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The early-life gut microbiome and vaccine efficacy

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Vaccines are one of the greatest successes of public health, preventing millions of cases of disease and death in children each year. However, the efficacy of many vaccines can vary greatly between infants from geographically and socioeconomically distinct locations. Differences in the composition of the intestinal microbiome have emerged as one of the main factors that can account for variations in immunisation outcomes. In this Review, we assess the influence of the gut microbiota upon early life immunity, focusing on two important members of the microbiota with health-promoting and immunomodulatory properties: *Bifidobacterium* and *Bacteroides*. Additionally, we discuss their immune stimulatory microbial properties, interactions with the host, and their effect on vaccine responses and efficacy in infants. We also provide an overview of current microbiota-based approaches to enhance vaccine outcomes, and describe novel microbe-derived components that could lead to safer, more effective vaccines and vaccine adjuvants.

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Introduction

The efficacy of many licensed vaccines varies within and between populations,[1] and in some cases, translates to little effectiveness.[2] Many intrinsic factors contribute to this variability in vaccine responses, including age, genetics (accounting for 20–40% of the variation between recipients), anaemia,[3] and gender.[4] In addition, a wide range of external factors can greatly affect vaccination outcomes, including vaccine composition and immunisation regimen,[5] pre-exposure to pathogens and chronic inflammation,[6] exposure to maternal antibodies,[7] nutritional status, and geographical location.[3]

Crucially, those at most risk of morbidity and mortality from infectious diseases are infants (aged 0–5 years) residing in low-income and middle-income countries. Low immunisation rates, coupled with poor immune responses to vaccines in these regions, are of concern, particularly for mucosal-delivered vaccines.[1] For instance, although 98% of Finnish children (aged 0·5–2 years) respond to oral rotavirus vaccination, only 58% of children from Nicaragua and 46% of infants from Bangladesh develop protective immunity.[8] For parenterally delivered vaccines, such as the BCG vaccine, protection ranges between 0–51% and 88–100% when comparing responses in African and European children.[9] There is therefore a pressing need to understand how vaccine responses in infants, particularly for those most at risk, can be improved.

The gastrointestinal tract microbiota in early life is key for the development and maturation of the infant mucosal and systemic immune system.[2] Intestinal microbial perturbation caused by hygiene, diet, socioeconomic, and environmental circumstances of both mothers and newborn babies is associated with distinct microbiota profiles in infants from Africa versus those in western Europe.[2] These differences in microbiota profiles in children (or patients) from low-income and middle-income countries are associated with decreased antibody-specific immune responses after vaccination, potentially explaining geographic and individual variability in vaccine efficacy.[2] However, other factors contributing to this discrepancy cannot be excluded.

Adjuvants can boost immunogenicity and vaccine efficacy. However, many adjuvants are considered unsafe,[10] with adverse effects that could contribute to vaccination hesitancy. Additionally, different types of adjuvants are required for parenteral and mucosal (oral) vaccines, driving vaccine costs higher and necessitating further research. A 2021 study by Yakabe and colleagues[10] reviewed the advantages and disadvantages of commonly used adjuvants, highlighting potential alternatives originating from the interaction between nutrition and the gastrointestinal tract microbiome.

Early-life colonisation and development of a beneficial gut microbiota–immune relationship

The gastrointestinal tract microbiome

The human gastrointestinal tract microbiome comprises a complex community of bacteria, viruses, archaea, and fungi, which varies in composition along the length of the gastrointestinal tract.[11] Microbial colonisation is initiated during birth, and changes continuously across the life course, with the first 1000 days of life representing the most vulnerable and unstable period with respect to ecosystem structure.[2,10] The gastrointestinal tract microbiota is essential for health, contributing to the maturation of the mucosal and systemic immune system,[12] maintaining tolerance,[13] resisting pathogen colonisation,[2] digesting dietary components, and providing micronutrients.[2] Structural and functional perturbations of the gastrointestinal tract microbiota are associated with various diseases, including metabolic disorders,[14] neurodegenerative conditions,[15] allergies,[16] autoimmune disorders,[17] and cancers.[18]

Notably, factors influencing the microbiome are similar to those impacting vaccine immune responses, underlining the mutual relationship between immunity and the gastrointestinal tract microbiota. During early life, additional factors that affect the developing gastrointestinal tract microbiota are the method of delivery,[19] preterm birth,[20] nutrition (breastmilk *vs* formula milk),[19] early use of antibiotics or probiotics,[21] and hygiene,[22] as discussed in the following sections.

