. Could the possible NSEs of COVID-19 mRNA vaccines include IgG4-mediated immune suppression?

People who received 2 or more shots of the COVID-19 mRNA vaccines have been reported to have unusually elevated concentrations of IgG4 antibodies, according to recent studies [176,177]. It has also been shown that the HIV, malaria, and pertussis vaccines elicited higher-than-normal IgG4 production, which has been related to decreased protection against infections [178–180]. A rise in IgG4 levels has been hypothesized to provide protection by reducing immunological hyperactivation, similar to that seen following effective allergen-specific immunotherapy, by blocking the effects of IgE [176].

The described rise in IgG4 levels found following repeated administration of the mRNA vaccines, nevertheless, might not correspond to a protective mechanism, according to new findings. Instead, it may represent an immune tolerance mechanism to the spike protein, which might encourage unrestrained SARS-CoV2 infection and replication by inhibiting natural antiviral responses [181]. Excessive antigen concentration, repeated vaccination, and the particular kind of vaccine administered are three important variables that influence the class switch to IgG4 antibodies, according to an extensive analysis of the literature [181].

It is important to mention that while adenovirus-based vaccines did not result in long-lasting IgG4 responses, mRNA vaccines did [177]. Detailed examination of the literature revealed that only vaccines utilizing a portion of the virus—the spike protein for mRNA vaccines, the gp120 protein for HIV, and the EBA-175 antigen for the malaria vaccine, respectively—produced an increase in IgG4 levels [176,178,179]. Additionally, other investigations have demonstrated that IgG4 antibody production was stimulated by acellular (aP) but not whole Pertussis (wP) vaccines, which was similarly associated with compromised immunity [180,182].

Is it possible that the excessive mortality reported in highly COVID-19-vaccinated countries is related to the deleterious NSEs induced by the mRNA vaccines?

There is not enough information regarding the NSEs of COVID-19 mRNA vaccines. By analyzing the randomized control trials (RCTs) of mRNA and adenovirus-vector COVID-19 vaccines, a recent investigation evaluated the potential non-specific outcomes of these vaccines [183]. Researchers estimated mortality risk ratios (RRs) for COVID-19 vaccines in participants receiving mRNA vaccines in comparison to placebo receivers and contrasted them to RRs for COVID-19 vaccine recipients receiving adenovirus-vector vaccines compared to controls. The risk ratio (RR) of mRNA vaccines compared to placebo for overall mortality was 1.03 (95 % confidence range [CI]: 0.63–1.71). The RR for overall mortality in the adenovirus-vector vaccination RCTs was 0.37 (0.19–0.70). The mortality risk ratio was 3 times higher for the mRNA vaccines than that of the adenovirus-vector vaccine. Unfortunately, the COVID-19 RCTs were quickly unblinded, and controls received vaccinations; as a result, the chance to conduct extensive RCTs vaccine-vs.-placebo trials that would have offered significant data was eliminated [183].

Since the canonical view is that vaccines only provide immunity against the disease they were designed for, then the significant mortality decrease linked to adenovirus-vector vaccines may be challenging to accept. It is crucial to remember that these non-specific outcomes and their immunological underpinnings have been demonstrated for many different vaccines [16,22,23,26,184]. It was recently demonstrated that the AstraZeneca vaccine enhanced monocyte counts and frequency up to 3 months after immunization in comparison to their pre-vaccine levels [169]. In addition to having improved antigen presentation abilities, monocytes were more competent to create important cytokines and chemokines in reacting to unrelated pathogens. Therefore, it appears that the adenovirus-based vaccine promoted trained immunity [169]. The viral vector may stimulate the immune system similar to a "live" vaccine, even though it lacks replication [183].

To sum up, in view of the urgency due to COVID-19, mRNA vaccines were used as soon as they showed promise and saved many lives. Like any other therapy, the decision must have been based upon benefits outweighing risks. As a new kind of vaccines, their clearances were fastest as compared to any other technology platform for vaccines. Now

that kind of urgency is not there, it may be good to more closely look at their both short range and long range effects. These studies should also include looking at their NSEs.

A recently published research investigated heterologous and specific immunological effects of BNT162b2 COVID-19 vaccination in children [185]. The *in vitro* cytokine responses to heterologous stimulants (killed pathogens, Toll-like receptor ligands, and SARS-CoV-2 antigens) were examined using a whole blood stimulation test. 29 children between the ages of 5 and 11 years old had samples taken before and 28 days after receiving a second BNT162b2 vaccination (V2 + 28). Samples of eight children were examined six months after receiving the BNT162b2 vaccine. The main findings of this study were that SARS-CoV-2 mRNA vaccine reduces the production of inflammatory cytokines (IFN-γ, monocyte chemoattractant protein-1 (MCP-1), IL-6, IL-8, and IL-15) against heterologous bacterial, fungal, and viral stimulation [185]. These findings suggest that SARS-CoV-2 mRNA vaccination may impair immune responses to other pathogens that cause both vaccine-preventable and non-vaccine-preventable illnesses, and draw attention to the importance of more research and consideration of heterologous effects in vaccination programs because of the wide-ranging public health repercussions [185].

11. Proposed solutions

Some proposals have been made to diminish the harmful NSEs of non-live vaccines: First and foremost, it has been recommended that every child in Africa needs to be immunized against BCG at birth [154]. However, less than 50 % of children in Africa currently receive the BCG vaccine during the first month of life, although this has been demonstrated to reduce newborn mortality by more than one-third [15]. To strengthen the infant's immune system, the BCG vaccine should be marketed as a non-specific shot. Second, it might be desirable to reverse the intention to discontinue the live OPV [154]. With only 68 children needing to receive the OPV vaccine to save the life of one kid, vaccination programs with this vaccine have significantly decreased infant mortality in low-income nations [43].

Therefore, the positive effects of OPV exceed the relatively low risk of polio infection caused by vaccination [154]. Third, children should receive a live vaccine soon after getting a non-live vaccine [154]. For instance, all of the investigations found that inoculating the DTP vaccine after MV resulted in a higher rate of female mortality than giving MV after the DTP vaccine [7]. Because HTMV was administered at such a young age and most kids received the DTP vaccine after HTMV, this may also help to explain why the live, high-titer measles vaccine (HTMV) had been linked to higher female mortality [3]. At the same time, with more powerful tools like CRISPR emerging, efforts should be continued/renewed to further eliminate the risk of polio due to OPV.

12. Conclusions and future perspectives

The current vaccination model presupposes that vaccines only provide protection against a specific infection, that effective vaccines diminish mortality concerning the proportion of all deaths attributable to the target infection, and that the outcomes of vaccines are the same for both boys and girls. Epidemiological vaccine investigation, nonetheless, has produced findings that defy these presumptions and imply that vaccines have significant non-specific impacts on population health [26]. It can be understandable that, in a time of increasing vaccination hesitancy, numerous researchers are reluctant to even contemplate the possibility that such harmful NSEs might occur [186]. Recognizing that non-live vaccines have negative effects does not mean that they should stop being used, and should not encourage people who believe that vaccines only cause harm to continue to refuse them. Like any medicine, non-live vaccines can in some circumstances induce iatrogenic effects, which can be effectively neutralized when the last to be applied is a live vaccine. Since live vaccines promote immune training and enhance resistance to unrelated infections, they should not be replaced by non-live vaccines.