Establishment of a microbial profile in early life

During and after birth, the infant is exposed to environmental antigens and microbes that promote the maturation of the immune system, the nature of which is dependent on the method of delivery.[19] Vaginally delivered infants are exposed to the maternal vaginal and faecal microbiota, leading to microbial profiles dominated by *Escherichia*, *Lactobacillus*, *Bacteroides*, and *Bifidobacterium*.[10] By contrast, newborn babies delivered by caesarean section have more contact with maternal skin and hospital-derived microbes, and are often colonised by *Streptococcus*, *Staphylococcus*, and *Enterococcus* species.[10] Early treatment with antibiotics negatively impacts this initial colonisation and subsequent immune system development. These early-life disturbances can lead to an increased risk of infections in the short term, and in the longer term are associated with increased risk of developing diseases associated with immunity and metabolism, such as atopy.[14] Thus, colonisation by the right microbes at the right time is crucial for effective establishment of immune defences and homoeostasis.[19]

Breastfeeding provides a supply of antimicrobial peptides, maternal antibodies, and innate immunity factors (eg, soluble TLR2 and 4, CD14, and MD2), which promote passive protection of the newborn baby and provide key dietary components that shape the infant's microbiota.[19] Human milk oligosaccharides enhance *Bifidobacterium* colonisation and persistence (making up to 80% of the total microbial community), which is less pronounced in infants fed by formula milk (5–30%).[23] Microbial metabolism of human milk oligosaccharides leads to the production of short-chain fatty acids (SCFAs), which are recognised by specific G-protein-coupled receptors bound to membranes, expressed by immune cells both systemically and in the gastrointestinal tract, and are key for developing immune tolerance.[19] Breastmilk also introduces microbes directly via the breastmilk microbiome, allowing further seeding of the infant intestine.[19,23]

Weaning and transitioning from breastmilk or formula-based nutrition to solid foods drives major changes in the gastrointestinal tract microbiota, with reductions in *Bifidobacterium* species, and introduction of *Ruminococcus*, *Akkermansia*, and *Prevotella* species.[24] Zmora and colleagues[25] provide a detailed overview of the influence of nutrition on the gut microbiota and its composition.

Influence of the infant gastrointestinal tract microbiome on immunity and vaccination

There is a growing appreciation for the role of the infant gastrointestinal tract microbiota in immunity from vaccines.[26] The gastrointestinal tract microbiota has been shown to promote the efficient stimulation of both humoral and cellular immune responses to vaccines via a variety of mechanisms (figure).[27] Microbiota stimulation is essential for the development and maturation of B cells that produce immunoglobulin (IgA), memory plasma cells through promotion of IgA class switching, and the development of germinal centres within the Peyer's patches in the lumen.[2] Additionally, antigen-specific T-cell responses are enhanced by the microbiota via mediation of type I interferon production by plasmacytoid dendritic cells.[28]

The mutualistic relationship between the gut microbiota and the immune system, together with genetic and environmental influences, could explain the variability of individual immune responses to vaccines.[2] Antibiotic-induced disturbances of the neonatal mouse microbiome, and use of immune-deficient germ-free pups, resulted in impairment of humoral responses to different adjuvanted and live-attenuated vaccines, characterised by a reduction in Th1 and Th17 response, and lower IgG and IgM production. Notably, this impairment was reversible after restoration of the microbiota via administration of specific flagellated *Escherichia coli* strains or faecal microbiota transfer.[29] The abundance of certain bacterial families, genera, and species have been linked to differences in immune responses to vaccines in humans, both positively and negatively. Huda and colleagues[30] observed a positive effect of Actinobacteria, but a negative correlation if Enterobacteriaceae predominated in response to systemic BCG, tetanus, and hepatitis B vaccines, and oral polio vaccine, in Bangladeshi infants. A follow-up study of Bangladeshi infants at 2 years of age showed that a high abundance of *Bifidobacterium* species in early life was positively associated to BCG, tetanus, and polio vaccines, with $CD4^+$ T-cell responses and detectable IgG and IgA at both 15 weeks and 2 years.[31] Comparative studies of microbial profiles in responding and non-responding children given the rotavirus vaccine, from Ghana, Pakistan, Bangladesh, and the Netherlands, revealed that the microbiota composition of responders from different low-income and middle-income countries showed more similarities to Dutch infant microbiota (ie, higher abundance of *Clostridium* cluster XI and Proteobacteria, and lowered amounts of the Bacteroidetes phylum) than to non-responders from the same cohort.[32] As the gastrointestinal tract microbiome has a multifactorial role in terms of activation and suppression of immune responses and subsequent effects on vaccine immunity, different microbiota-modulating interventions have been investigated to maximise vaccine efficacy.

Modulating the gastrointestinal tract microbiota to enhance immune protection

Use of prebiotics, probiotics, and antibiotics in vaccine trials

Studies of influenza and cholera vaccination in mice reported a positive association between different prebiotics (defined by Gibson and colleagues[33] for the International Scientific Association for Probiotics and Prebiotics as "a substrate that is selectively utilized by host microorganisms conferring a health benefit") and systemic immune responses after vaccination, linked to increases in the

abundance of *Bifidobacterium* and *Lactobacillus*, and to SCFA production.[34] As these bacterial genera are often used in commercial probiotic foods (defined by WHO[35] as "live microorganisms, which when consumed in adequate amounts, confer a health effect on the host"), their effects on vaccination responses have been investigated. A systematic review by Zimmerman and Curtis[36] in 2018 summarised the results of 26 interventional studies in humans using probiotics to enhance efficacy of 17 different vaccines, revealing positive outcomes in half of the studies. Trials of the use of probiotic *Lactobacillus* and *Bifidobacterium* strains in neonates and young children (aged 0–16 weeks) showed an elevated success rate when compared with adults, with the highest effects on humoral immunity occurring after vaccination against influenza, diphtheria, rotavirus, and polio. However, the lack of conformity in the design of the different studies (including bacterial strain used) makes it difficult to draw robust conclusions.[36]

Human studies have investigated the effect of antibiotic-mediated microbial depletion on the immune response to influenza, polio, rotavirus, tetanus, and BCG vaccination (due to ethical reasons, only the polio trial was performed in infants). Either no improvement or markedly reduced vaccine immunogenicity was observed, which links to the negative associations of antibiotic usage, microbiota disturbances, and an increase of immune-mediated diseases.[32]

Taken together, certain bacterial species are positively correlated with, or directly induce, enhanced humoral or cellular immunity in infant vaccine responders. *Bifidobacterium* is a good candidate for augmenting vaccination responses, and is also a key member of the healthy early-life gut microbiota. Other core gut microbiota members, such as *Bacteroides*, that can stimulate innate and adaptive immune responses, are emerging as novel microbiota therapeutics in vaccine research.

Immune modulatory features of *Bifidobacterium* and *Bacteroides*

In a murine obesity model, administration of *Bifidobacterium pseudocatenulatum* CECT7765 reduces systemic inflammation via restoration of a balanced state of regulatory T cells (Treg) and B lymphocytes, and lowers concentrations of proinflammatory cytokines interleukin 17a (IL-17A) and TNF (tumour necrosis factor).[37] Similar immune homoeostatic properties have been attributed to *Bifidobacterium bifidum* strains, which, via stimulation of dendritic cells in vitro, induce Th17 profiles and enhance the differentiation of Treg cells from naive lymphocytes.[12] Stimulation of peripheral T cells with lysates of *B bifidum* DSM 20082 increases $CD8^+$ T-cell cytotoxic activity without any affect on $CD4^+$ T-cell activity.[38] Although the mechanisms underpinning these immunomodulatory properties are unclear, several targets have been proposed. These include dietary fermentation products (eg, after metabolism of human milk oligosaccharides and other complex carbohydrates) leading to production of SCFAs (ie, acetate) and other metabolite byproducts that directly interact with host immune-cell receptors and facilitate cross-feeding of other commensal bacteria such as *Bacteroides* and *E coli*.[39] *Bifidobacterium longum* subspecies *longum* produces immunomodulatory compounds and proteins, such as extracellular serpin that irreversibly inactivates proinflammatory proteases. *B bifidum* MIMBb75 has previously been shown to have peptidoglycan hydrolase TgaA on its surface, which, via initiation of IL-2 production and monocyte-derived dendritic cell activation, promotes Treg expansion.[40]

In neonatal piglets, *B longum* strain AH1206 enhanced production of intestinal IL-10, whereas *Bifidobacterium animalis* subspecies *lactis* Bb12, *Bifidobacterium infantis* MCC12, and *Bifidobacterium breve* MCC1274 promoted immune maturation and immune homoeostasis.[41,42,43] More importantly, MCC12 and MCC1274 strains boosted B-cell and antiviral responses after rotavirus vaccination, showing how the immune-altering features of *Bifidobacterium* are specific to some strains. In humans, several studies have highlighted a positive association between a high abundance of the Actinobacteria phylum and certain *Bifidobacterium* strains with increased immune responses to different vaccines. High concentrations of *B longum* subspecies *infantis* in the gastrointestinal tract microbiome of Bangladeshi infants were correlated with increased $CD8^+$ T-cell and $CD4^+$