Regarding the detrimental NSEs of non-live vaccines, an inevitable question arises: why is there higher mortality in low-income countries linked with the administration of such vaccines? Apparently, the same vaccines had been also used in high-income countries, where such higher mortality has not been reported. We suggest that malnutrition, lack of medical doctors and well-equipped hospitals in rural areas, and an inadequate supply of antibiotics and antivirals have caused higher mortality from diseases that are promptly and appropriately treated in high-income countries.

A final suggestion is that the high IgG4 antibody levels generated in response to repeated inoculation with COVID-19 mRNA vaccines are associated with a higher mortality rate from unrelated diseases and infections by suppressing the immune system. Since most vaccinated countries are reporting high percentages of excess mortality not directly attributable to deaths from COVID-19 [187], the NSEs of these vaccines on global mortality should be studied in depth.

Peter Aaby and Christine Benn [51] made an interesting point that in view of the beneficial NSE of live vaccines, one should think of continuation of their vaccination programs even after the disease for which the vaccine is originally designed has been eradicated. They cite a retrospective study related to small pox vaccine which has examined the negative consequence of stopping vaccination for small pox. Benn *et al.* (2016) found that booster doses also result in further benefits even in terms of NSE and lead to reduction of mortality [70].

Aaby and Benn [12] have advocated that vaccination programs now should factor in the beneficial NSE of live vaccines in view of such effects clearly established at least for small pox, BCG, OPV and MV. Notably, they also have tried to cast doubts on the positive NSE reported for DTP and rabies, while underplaying few adverse NSE reported with some preparations of few live vaccines especially increased mortality reported for females [12]. While such debates can continue, based upon risks overweighing benefits, attenuated live vaccines are superior to the vaccines based upon inactivated viruses even from the perspective of beneficial NSE.

One issue which is now garnering increasing attention is the usefulness of vaccines in replacing antibiotics as their misuse and other factors have led to serious issue of antibiotic resistant pathogens [188,189]. Such optimism needs to be balanced in view of the fact that while BCG has been around for a long time that has not lessened the search for effective antibiotics for treatment of tuberculosis. There are different ways vaccinations can help in combating the challenges of antibiotic resistance [190–193]. Kennedy and Read (2018) have discussed why vaccine resistance has lower chance of developing as compared to the antibiotic resistance [194]. On the other hand, there is a report that an imperfect vaccine, which did not prevent virus transmission by vaccinated birds, led to evolution of more virulent strains of the virus [195]. Mike Boots (2015) have also discussed the issue that the ""imperfect" vaccination can select for higher virulence" [196].

While the role of vaccination in limiting spread of antibiotic resistance has attracted considerable attention, surprisingly how NSE of vaccines broadens this for a larger set of infections for which even no vaccine may be available seems to have gone unnoticed. NSE of vaccinations also help in preventing the necessity of use/misuse of antibiotics as even NSE are prophylactic in nature. Drug repurposing is already an established approach in combating diseases [6,7].

Perhaps, a deeper understanding of NSE of vaccines will lead us think of vaccine repurposing. Efforts to look at the BCG as a prophylactic or for minimizing pathogenesis due to SARS-CoV-2 infection point out to such a possibility [175].

CRediT authorship contribution statement

Alberto Rubio-Casillas: Conceptualization, Validation, Formal analysis, Data curation, Writing – original draft, Writing – review &

editing, Supervision. **Cesar Manuel Rodriguez-Quintero:** . **Elrashdy M. Redwan:** Validation, Formal analysis, Data curation, Writing – review & editing. **Munishwar Nath Gupta:** . **Vladimir N. Uversky:** Conceptualization, Validation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Supervision. **Mikolaj Raszek:** Formal analysis, Data curation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

[1] Riedel S. Edward Jenner and the history of smallpox and vaccination. Proc (Bayl Univ Med Cent) 2005;18:21–5. https://doi.org/10.1080/08998280.2005.11928028.
[2] Lakhani S. Early clinical pathologists: Edward Jenner (1749–1823). J Clin Pathol 1992;45:756–8. https://doi.org/10.1136/jcp.45.9.756.
[3] Ehrlich, P. Experimental researches on specific therapy: on immunity with special reference to the relationship between distribution and action of antigens: FIRST HARBEN LECTURE. In *The collected papers of Paul Ehrlich*; Elsevier: 1960; pp. 106-117.
[4] Gupta MN, Uversky VN. Structure and Intrinsic Disorder in Enzymology. Elsevier; 2022.
[5] Gupta MN, Kapoor M, Majumder AB, Singh V. Isozymes, moonlighting proteins and promiscous enzymes. Curr Sci 2011:1152–62.
[6] Gupta MN, Alam A, Hasnain SE. Protein promiscuity in drug discovery, drug-repurposing and antibiotic resistance. Biochimie 2020;175:50–7. https://doi.org/10.1016/j.biochi.2020.05.004.
[7] Gupta MN, Roy I. Drugs, host proteins and viral proteins: how their promiscuities shape antiviral design. Biol Rev Camb Philos Soc 2021;96:205–22. https://doi.org/10.1111/brv.12652.
[8] Gupta MN, Uversky VN. Moonlighting enzymes: when cellular context defines specificity. Cell Mol Life Sci 2023;80:130. https://doi.org/10.1007/s00018-023-04781-0.
[9] Mayr A. Taking advantage of the positive side-effects of smallpox vaccination. J Vet Med B 2004;51:199–201.
[10] Calmette A. Preventive Vaccination Against Tuberculosis with BCG. Proc R Soc Med 1931;24:1481–90.
[11] Voroshilova MK. Potential use of nonpathogenic enteroviruses for control of human disease. Prog Med Virol 1989;36:191–202.
[12] Aaby P, Benn CS. Developing the concept of beneficial non-specific effect of live vaccines with epidemiological studies. Clin Microbiol Infect 2019;25:1459–67.
[13] Aaby P, Samb B, Simondon F, Seck AMC, Knudsen K, Whittle H. Non-specific beneficial effect of measles immunisation: analysis of mortality studies from developing countries. BMJ 1995;311:481–5.
[14] Kristensen I, Fine P, Aaby P, Jensen H. Routine vaccinations and child survival: follow up study in Guinea-Bissau, West AfricaCommentary: an unexpected finding that needs confirmation or rejection. BMJ 2000;321:1435.
[15] Aaby P, Jensen H, Samb B, Cisse B, Sodemann M, Jakobsen M, et al. Differences in female-male mortality after high-titre measles vaccine and association with subsequent vaccination with diphtheria-tetanus-pertussis and inactivated poliovirus: reanalysis of West African studies. Lancet 2003;361:2183–8.
[16] Benn CS, Netea MG, Selin LK, Aaby P. A small jab–a big effect: nonspecific immunomodulation by vaccines. Trends Immunol 2013;34:431–9.
[17] Kleinnijenhuis J, Quintin J, Preijers F, Joosten LA, Ifrim DC, Saeed S, et al. Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. Proc Natl Acad Sci 2012;109:17537–42.
[18] Arts RJ, Moorlag SJ, Novakovic B, Li Y, Wang S-Y, Oosting M, et al. BCG vaccination protects against experimental viral infection in humans through the induction of cytokines associated with trained immunity. Cell Host Microbe 2018;23(89–100):e105.
[19] Organization, W.H. Child mortality and causes of death https://www. who. int/data/gho/data/themes/topics/topic-details. *GHO/childmortality-and-causes-of-death2020 [Accessed on 30 January 2022]* **2019**.
[20] Feikin DR, Flannery B, Hamel MJ, Stack M, Hansen PM. Vaccines for children in low-and middle-income countries. *Reproductive, maternal, newborn, and child health: disease control priorities* **2016**, *2*, 187-204.
[21] Plotkin S. History of vaccination. Proc Natl Acad Sci USA 2014;111:12283–7.
[22] Aaby P, Kollmann TR, Benn CS. Nonspecific effects of neonatal and infant vaccination: public-health, immunological and conceptual challenges. Nat Immunol 2014;15:895–9.
[23] Aaby P, Benn CS. Non-specific and sex-differential effects of routine vaccines: what evidence is needed to take these effects into consideration in low-income countries? Hum Vaccin 2011;7:120–4.
[24] Higgins, J.P.; Soares-Weiser, K.; López-López, J.A.; Kakourou, A.; Chaplin, K.; Christensen, H.; Martin, N.K.; Sterne, J.A.; Reingold, A.L. Association of BCG, DTP, and measles containing vaccines with childhood mortality: systematic review. *bmj* **2016**, *355*.
[25] Thysen SM, Rodrigues A, Aaby P, Fisker AB. Out-of-sequence DTP and measles vaccinations and child mortality in Guinea-Bissau: a reanalysis. BMJ Open 2019;9:e024893.
[26] Benn CS, Fisker AB, Rieckmann A, Sørup S, Aaby P. Vaccinology: time to change the paradigm? Lancet Infect Dis 2020;20:e274–83.
[27] Male D. *Immunology: an illustrated outline*; Garland Science, Taylor & Francis Group. New York: LLC; 2014.
[28] Lydyard P, Whelan A, Fanger M. BIOS Instant notes in immunology. Taylor & Francis; 2011.
[29] Biering-Sørensen S, Aaby P, Lund N, Monteiro I, Jensen KJ, Eriksen HB, et al. Early BCG-Denmark and neonatal mortality among infants weighing< 2500 g: a randomized controlled trial. Clin Infect Dis 2017;65:1183–90.
[30] Schaltz-Buchholzer F, Aaby P, Monteiro I, Camala L, Faurholt Simonsen S, Nørtoft Frankel H, et al. Immediate Bacille Calmette-Guérin vaccination to neonates requiring perinatal treatment at the maternity ward in Guinea-Bissau: a randomized controlled trial. J Infect Dis 2021;224:1935–44.
[31] Prentice S, Nassanga B, Webb EL, Akello F, Kiwudhu F, Akurut H, et al. BCG-induced non-specific effects on heterologous infectious disease in Ugandan neonates: an investigator-blind randomised controlled trial. Lancet Infect Dis 2021;21:993–1003.
[32] Aaby P, Martins CL, Garly M-L, Balé C, Andersen A, Rodrigues A, et al. Non-specific effects of standard measles vaccine at 4.5 and 9 months of age on childhood mortality: randomised controlled trial. BMJ 2010;341.
[33] Berendsen ML, Silva I, Balé C, Nielsen S, Hvidt S, Martins CL, et al. The effect of a second dose of measles vaccine at 18 months of age on nonaccidental deaths and hospital admissions in Guinea-Bissau: interim analysis of a randomized controlled trial. Clin Infect Dis 2022;75:1370–8.
[34] Lund N, Andersen A, Hansen ASK, Jepsen FS, Barbosa A, Biering-Sørensen S, et al. The effect of oral polio vaccine at birth on infant mortality: a randomized trial. Clin Infect Dis 2015;61:1504–11.
[35] Blume S, Geesink I. A brief history of polio vaccines. Science 2000;288:1593–4. https://doi.org/10.1126/science.288.5471.1593.
[36] Vashishtha VM, Kamath S. A Brief History of Vaccines Against Polio. Indian Pediatr 2016;53(Suppl 1):S20–7.
[37] Robbins FC. The History of Polio Vaccine Development. In: Plotkin SA, Orenstein WA, editors. Vaccines. PA Saunders: Phyladelphia; 2004. p. 17–30.
[38] Contreras G. Sabin's vaccine used for nonspecific prevention of infant diarrhea of viral etiology. Bull Pan Am Health Organ (PAHO) 1974;8(2):1974.
[39] Chumakov M, Voroshilova M, Antsupova A, Boiko V, Blinova M, Rodin V. Live enterovirus vaccines for emergency non-specific prevention of mass respiratory diseases during autumn-winter outbreaks of influenza and other acute respiratory diseases. Zh Mikrobiol Epidemiol Immunobiol 1992;11:37–40.
[40] Nielsen S, Khalek MA, Benn CS, Aaby P, Hanifi SMA. National immunisation campaigns with oral polio vaccine may reduce all-cause mortality: Analysis of 2004–2019 demographic surveillance data in rural Bangladesh. *EClinicalMedicine* **2021**, *36*.
[41] Schoeps A, Nebié E, Fisker AB, Sié A, Zakane A, Müller O, et al. No effect of an additional early dose of measles vaccine on hospitalization or mortality in children: a randomized controlled trial. Vaccine 2018;36:1965–71.
[42] Welaga P, Oduro A, Debpuur C, Aaby P, Ravn H, Andersen A, et al. Fewer out-of-sequence vaccinations and reduction of child mortality in Northern Ghana. Vaccine 2017;35:2496–503.
[43] Andersen A, Fisker AB, Rodrigues A, Martins C, Ravn H, Lund N, et al. National immunization campaigns with oral polio vaccine reduce all-cause mortality: a natural experiment within seven randomized trials. Front Public Health 2018;6:13.
[44] Nielsen S, Sujan HM, Benn CS, Aaby P, Hanifi SMA. Oral polio vaccine campaigns may reduce the risk of death from respiratory infections. Vaccines 2021;9:1133.
[45] Sørup S, Stensballe LG, Krause TG, Aaby P, Benn CS, Ravn H. Oral polio vaccination and hospital admissions with non-polio infections in Denmark: nationwide retrospective cohort study. In: In Proceedings of the Open forum infectious diseases; 2016. p. ofv204..
[46] Seppälä E, Viskari H, Hoppu S, Honkanen H, Huhtala H, Simell O, et al. Viral interference induced by live attenuated virus vaccine (OPV) can prevent otitis media. Vaccine 2011;29:8615–8.
[47] Upfill-Brown A, Taniuchi M, Platts-Mills JA, Kirkpatrick B, Burgess SL, Oberste MS, et al. Nonspecific effects of oral polio vaccine on diarrheal burden and etiology among Bangladeshi infants. Clin Infect Dis 2017;65:414–9.
[48] Velema JP, Alihonou EM, Gandaho T, Hounye FH. Childhood mortality among users and non-users of primary health care in a rural west African community. Int J Epidemiol 1991;20:474–9.
[49] Aaby P, Jensen H, Garly M-L, Balé C, Martins C, Lisse I. Routine vaccinations and child survival in a war situation with high mortality: effect of gender. Vaccine 2002;21:15–20.
[50] Aaby P, Rodrigues A, Biai S, Martins C, Veirum JE, Benn CS, et al. Oral polio vaccination and low case fatality at the paediatric ward in Bissau. Guinea-Bissau *Vaccine* 2004;22:3014–7.