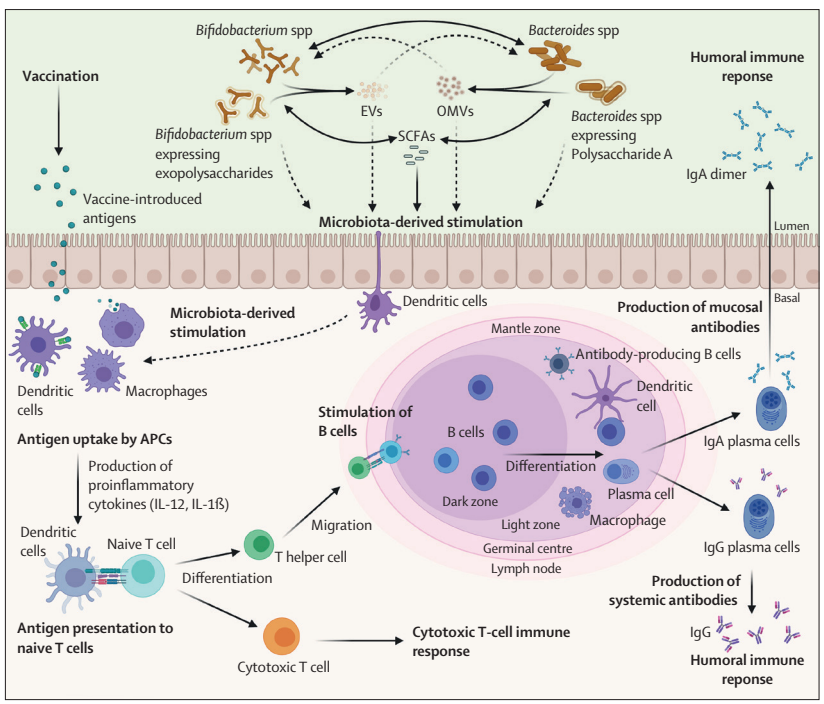

Figure: **Commensal gut microbiota members *Bifidobacterium*, *Bacteroides*, and their microbial products have immunomodulatory properties that can affect early-life vaccination outcomes**
APCs=antigen-presenting cells. EV=extracellular vesicle. Ig=immunoglobulin. IL=interleukin. OMV=outer membrane vesicle. SCFA=short chain fatty acid.

T-cell responses, and higher titres of IgG after vaccination against BCG, tetanus, and hepatitis B.[30,31] Chinese infants with high abundance of *B breve* showed increased polio-specific IgA responses after oral polio vaccination.[44] In vaccine supplementation studies, *B longum* BB536 was shown to enhance Th1 responses in infants via induction of interferon-γ (IFN-γ) secretion.[37]

Bacteroides is a major genus colonising the intestinal tract in infancy, and it is dominant throughout adult life. Certain species and strains have important mutualistic roles, from production of antimicrobial molecules to nutrient provision through breakdown of different glycans.[10]

With respect to *Bacteroides* and vaccine responsiveness, contradictory results have been reported. Rotavirus trials in Ghana found a negative association between elevated prevalence of members of the Bacteroidetes phylum,[45] whereas a similar study of Pakistani infants revealed increased concentrations of this phyla in vaccine responders.[32] Fix and colleagues[46] observed differences in the abundances of different *Bacteroides* strains in vaccine responders and non-responders in Nicaraguan infants. However, these findings were not statistically significant after multiple adjustments due to the small sample size, emphasising the need for larger studies to explore these associations.

A link between *Bacteroides* and vaccine responses can be expected given the prominent role they have in induction of homoeostatic immune priming. The unique lipoligosaccharide structure in *Bacteroides thetaiotaomicron* has adjuvant properties, inducing elevated concentrations of hepatitis B virus antigen-specific antibodies initiated by the hepatitis B virus vaccine, in a manner dependent on $CD4^+$ T cells, suggesting this lipoligosaccharide as an alternative adjuvant to the more toxic lipopolysaccharides of *E coli*.[47]

Immune stimulatory properties of different microbial products

SCFAs

SCFAs are produced by different members of the gut microbiota through fermentation of dietary complex carbohydrates, including those in breastmilk or prebiotics.[19] SCFAs contain less than six carbon atoms in their monocarboxylic acid carbon chain, with acetate (C2), propionate (C3), and butyrate (C4) being the most prevalent.[48] They are water soluble and can be directly absorbed by, transported into, or interact with, different cells including intestinal epithelial cells, sympathetic neurons, and immune cells.[49] They provide numerous beneficial health effects including an energy source for enterocytes, strengthening the epithelial barrier, changing metabolic processes, inhibiting enteric pathogen growth, mediators for ion absorption, and as signalling molecules in regulatory pathways of intestinal and systemic immunity.[49] Acetate produced by both *Bifidobacterium* and *Bacteroides*, and propionate production by *Bacteroides*,[49] will be discussed in more detail in the following sections.

Acetate can be enzymatically converted to acetyl-coenzyme A (acetyl-coA), and is used by many different microbiota members to produce butyrate, and as a major energy source in the tricarboxylic acid cycle.[49] Increased intracellular acetyl-coA in T cells activates mTOR, which drives differentiation of Th1 and Th17 T cells.[50] Acetate can also activate G-protein-coupled receptor 43 (GPR43) expressed on B cells, subsets of T cells, neutrophils, macrophages, dendritic cells, and intestinal epithelial cells, leading to proliferation of lamina propria Treg cells,[50] and regulation of autoantibody production and marginal zone B cells.[51] Activation of GPR43 influences neutrophil chemotaxis and degranulation, and TNF production by macrophages in adipose tissue.[52] Furthermore, dendritic cells lacking GPR43 are unable to induce class switching in B cells.[53] Mice with no SCFA-producing gastrointestinal tract bacteria have decreased plasma cell differentiation and deficiencies in homoeostatic and pathogen-specific antibody responses.[54] Acetate can potentiate vaccine responses through an enhanced production of antigen-specific IgA and IgG against cholera toxin in vitro, and stimulation of signalling molecules in dendritic cells essential for plasma cell differentiation.[55]