[51] Aaby P, Benn CS. Beneficial nonspecific effects of oral polio vaccine (OPV): Implications for the cessation of OPV? Clin Infect Dis 2017;65:420–1. https://doi.org/10.1093/cid/cix340.
[52] Moorlag S, Arts R, Van Crevel R, Netea M. Non-specific effects of BCG vaccine on viral infections. Clin Microbiol Infect 2019;25:1473–8.
[53] Shann F. The non-specific effects of vaccines. Arch Dis Child 2010;95:662–7.
[54] Shann F. Nonspecific effects of vaccines and the reduction of mortality in children. Clin Ther 2013;35:109–14.
[55] Garly M-L, Martins CL, Balé C, Baldé MA, Hedegaard KL, Gustafson P, et al. BCG scar and positive tuberculin reaction associated with reduced child mortality in West Africa: a non-specific beneficial effect of BCG? Vaccine 2003;21:2782–90.
[56] Roth A, Gustafson P, Nhaga A, Djana Q, Poulsen A, Garly M-L, et al. BCG vaccination scar associated with better childhood survival in Guinea-Bissau. Int J Epidemiol 2005;34:540–7.
[57] Aaby P, Ravn H, Benn CS, Rodrigues A, Samb B, Ibrahim SA, et al. Randomized trials comparing inactivated vaccine after medium-or high-titer measles vaccine with standard titer measles vaccine after inactivated vaccine. Pediatr Infect Dis J 2016;35:1232–41.
[58] Biering-Sørensen S, Aaby P, Napirna BM, Roth A, Ravn H, Rodrigues A, et al. Small randomized trial among low–birth-weight children receiving Bacillus Calmette-Guérin vaccination at first health center contact. Pediatr Infect Dis J 2012;31:306–8.
[59] Aaby P, Roth A, Ravn H, Napirna BM, Rodrigues A, Lisse IM, et al. Randomized trial of BCG vaccination at birth to low-birth-weight children: beneficial nonspecific effects in the neonatal period? J Infect Dis 2011;204:245–52.
[60] Aaby P, Nielsen J, Benn CS, Trape J-F. Sex-differential and non-specific effects of routine vaccinations in a rural area with low vaccination coverage: an observational study from Senegal. Trans R Soc Trop Med Hyg 2015;109:77–84.
[61] Breiman RF, Streatfield PK, Phelan M, Shifa N, Rashid M, Yunus M. Effect of infant immunisation on childhood mortality in rural Bangladesh: analysis of health and demographic surveillance data. Lancet 2004;364:2204–11.
[62] Roth A, Sodemann M, Jensen H, Poulsen A, Gustafson P, Weise C, et al. Tuberculin reaction, BCG scar, and lower female mortality. Epidemiology 2006:562–8.
[63] Brook B, Harbeson DJ, Shannon CP, Cai B, He D, Ben-Othman R, et al. BCG vaccination–induced emergency granulopoiesis provides rapid protection from neonatal sepsis. Sci Transl Med 2020;12:eaax4517.
[64] Kollmann TR, Marchant A, Way SS. Vaccination strategies to enhance immunity in neonates. Science 2020;368:612–5.
[65] Aaby P, Bukh J, Lisse IM, Smits A. Measles vaccination and child mortality. Measles vaccination and child mortality 1981;2.
[66] Aaby P, Bukh J, Lisse IM, Smits AJ. Measles vaccination and reduction in child mortality: a community study from Guinea-Bissau. J Infect 1984;8:13–21.
[67] Roth AE, Benn CS, Ravn H, Rodrigues A, Lisse IM, Yazdanbakhsh M, et al. Effect of revaccination with BCG in early childhood on mortality: randomised trial in Guinea-Bissau. BMJ 2010;340.
[68] Andersen A, Fisker AB, Nielsen S, Rodrigues A, Benn CS, Aaby P. National immunization campaigns with oral polio vaccine may reduce all-cause mortality: An analysis of 13 years of demographic surveillance data from an urban African area. Clin Infect Dis 2021;72:e596–603.
[69] Aaby P, Martins CL, Garly M-L, Andersen A, Fisker AB, Claesson MH, et al. Measles vaccination in the presence or absence of maternal measles antibody: impact on child survival. Clin Infect Dis 2014;59:484–92.
[70] Benn CS, Fisker AB, Whittle HC, Aaby P. Revaccination with live attenuated vaccines confer additional beneficial nonspecific effects on overall survival: a review. EBioMedicine 2016;10:312–7.
[71] Berendsen ML, Øland CB, Bles P, Jensen AKG, Kofoed P-E, Whittle H, et al. Maternal priming: Bacillus Calmette-Guérin (BCG) vaccine scarring in mothers enhances the survival of their child with a BCG vaccine scar. J Pediatric Infect Dis Soc 2020;9:166–72.
[72] Hartfield J, Morley D. Efficacy of measles vaccine. Epidemiol Infect 1963;61:143–7.
[73] Team KP. Influence of measles vaccination on survival pattern of 7–35-month-old children in Kasongo. Zaire *The Lancet* 1981;317:764–7.
[74] Aaby P, Bhuiya A, Nahar L, Knudsen K, de Francisco A, Strong M. The survival benefit of measles immunization may not be explained entirely by the prevention of measles disease: a community study from rural Bangladesh. Int J Epidemiol 2003;32:106–15.
[75] Aaby P, Samb B, Simondon F, Knudsen K, Seck AMC, Bennett J, et al. Divergent mortality for male and female recipients of low-titer and high-titer measles vaccines in rural Senegal. Am J Epidemiol 1993;138:746–55.
[76] du Loû AD, Pison G, Aaby P. Role of immunizations in the recent decline in childhood mortality and the changes in the female/male mortality ratio in rural Senegal. Am J Epidemiol 1995;142:643–52.
[77] Holt EA, Boulos R, Halsey NA, Boulos L-M, Boulos C. Childhood survival in Haiti: protective effect of measles vaccination. Pediatrics 1990;85:188–94.
[78] Aaby P. Assumptions and contradictions in measles and measles immunization research: Is measles good for something? Soc Sci Med 1995;41:673–86.
[79] Veirum JE, Sodemann M, Biai S, Jakobsen M, Garly M-L, Hedegaard K, et al. Routine vaccinations associated with divergent effects on female and male mortality at the paediatric ward in Bissau. Guinea-Bissau *Vaccine* 2005;23:1197–204.
[80] Cutts F, Steinglass R. Should measles be eradicated? BMJ 1998;316:765–7.
[81] Shann F. A little bit of measles does you good: Even if measles is eradicated, immunisation may still be desirable in developing countries. BMJ 1999;319:4–5. https://doi.org/10.1136/bmj.319.7201.4.
[82] Strassburg MA. The global eradication of smallpox. Am J Infect Control 1982;10:53–9.
[83] Aaby P, Gustafson P, Roth A, Rodrigues A, Fernandes M, Sodemann M, et al. Vaccinia scars associated with better survival for adults: an observational study from Guinea-Bissau. Vaccine 2006;24:5718–25.
[84] Jensen ML, Dave S, van der Loeff MS, da Costa C, Vincent T, Leligdowicz A, et al. Vaccinia scars associated with improved survival among adults in rural Guinea-Bissau. PLoS One 2006;1:e101.
[85] Rieckmann A, Villumsen M, Sørup S, Haugaard LK, Ravn H, Roth A, et al. Vaccinations against smallpox and tuberculosis are associated with better long-term survival: a Danish case-cohort study 1971–2010. Int J Epidemiol 2017;46:695–705.
[86] Rieckmann A, Villumsen M, Jensen ML, Ravn H, Da Silva ZJ, Sørup S, et al. The effect of smallpox and Bacillus Calmette-Guérin vaccination on the risk of human immunodeficiency virus-1 infection in Guinea-Bissau and Denmark. In: In Proceedings of the Open forum infectious diseases; 2017. p. ofx130..
[87] Aaby P, Benn CS. Stopping live vaccines after disease eradication may increase mortality. Vaccine 2020;38:10–4.
[88] Aaby P, Ravn H, Fisker AB, Rodrigues A, Benn CS. Is diphtheria-tetanus-pertussis (DTP) associated with increased female mortality? A meta-analysis testing the hypotheses of sex-differential non-specific effects of DTP vaccine. Trans R Soc Trop Med Hyg 2016;110:570–81.
[89] Fisker AB, Ravn H, Rodrigues A, Østergaard MD, Bale C, Benn CS, et al. Co-administration of live measles and yellow fever vaccines and inactivated pentavalent vaccines is associated with increased mortality compared with measles and yellow fever vaccines only. An observational study from Guinea-Bissau *Vaccine* 2014;32:598–605.
[90] Garly M-L, Jensen H, Martins CL, Balé C, Baldé MA, Lisse IM, et al. Hepatitis B vaccination associated with higher female than male mortality in Guinea-Bissau: an observational study. Pediatr Infect Dis J 2004;23:1086–92.
[91] Aaby P, Garly M-L, Nielsen J, Ravn H, Martins C, Balé C, et al. Increased female-male mortality ratio associated with inactivated polio and diphtheria-tetanus-pertussis vaccines: observations from vaccination trials in Guinea-Bissau. Pediatr Infect Dis J 2007;26:247–52.
[92] Klein SL, Shann F, Moss WJ, Benn CS, Aaby PRTS. S malaria vaccine and increased mortality in girls. MBio 2016;7. https://doi.org/10.1128/mbio.00514-16. e00514–00516.
[93] Fine PE, Smith PG. 'Non-specific effects of vaccines'–an important analytical insight, and call for a workshop. Trop Med Int Health 2007;12:1–4. https://doi.org/10.1111/j.1365-3156.2006.01794.x.
[94] Usher NT, Chang S, Howard RS, Martinez A, Harrison LH, Santosham M, et al. Association of BCG vaccination in childhood with subsequent cancer diagnoses: a 60-year follow-up of a clinical trial. JAMA Netw Open 2019;2:e1912014–.
[95] Sørup S, Benn CS, Poulsen A, Krause TG, Aaby P, Ravn H. Live vaccine against measles, mumps, and rubella and the risk of hospital admissions for nontargeted infections. JAMA 2014;311:826–35.
[96] Bardenheier BH, McNeil MM, Wodi AP, McNicholl JM, DeStefano F. Risk of nontargeted infectious disease hospitalizations among US children following inactivated and live vaccines, 2005–2014. Clin Infect Dis 2017;65:729–37.
[97] Benn CS, Amenyogbe N, Björkman A, Domínguez-Andrés J, Fish EN, Flanagan KL, et al. Implications of non-specific effects for testing, approving, and regulating vaccines. Drug Saf 2023:1–10.
[98] Goodridge HS, Ahmed SS, Curtis N, Kollmann TR, Levy O, Netea MG, et al. Harnessing the beneficial heterologous effects of vaccination. Nat Rev Immunol 2016;16:392–400.
[99] Aaby P, Benn C, Nielsen J, Lisse IM, Rodrigues A, Ravn H. Testing the hypothesis that diphtheria–tetanus–pertussis vaccine has negative non-specific and sex-differential effects on child survival in high-mortality countries. BMJ Open 2012;2:e000707.
[100] Aaby P, Ravn H, Benn CS. The WHO review of the possible nonspecific effects of diphtheria-tetanus-pertussis vaccine. Pediatr Infect Dis J 2016;35:1247–57.
[101] Rodrigues A, Fischer TK, Valentiner-Branth P, Nielsen J, Steinsland H, Perch M, et al. Community cohort study of rotavirus and other enteropathogens: are routine vaccinations associated with sex-differential incidence rates? Vaccine 2006;24:4737–46.
[102] Valentiner-Branth P, Perch M, Nielsen J, Steinsland H, Garly M-L, Fischer TK, et al. Community cohort study of Cryptosporidium parvum infections: sex-differential incidences associated with BCG and diptheria–tetanus–pertussis vaccinations. Vaccine 2007;25:2733–41.
[103] Noho-Konteh F, Adetifa JU, Cox M, Hossin S, Reynolds J, Le MT, et al. Sex-differential non-vaccine-specific immunological effects of diphtheria-tetanus-pertussis and measles vaccination. Clin Infect Dis 2016;63:1213–26.
[104] Aaby P, Jensen H, Gomes J, Fernandes M, Lisse IM. The introduction of diphtheria-tetanus-pertussis vaccine and child mortality in rural Guinea-Bissau: an observational study. Int J Epidemiol 2004;33:374–80.
[105] Mogensen SW, Andersen A, Rodrigues A, Benn CS, Aaby P. The introduction of diphtheria-tetanus-pertussis and oral polio vaccine among young infants in an urban African community: a natural experiment. EBioMedicine 2017;17:192–8.
[106] Aaby P, Mogensen SW, Rodrigues A, Benn CS. Evidence of increase in mortality after the introduction of diphtheria–tetanus–pertussis vaccine to children aged 6–35 months in Guinea-Bissau: a time for reflection? Front Public Health 2018;79.
[107] Welaga P, Nielsen J, Adjuik M, Debpuur C, Ross DA, Ravn H, et al. Non-specific effects of diphtheria-tetanus-pertussis and measles vaccinations? An analysis of surveillance data from Navrongo. Ghana *Tropical medicine & international health* 2012;17:1492–505.