Propionate induces the differentiation and proliferation of murine Treg cells and expression of IL-10.[49] Activation of GPR15 and GPR43 by propionate also increases concentrations of colonic Treg cells via reduced expression of histone deacetylase 6 and 9 and inhibition of NF-κB signalling.[49] GPR41 activation by propionate alters bone marrow haematopoiesis, resulting in elevated concentrations of macrophage and dendritic cell precursors, and skews Th2 differentiation.[10] A study investigating the affect of SCFAs on influenza A virus infection revealed an essential role for SCFAs, including propionate, via activation of GPR43 to limit infection severity and concomitant pneumococcal superinfection.[56] GPR43 was proposed to be a coreceptor for influenza A virus entry, meaning that binding of SCFAs to this receptor inhibited virus entry and replication, which indicates potential adjuvant properties for influenza vaccines.

Exopolysaccharides

Exopolysaccharides are clusters of monosaccharides or oligosaccharides, comprising glucose, fructose, galactose, fucose, and rhamnose, which form either homopolysaccharides or heteropolysaccharides.[39] These can be secreted into the intestinal environment or are associated with the cell wall of the parent bacteria.[57] Expression of exopolysaccharides enhances adhesion to host cells, provides protection from digestion and environmental stress, and facilitates biofilm formation and longer-term colonisation in the gastrointestinal

tract.[57] Exopolysaccharides derived from different *Bifidobacterium* strains can be fermented by other microbes, altering the metabolite milieu and SCFA concentrations.[57] Exopolysaccharides can be recognised as microbe-associated molecular patterns via specific pattern-recognition receptors, such as TLR1, TLR2, or TLR6, expressed on the surface of macrophages and dendritic cells. The activation of these receptors, depending on the chemophysical properties of exopolysaccharides (eg, molecular weight or charge[39]), leads to the production of distinct proinflammatory and anti-inflammatory cytokine profiles, and respective differentiation of naive T cells in a strain-specific manner.[12]

The surface polysaccharide A of *Bacteroides fragilis* activates TLR2 on macrophages, and induces the Foxp3 Treg expansion and production of anti-inflammatory IL-10, which promotes robust anti-inflammatory responses during virus infection. Moreover, polysaccharide A activates TLR4 and secretion of TNF by colonic dendritic cells, providing increased natural resistance to viral infections.[58] Exopolysaccharides derived from *B longum* BCRC14634 were shown to enhance IL-10 production by macrophages,[59] with exopolysaccharides from *B longum* subspecies *longum* 35624 shown to suppress expansion of proinflammatory Th17 cells through the dampening of proinflammatory cytokine production, compared with an isogenic exopolysaccharide-negative mutant.[60] In some cases, the presence of exopolysaccharide has been associated with an immune silencing effect and an evasion of adaptive B-cell responses, as observed in *B breve* UCC2003. Moreover, this bifidobacterial strain and presence of exopolysaccharides were also associated with a lower abundance of proinflammatory IFN-γ, TNF, and IL-12.[61] Yu and colleagues[12] showed that *Bifidobacterium adolescentis* IF1-03 increased anti-inflammatory immune responses via increased IL-10 secretion through macrophages, with enhanced Treg concentrations, which required activation of TLR2 and signal transduction via the extracellular signal-regulated kinase, or p38 mitogen-activated protein kinase, and NF-κB pathway. Notably, an opposite effect was seen in the case of exopolysaccharides produced by *B adolescentis* IF1-11, which simulated macrophages to secrete high concentrations of proinflammatory IL-6, IL-17A, and TGF-β, and low amounts of IL-10, subsequently skewing T cells to Th17 cells. These studies highlight the immune-modulatory abilities of exopolysaccharides, dependent on strain. Previous work using *Lactobacillus*-derived exopolysaccharides has indicated they can act as novel vaccine adjuvants,[62] therefore further tests focusing on the role of *Bacteroides* and *Bifidobacterium*-associated exopolysaccharides after specific vaccinations are required.

Bacterial extracellular vesicles (BEVs)

BEVs are spherical, membrane-derived structures, ranging in size from 10 nm to 400 nm, which contain various components from the membrane and periplasm of the parent cell.[63] They are distinguished according to their membrane composition and structure, with their contents being influenced by environmental factors (eg, culture conditions or nutrient stress). This influence can result in quantitative and qualitative differences in DNA, RNA, lipopolysaccharides, enzymes, peptidoglycan, toxins, signalling molecules, metabolites, and virulence factors.[63] BEVs are not restricted to the gastrointestinal tract and have been detected in the bloodstream, from where they can easily access different tissues, including the brain.[64] A study by Aytar Çelik and colleagues[65] gives a detailed overview about different bacterial membrane vesicles, their characteristics, functions, and potential applications. BEVs generated by commensal bacteria contribute to cooperation and syntrophic interactions between members of complex microbial communities, and as mediators of interkingdom cross-talk between members of the gastrointestinal tract microbiota and the host.[63]

BEVs can activate immune cells and promote immune responses against the vesicles themselves and the parental cells.[66] The membrane lipids, proteins (including microbe-associated molecular patterns), danger signals, and exopolysaccharides can bind to and activate pattern recognition receptors and toll-like receptors, triggering different immune responses beyond the epithelium.[63] Vesicles from *B fragilis* carry capsular polysaccharide A, which, as stated previously, activates TLR2 on submucosal dendritic cells, and the extracellular vesicles are subsequently internalised in an actin-dependent manner, leading to increased concentrations of IL-10 and T-cell polarisation skewed towards Treg differentiation.[64] Findings by Durant and colleagues[67] showed that BEVs derived from *Bacteroides thetaiotaomicron* induce IL-10-dependent immunoregulatory responses in human mucosal and blood dendritic cells in a species-specific manner.

The non-replicative nature of BEVs and their inbuilt adjuvanticity, thermostability, and resistance to low pH and enzymatic degradation, offers multiple possibilities for vaccination design and delivery.[64] In addition, they can be administered without injections and directly to mucosal sites (eg, gastrointestinal and respiratory tract), reducing administration costs and reducing the risk of potential adverse effects linked to parental delivery.[64] Also, BEVs containing immunogenic components can promote strong innate and adaptive immune responses, and provide high amounts of protection against infectious diseases.[68]

BEVs from pathogenic bacteria have been successfully used in vaccine formulations, and several extracellular vesicles vaccines against *Vibrio cholerae* and serogroup B *Neisseria meningitidis* are licensed, with those for serogroup B *N meningitidis* showing potential cross-species protection against *Neisseria gonorrhoeae*.[69] Other studies have shown that BEVs produced by microbiota

Search strategy and selection criteria

Between May 5, 2021, and Sept 30, 2021 we searched PubMed (1996 to present) with the search terms: (microbiome OR microbiota OR *Bacteroides* OR *Bifidobacterium* OR short chain fatty acid OR acetate OR propionate OR exopolysaccharides OR extracellular membrane vesicles OR probiotics OR prebiotics OR antibiotics) AND (vaccine OR vaccination OR humoral, cellular, vaccine, immune, mucosal response OR immunisation OR humoral, cellular, mucosal immunity OR antibodies OR immunoglobulin OR adjuvants) AND/OR (breast milk OR delivery OR antibiotics OR nutrition), disregarding language limitations. A publication year filter of 2019 and onwards was applied at first, but was disregarded for fundamental publications and essential studies looking at the microbiota–vaccination relationship. 328 studies and reviews were identified and taken into consideration. We limited the number of reviews based on the focus of our Review and their respective publication year.

members, including bioengineered BEVs, have been used to deliver antigens from pathogens. Carvalho and colleagues[70] showed that BEVs deriving from *Bacteroides thetaiotaomicron* and expressing different *Yersinia pestis* antigens elicited specific and strong immune responses in vivo, including serum IgG and mucosal IgA, which were able to clear plague infection. Lee and colleagues[71] showed that BEVs from mutated, non-pathogenic *E coli* have adjuvant properties on antigen-specific T-cell responses with decreased toxicity.

Conclusion and future perspectives

The human gut microbiota is emerging as an important determinant of vaccine responsiveness, with two prominent members, *Bifidobacterium* and *Bacteroides*, being able to influence immunity and individual vaccine immune responses. This factor is particularly important in early life as these two genera are important constituents of the developing, healthy infant gut microbiota, but are highly susceptible to early-life disturbances, such as caesarean-section birth, formula milk versus breastmilk, and antibiotic usage.[19]

Focusing on these health-promoting genera and exploiting their immunomodulatory properties could lead to safer approaches to enhancing infant immunity and vaccine efficacy. Novel strategies, with either whole bacteria or their products and metabolites to modulate immune responses, are possible, such as observed in the augmentation of responses to immune checkpoint inhibitors in cancer.[72] Given that some *Bifidobacterium* species are generally recognised as safe microorganisms,[73] preclinical studies can rapidly advance to the clinic. Focusing on specific microbial products, such as BEVs, represents a more refined approach. The success of the already established BEV vaccine against cholera and type B meningitis,[69] and the promising results with commensal BEV antigen carriers against plague and influenza,[70] could give rise to a novel vaccine generation based on immunomodulatory BEVs of commensal origin with high efficacy and biosafety standards on a global scale. Nevertheless, further studies will be required to identify which strains produce novel adjuvant-like BEVs from early-life microbiota members such as *Bifidobacterium* and *Bacteroides*. For instance, few studies have investigated BEVs from *Bifidobacterium* strains that might represent a promising avenue for future potent adjuvants.[74] Use of BEVs as a vaccine–antigen delivery vehicle that possesses inherent adjuvanticity could also potentially result in safe, effective mucosal vaccines. Thus, a greater understanding and characterisation of key strains from the healthy infant microbiome, and their byproducts and metabolites, could give rise to a new generation of safe, needle-free, and economical vaccine-boosting therapies, ideally suited to use in low-income and middle-income countries. Key features, like dosage, vaccine design, and time of administration, will be important considerations,[2] with in-depth studies to provide underlying immune-associated mechanisms representing a substantial challenge because of their molecular complexity. With the launch of the Immunisation Agenda 2030 by WHO,[75] the need for full understanding has become even more important.