[108] Agergaard J, Nante E, Poulstrup G, Nielsen J, Flanagan K, Østergaard L, et al. Diphtheria–tetanus–pertussis vaccine administered simultaneously with measles vaccine is associated with increased morbidity and poor growth in girls. A randomised trial from Guinea-Bissau. *Vaccine* 2011;29:487–500.
[109] WHO. World Health Organization Summary of the SAGE April 2014 meeting. **2014**.
[110] Aaby P, Jensen H, Walraven G. Age-specific changes in the female–male mortality ratio related to the pattern of vaccinations: an observational study from rural Gambia. Vaccine 2006;24:4701–8.
[111] Aaby P, Vessari H, Nielsen J, Maleta K, Benn CS, Jensen H, et al. Sex differential effects of routine immunizations and childhood survival in rural Malawi. Pediatr Infect Dis J 2006;25:721–7.
[112] Aaby P, Andersen A, Ravn H, Zaman K. Co-administration of BCG and diphtheria-tetanus-pertussis (DTP) vaccinations may reduce infant mortality more than the WHO-schedule of BCG first and then DTP. A re-analysis of demographic surveillance data from rural Bangladesh. EBioMedicine 2017;22:173–80.
[113] Hirve S, Bavdekar A, Juvekar S, Benn CS, Nielsen J, Aaby P. Non-specific and sex-differential effects of vaccinations on child survival in rural western India. Vaccine 2012;30:7300–8.
[114] Clipet-Jensen C, Andersen A, Jensen A, Peter A, Khalequzzaman M. Out-of-sequence measles and dtp vaccinations. A re-analysis of demographic surveillance data from rural bangladesh. *CID* **2021**.
[115] Hanifi SMA, Biering-Sørensen S, Jensen AKG, Aaby P, Bhuiya A. Penta is associated with an increased female-male mortality ratio: cohort study from Bangladesh. Hum Vaccin Immunother 2021;17:197–204.
[116] Park JJ, Brondi L. Why are girls still dying unnecessarily? The need to address gender inequity in child health in the post–2015 development agenda. J Glob Health 2015;5.
[117] Alkema L, Chao F, You D, Pedersen J, Sawyer CC. National, regional, and global sex ratios of infant, child, and under-5 mortality and identification of countries with outlying ratios: a systematic assessment. Lancet Glob Health 2014;2: e521–30.
[118] Ram U, Jha P, Ram F, Kumar K, Awasthi S, Shet A, et al. Neonatal, 1–59 month, and under-5 mortality in 597 Indian districts, 2001 to 2012: estimates from national demographic and mortality surveys. Lancet Glob Health 2013;1: e219–26.
[119] Pande RP. Selective gender differences in childhood nutrition and immunization in rural India: the role of siblings. Demography 2003;40:395–418.
[120] Krishnan A, Ng N, Kapoor SK, Pandav CS, Byass P. Temporal trends and gender differentials in causes of childhood deaths at Ballabgarh, India: need for revisiting child survival strategies. BMC Public Health 2012;12:555.
[121] Organization, W.H. Report of the WHO pandemic influenza A (H1N1) vaccine deployment initiative. **2012**.
[122] Girard MP, Tam JS, Assossou OM, Kieny MP. The 2009 A (H1N1) influenza virus pandemic: A review. Vaccine 2010;28:4895–902.
[123] WHO. World Health Organization. Flunet. www.who.int/flunet. Accessed June 2023. **2023**.
[124] Hansen OB, Rodrigues A, Martins C, Rieckmann A, Benn CS, Aaby P, et al. Impact of H1N1 influenza vaccination on child morbidity in Guinea-Bissau. J Trop Pediatr 2019;65:446–56.
[125] Njuguna H, Ahmed J, Oria PA, Arunga G, Williamson J, Kosgey A, et al. Uptake and effectiveness of monovalent influenza A (H1N1) pandemic 2009 vaccine among healthcare personnel in Kenya, 2010. Vaccine 2013;31:4662–7.
[126] Cowling BJ, Chan K-H, Feng S, Chan EL, Lo JY, Peiris JM, et al. The effectiveness of influenza vaccination in preventing hospitalizations in children in Hong Kong, 2009–2013. Vaccine 2014;32:5278–84.
[127] Welsh RM, Selin LK. No one is naive: the significance of heterologous T-cell immunity. Nat Rev Immunol 2002;2:417–26.
[128] Leentjens J, Kox M, Stokman R, Gerretsen J, Diavatopoulos DA, van Crevel R, et al. BCG vaccination enhances the immunogenicity of subsequent influenza vaccination in healthy volunteers: a randomized, placebo-controlled pilot study. J Infect Dis 2015;212:1930–8.
[129] Organization WH. World malaria report 2022. World Health Organization; 2022.
[130] Rts S. Efficacy and safety of RTS, S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. Lancet 2015;386:31–45.
[131] Aaby P, Rodrigues A, Kofoed P-E, Benn CS. RTS, S/AS01 malaria vaccine and child mortality. Lancet 2015;386:1735–6.
[132] Hanifi SMA, Fisker AB, Welaga P, Rieckmann A, Jensen AG, Benn CS, et al. Diphtheria-tetanus-pertussis (DTP) vaccine is associated with increased female-male mortality. Studies of DTP administered before and after measles vaccine. J Infect Dis 2021;223:1984–91.
[133] Benn CS, Aaby P. Diphtheria-tetanus-pertussis vaccination administered after measles vaccine: increased female mortality? Pediatr Infect Dis J 2012;31: 1095–7.
[134] Shann F. The heterologous (non-specific) effects of vaccines: implications for policy in high-mortality countries. Trans R Soc Trop Med Hyg 2015;109:5–8.
[135] Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol 2016;16:626–38. https://doi.org/10.1038/nri.2016.90.
[136] Ortona E, Pierdominici M, Rider V. Editorial: Sex Hormones and Gender Differences in Immune Responses. Front Immunol 2019;10:1076. https://doi.org/10.3389/fimmu.2019.01076.
[137] Sciarra F, Campolo F, Franceschini E, Carlomagno F, Venneri MA. Gender-Specific Impact of Sex Hormones on the Immune System. Int J Mol Sci 2023;24. https://doi.org/10.3390/ijms24076302.
[138] Poy A, van den Ent MM, Sosler S, Hinman AR, Brown S, Sodha S, et al. Monitoring results in routine immunization: development of routine immunization dashboard in selected African countries in the context of the Polio Eradication Endgame Strategic Plan. J Infect Dis 2017;216:S226–36.
[139] Shearer JC, Walker DG, Risko N, Levine OS. The impact of new vaccine introduction on the coverage of existing vaccines: a cross-national, multivariable analysis. Vaccine 2012;30:7582–7.
[140] WHO. Global Vaccine Action Plan.
[141] Hanifi SMA. Non-specific effects of vaccines and sex-differential child mortality in rural Bangladesh. Denmark: Southern Danish University Odense; 2017.
[142] Fisker A, Bale C, Rodrigues A, Balde I, Fernandes M, Jørgensen MJ, et al. A Randomised Trial of High-dose Vitamin A at Vaccination Contacts After 6 Months of Age: Sex-differential Effects on. Mortality 2015.
[143] Aaby P, Benn CS, Nielsen J, Lisse IM, Rodrigues A, Jensen H. DTP vaccination and child survival in observational studies with incomplete vaccination data. Trop Med Int Health 2007;12:15–24.
[144] Fisker AB, Biering-Sørensen S, Lund N, Djana Q, Rodrigues A, Martins CL, et al. Contrasting female-male mortality ratios after routine vaccinations with pentavalent vaccine versus measles and yellow fever vaccine. A cohort study from urban Guinea-Bissau. Vaccine 2016;34:4551–7.
[145] Expanded programme on immunization. Safety and efficacy of high titre measles vaccine at 6 months of age. Wkly Epidemiol Rec 1991;66:249–51.
[146] Aaby P, Hansen H, Thårup J, Sodemann M, Jensen T, Kristiansen H, et al. Trial of high-dose Edmonston-Zagreb measles vaccine in Guinea-Bissau: protective efficacy. Lancet 1988;332:809–11.
[147] Samb B, Aaby P, Whittle H, Coll Seck AM, Simondon F. Protective efficacy of high-titre measles vaccines administered from the age of five months: a community study in rural Senegal. Trans R Soc Trop Med Hyg 1993;87:697–701.
[148] Aaby P, Ravn H, Benn C, Rodrigues A, Samb B, Ibrahim S, et al. Child mortality after inactivated vaccines versus standard measles vaccine at nine months of age: a combined analysis of five cross-over randomised trials. Pediatr Infect Dis J 2016;35:1232–41.
[149] Billeskov R, Beikzadeh B, Berzofsky JA. The effect of antigen dose on T cell-targeting vaccine outcome. Hum Vaccin Immunother 2019;15:407–11. https://doi.org/10.1080/21645515.2018.1527496.
[150] Sridhar S, Brokstad KA, Cox RJ. Influenza Vaccination Strategies: Comparing Inactivated and Live Attenuated Influenza Vaccines. Vaccines (Basel) 2015;3: 373–89. https://doi.org/10.3390/vaccines3020373.
[151] Lund N, Andersen A, Hansen AS, Jepsen FS, Barbosa A, Biering-Sorensen S, et al. The Effect of Oral Polio Vaccine at Birth on Infant Mortality: A Randomized Trial. Clin Infect Dis 2015;61:1504–11. https://doi.org/10.1093/cid/civ617.
[152] Bhardwaj, S. Vaccines. In *Pharmaceutical Medicine and Translational Clinical Research*, Vohora, D., Singh, G., Eds.; Academic Press: London, UK; San Diego, USA; Cambridge, USA; Oxford, UK, 2017; pp. 341-353.
[153] Blok BA, de Bree LCJ, Diavatopoulos DA, Langereis JD, Joosten LA, Aaby P, et al. Interacting, nonspecific, immunological effects of Bacille Calmette-Guerin and tetanus-diphtheria-pertussis inactivated polio vaccinations: an explorative, randomized trial. Clin Infect Dis 2020;70:455–63.
[154] Janeway CJ, Travers P, Walport M, Shlomchik M. Immunobiology. New York: Garland Publishing; 2001.
[155] Plotkin SA, Orenstein WA, Offit PA. Vaccines. 5th ed. Philadelphia: Saunders; 2008.
[156] Czerkinsky C, Anjuere F, McGhee JR, George-Chandy A, Holmgren J, Kieny MP, et al. Mucosal immunity and tolerance: relevance to vaccine development. Immunol Rev 1999;170:197–222. https://doi.org/10.1111/j.1600-065x.1999.tb01339.x.
[157] Shakya AK, Chowdhury MYE, Tao W, Gill HS. Mucosal vaccine delivery: Current state and a pediatric perspective. J Control Release 2016;240:394–413. https://doi.org/10.1016/j.jconrel.2016.02.014.
[158] Tarancon R, Dominguez-Andres J, Uranga S, Ferreira AV, Groh LA, Domenech M, et al. New live attenuated tuberculosis vaccine MTBVAC induces trained immunity and confers protection against experimental lethal pneumonia. PLoS Pathog 2020;16:e1008404.
[159] Dominguez-Andres J, Netea MG. Long-term reprogramming of the innate immune system. J Leukoc Biol 2019;105:329–38. https://doi.org/10.1002/JLB.MR0318-104R.
[160] Moorman CD, Sohn SJ, Phee H. Emerging Therapeutics for Immune Tolerance: Tolerogenic Vaccines, T cell Therapy, and IL-2 Therapy. Front Immunol 2021;12: 657768. https://doi.org/10.3389/fimmu.2021.657768.
[161] Pilat N, Sprent J. Treg Therapies Revisited: Tolerance Beyond Deletion. Front Immunol 2020;11:622810. https://doi.org/10.3389/fimmu.2020.622810.
[162] Pulendran B, P, S.A.; O'Hagan, D.T. Emerging concepts in the science of vaccine adjuvants. *Nat Rev Drug Discov* **2021**, *20*, 454-475, doi:10.1038/s41573-021-00163-y.
[163] Walk J, de Bree LCJ, Graumans W, Stoter R, van Gemert G-J, van de Vegte-Bolmer M, et al. Outcomes of controlled human malaria infection after BCG vaccination. Nat Commun 2019;10:874.
[164] Clark I, Allison A, Cox F. Protection of mice against Babesia, and Plasmodium with BCG. Nature 1976;259:309–11.
[165] Matsumoto S, Yukitake H, Kanbara H, Yamada H, Kitamura A, Yamada T. Mycobacterium bovis bacillus calmette-guerin induces protective immunity against infection by Plasmodium yoelii at blood-stage depending on shifting immunity toward Th1 type and inducing protective IgG2a after the parasite infection. Vaccine 2000;19:779–87.