Contributors

AJ and LJH conceptualised the Review and methodology. AJ did the formal analysis (literature review) and generated the figure with BioRender. All authors prepared the original draft, and reviewed and edited it. SRC and LJH provided supervision and acquired funding.

Declaration of interests

We declare no competing interests.

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References

1 Praharaj I, John SM, Bandyopadhyay R, Kang G. Probiotics, antibiotics and the immune responses to vaccines. *Philos Trans R Soc Lond B Biol Sci* 2015; **370:** 20140144.

2 Ciabattini A, Olivieri R, Lazzeri E, Medaglini D. Role of the microbiota in the modulation of vaccine immune responses. *Front Microbiol* 2019; **10:** 1305.

3 Hill DL, Carr EJ, Rutishauser T, et al. Immune system development varies according to age, location, and anemia in African children. *Sci Transl Med* 2020; **12:** eaaw9522.

4 Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol* 2016; **16:** 626–38.

5 Nascimento Silva JR, Camacho LAB, Siqueira MM, et al. Mutual interference on the immune response to yellow fever vaccine and a combined vaccine against measles, mumps and rubella. *Vaccine* 2011; **29:** 6327–34.

6 Grassly NC, Jafari H, Bahl S, et al. Waning intestinal immunity after vaccination with oral poliovirus vaccines in India. *J Infect Dis* 2012; **205:** 1554–61.

7 Parker EPK, Bronowski C, Sindhu KNC, et al. Impact of maternal antibodies and microbiota development on the immunogenicity of oral rotavirus vaccine in African, Indian, and European infants. *Nat Commun* 2021; **12:** 7288.

8 Burnett E, Parashar UD, Tate JE. Real-world effectiveness of rotavirus vaccines, 2006–19: a literature review and meta-analysis. *Lancet Glob Health* 2020; **8:** e1195–202.

9 Lalor MK, Ben-Smith A, Gorak-Stolinska P, et al. Population differences in immune responses to BCG in infancy. *J Infect Dis* 2009; **199:** 795–800.

10 Yakabe K, Uchiyama J, Akiyama M, Kim YG. Understanding host immunity and the gut microbiota inspires the new development of vaccines and adjuvants. *Pharmaceutics* 2021; **13:** 163.

11 Ruan W, Engevik MA, Spinler JK, Versalovic J. Healthy human gastrointestinal microbiome: composition and function after a decade of exploration. *Dig Dis Sci* 2020; **65:** 695–705.

12 Yu R, Zuo F, Ma H, Chen S. Exopolysaccharide-producing *Bifidobacterium adolescentis* strains with similar adhesion property induce differential regulation of inflammatory immune response in Treg/Th17 axis of DSS-colitis mice. *Nutrients* 2019; **11:** 782.

13 Vlasova AN, Takanashi S, Miyazaki A, Rajashekara G, Saif LJ. How the gut microbiome regulates host immune responses to viral vaccines. *Curr Opin Virol* 2019; **37:** 16–25.

14 Valitutti F, Cucchiara S, Fasano A. Celiac disease and the microbiome. *Nutrients* 2019; **11:** e2403.

15 Ma Q, Xing C, Long W, Wang HY, Liu Q, Wang RF. Impact of microbiota on central nervous system and neurological diseases: the gut-brain axis. *J Neuroinflammation* 2019; **16:** 53.

16 Zhao W, Ho HE, Bunyavanich S. The gut microbiome in food allergy. *Ann Allergy Asthma Immunol* 2019; **122:** 276–82.

17 Brown EM, Kenny DJ, Xavier RJ. Gut microbiota regulation of T cells during inflammation and autoimmunity. *Annu Rev Immunol* 2019; **37:** 599–624.

18 Teng NMY, Price CA, McKee AM, Hall LJ, Robinson SD. Exploring the impact of gut microbiota and diet on breast cancer risk and progression. *Int J Cancer* 2021; **149:** 494–504.

19 Lyons KE, Ryan CA, Dempsey EM, Ross RP, Stanton C. Breast milk, a source of beneficial microbes and associated benefits for infant health. *Nutrients* 2020; **12:** e1039.

20 Humberg A, Fortmann I, Siller B, et al. Preterm birth and sustained inflammation: consequences for the neonate. *Semin Immunopathol* 2020; **42:** 451–68.