[166] Murphy J. Host defenses in murine malaria: nonspecific resistance to Plasmodium berghei generated in response to Mycobacterium bovis infection or Corynebacterium parvum stimulation. Infect Immun 1981;33:199–211.
[167] Parra M, Liu X, Derrick SC, Yang A, Tian J, Kolibab K, et al. Molecular analysis of non-specific protection against murine malaria induced by BCG vaccination. PLoS One 2013;8:e66115.
[168] van Puffelen JH, Novakovic B, van Emst L, Kooper D, Zuiverloon TC, Oldenhof UT, et al. Intravesical BCG in patients with non-muscle invasive bladder cancer induces trained immunity and decreases respiratory infections. J Immunother Cancer 2023:11.
[169] Murphy DM, Cox DJ, Connolly SA, Breen EP, Brugman AA, Phelan JJ, et al. Trained immunity is induced in humans after immunization with an adenoviral vector COVID-19 vaccine. J Clin Invest 2023;133.
[170] Blok BA, Arts RJ, van Crevel R, Aaby P, Joosten LA, Benn CS, et al. Differential effects of BCG vaccine on immune responses induced by vi polysaccharide typhoid fever vaccination: an explorative randomized trial. Eur J Clin Microbiol Infect Dis 2020;39:1177–84.
[171] Blok BA, Jensen KJ, Aaby P, Fomsgaard A, van Crevel R, Benn CS, et al. Opposite effects of Vaccinia and modified Vaccinia Ankara on trained immunity. Eur J Clin Microbiol Infect Dis 2019;38:449–56.
[172] Jensen KJ, Larsen N, Biering-Sørensen S, Andersen A, Eriksen HB, Monteiro I, et al. Heterologous immunological effects of early BCG vaccination in low-birth-weight infants in Guinea-Bissau: a randomized-controlled trial. J Infect Dis 2015; 211:956–67.
[173] Kleinnijenhuis J, Quintin J, Preijers F, Benn CS, Joosten LA, Jacobs C, et al. Long-lasting effects of BCG vaccination on both heterologous Th1/Th17 responses and innate trained immunity. J Innate Immun 2014;6:152–8.
[174] Luo J, Wang X, Ma F, Kang G, Ding Z, Ye C, et al. Long-term immunogenicity and immune persistence of live attenuated and inactivated hepatitis a vaccines: a report on additional observations from a phase IV study. Clin Microbiol Infect 2019;25:1422–7. https://doi.org/10.1016/j.cmi.2018.11.005.
[175] Gong W, Mao Y, Li Y, Qi Y. BCG Vaccination: A potential tool against COVID-19 and COVID-19-like Black Swan incidents. Int Immunopharmacol 2022;108: 108870. https://doi.org/10.1016/j.intimp.2022.108870.
[176] Irrgang P, Gerling J, Kocher K, Lapuente D, Steininger P, Habenicht K, et al. Class switch toward noninflammatory, spike-specific IgG4 antibodies after repeated SARS-CoV-2 mRNA vaccination. Sci Immunol 2022;8:eade2798.
[177] Buhre JS, Pongracz T, Künsting I, Lixenfeld AS, Wang W, Nouta J, et al. mRNA vaccines against SARS-CoV-2 induce comparably low long-term IgG Fc galactosylation and sialylation levels but increasing long-term IgG4 responses compared to an adenovirus-based vaccine. Front Immunol 2023:13.
[178] Chung AW, Ghebremichael M, Robinson H, Brown E, Choi I, Lane S, et al. Polyfunctional Fc-effector profiles mediated by IgG subclass selection distinguish RV144 and VAX003 vaccines. Sci Transl Med 2014;6. 228ra238-228ra238.
[179] Dobaño C, Quelhas D, Quintó L, Puyol L, Serra-Casas E, Mayor A, et al. Age-dependent IgG subclass responses to Plasmodium falciparum EBA-175 are differentially associated with incidence of malaria in Mozambican children. Clin Vaccine Immunol 2012;19:157–66.
[180] Diks AM, Versteegen P, Teodosio C, Groenland RJ, de Mooij B, Buisman A-M, et al. Age and primary vaccination background influence the plasma cell response to pertussis booster vaccination. Vaccines 2022;10:136.
[181] Uversky VN, Redwan EM, Makis W, Rubio-Casillas A. IgG4 Antibodies Induced by Repeated Vaccination May Generate Immune Tolerance to the SARS-CoV-2 Spike Protein. Vaccines 2023;11:991.
[182] Van der Lee S, Hendrikx LH, Sanders EA, Berbers GA, Buisman A-M. Whole-cell or acellular pertussis primary immunizations in infancy determines adolescent cellular immune profiles. Front Immunol 2018;9:51.
[183] Benn CS, Schaltz-Buchholzer F, Nielsen S, Netea MG, Aaby P. Randomized clinical trials of COVID-19 vaccines: Do adenovirus-vector vaccines have beneficial non-specific effects? Iscience 2023:26.
[184] Netea MG, Domínguez-Andrés J, Barreiro LB, Chavakis T, Divangahi M, Fuchs E, et al. Defining trained immunity and its role in health and disease. Nat Rev Immunol 2020;20:375–88.
[185] Noé A, Dang T, Axelrad C, Burrell, Germano S, Elia S, et al.. BNT162b2 COVID-19 vaccination in children alters cytokine responses to heterologous pathogens and Toll-like receptor agonists. *Frontiers in Immunology 14*, 1242380.
[186] Shann F. A live-vaccine-last schedule: saving an extra million lives a year? Clin Infect Dis 2021;72:1437–9. https://doi.org/10.1093/cid/ciaa292.
[187] OWD. Excess mortality during COVID-19. Available online: https:// ourworldindata.org/ (accessed on.
[188] Zaman SB, Hussain MA, Nye R, Mehta V, Mamun KT, Hossain N. A Review on Antibiotic Resistance: Alarm Bells are Ringing. Cureus 2017;9:e1403.
[189] Darby EM, Trampari E, Siasat P, Gaya MS, Alav I, Webber MA, et al. Molecular mechanisms of antibiotic resistance revisited. Nat Rev Microbiol 2023;21:280–95. https://doi.org/10.1038/s41579-022-00820-y.
[190] Rosini R, Nicchi S, Pizza M, Rappuoli R. Vaccines Against Antimicrobial Resistance *Front Immunol* 2020;11:1048. https://doi.org/10.3389/ fimmu.2020.01048.
[191] Micoli F, Bagnoli F, Rappuoli R, Serruto D. The role of vaccines in combatting antimicrobial resistance. Nat Rev Microbiol 2021;19:287–302. https://doi.org/ 10.1038/s41579-020-00506-3.
[192] Clift C, Salisbury DM. Enhancing the role of vaccines in combatting antimicrobial resistance. Vaccine 2017;35:6591–3. https://doi.org/10.1016/j. vaccine.2017.09.053.
[193] Costanzo V, Roviello GN. The Potential Role of Vaccines in Preventing Antimicrobial Resistance (AMR): An Update and Future Perspectives. Vaccines (Basel) 2023;11. https://doi.org/10.3390/vaccines11020333.
[194] Kennedy DA, Read AF. Why the evolution of vaccine resistance is less of a concern than the evolution of drug resistance. Proc Natl Acad Sci U S A 2018;115: 12878–86. https://doi.org/10.1073/pnas.1717159115.
[195] Read AF, Baigent SJ, Powers C, Kgosana LB, Blackwell L, Smith LP, et al. Imperfect Vaccination Can Enhance the Transmission of Highly Virulent Pathogens. PLoS Biol 2015;13:e1002198.
[196] Boots M. The Need for Evolutionarily Rational Disease Interventions: Vaccination Can Select for Higher Virulence. PLoS Biol 2015;13:e1002236.