21 Butel M-J, Waligora-Dupriet A-J, Wydau-Dematteis S. The developing gut microbiota and its consequences for health. *J Dev Orig Health Dis* 2018; **9:** 590–97.

22 Munyaka PM, Khafipour E, Ghia JE. External influence of early childhood establishment of gut microbiota and subsequent health implications. *Front Pediatr* 2014; **2:** 109.

23 Kordy K, Gaufin T, Mwangi M, et al. Contributions to human breast milk microbiome and enteromammary transfer of *Bifidobacterium breve*. *PLoS One* 2020; **15:** e0219633.

24 Herman DR, Rhoades N, Mercado J, Argueta P, Lopez U, Flores GE. Dietary habits of two to nine-year-old American children are associated with gut microbiome composition. *J Acad Nutr Diet* 2020; **120:** 517–34.

25 Zmora N, Suez J, Elinav E. You are what you eat: diet, health and the gut microbiota. *Nat Rev Gastroenterol Hepatol* 2019; **16:** 35–56.

26 Henneke P, Kierdorf K, Hall LJ, Sperandio M, Hornef M. Perinatal development of innate immune topology. *eLife* 2021; **10:** e67793.

27 Pulendran B, Ahmed R. Immunological mechanisms of vaccination. *Nat Immunol* 2011; **12:** 509–17.

28 Schaupp L, Muth S, Rogell L, et al. Microbiota-induced type I interferons instruct a poised basal state of dendritic cells. *Cell* 2020; **181:** 1080–96.

29 Kim D, Kim YG, Seo SU, et al. Nod2-mediated recognition of the microbiota is critical for mucosal adjuvant activity of cholera toxin. *Nat Med* 2016; **22:** 524–30.

30 Huda MN, Lewis Z, Kalanetra KM, et al. Stool microbiota and vaccine responses of infants. *Pediatrics* 2014; **134:** e362–72.

31 Huda MN, Ahmad SM, Alam MJ, et al. *Bifidobacterium* abundance in early infancy and vaccine response at 2 years of age. *Pediatrics* 2019; **143:** e20181489.

32 Harris V, Ali A, Fuentes S, et al. Rotavirus vaccine response correlates with the infant gut microbiota composition in Pakistan. *Gut Microbes* 2018; **9:** 93–101.

33 Gibson GR, Hutkins R, Sanders ME, et al. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol* 2017; **14:** 491–502.

34 Di Luccia B, Ahern PP, Griffin NW, et al. Combined prebiotic and microbial intervention improves oral cholera vaccination responses in a mouse model of childhood undernutrition. *Cell Host Microbe* 2020; **27:** 899–908.

35 FAO, WHO. Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk and live lactic acid bacteria. Geneva: World Health Organization, 2001: 1–4.

36 Zimmermann P, Curtis N. The influence of probiotics on vaccine responses—a systematic review. *Vaccine* 2018; **36:** 207–13.

37 Wu BB, Yang Y, Xu X, Wang WP. Effects of *Bifidobacterium* supplementation on intestinal microbiota composition and the immune response in healthy infants. *World J Pediatr* 2016; **12:** 177–82.

38 Pareek S, Kurakawa T, Das B, et al. Comparison of Japanese and Indian intestinal microbiota shows diet-dependent interaction between bacteria and fungi. *NPJ Biofilms Microbiomes* 2019; **20:** 37.

39 Hidalgo-Cantabrana C, López P, Gueimonde M, et al. Immune modulation capability of exopolysaccharides synthesised by lactic acid bacteria and *Bifidobacteria*. *Probiotics Antimicrob Proteins* 2012; **4:** 227–37.

40 Verma R, Lee C, Jeun EJ, et al. Cell surface polysaccharides of *Bifidobacterium bifidum* induce the generation of Foxp3+ regulatory T cells. *Science Immunology* 2018; published online Oct 26. https://www.science.org/doi/pdf/10.1126/sciimmunol.aat6975.

41 Herfel TM, Jacobi SK, Lin X, et al. Dietary supplementation of *Bifidobacterium longum* strain AH1206 increases its cecal abundance and elevates intestinal interleukin-10 expression in the neonatal piglet. *Food Chem Toxicol* 2013; **60:** 116–22.

42 Kandasamy S, Chattha KS, Vlasova AN, Rajashekara G, Saif LJ. *Lactobacilli* and *Bifidobacteria* enhance mucosal B cell responses and differentially modulate systemic antibody responses to an oral human rotavirus vaccine in a neonatal gnotobiotic pig disease model. *Gut Microbes* 2014; **5:** 639–51.

43 Ishizuka T, Kanmani P, Kobayashi H, et al. Immunobiotic *Bifidobacteria* strains modulate rotavirus immune response in porcine intestinal epitheliocytes via pattern recognition receptor signaling. *PLoS One* 2016; **11:** e0152416.

44 Zhao T, Li J, Fu Y, et al. Influence of gut microbiota on mucosal IgA antibody response to the polio vaccine. *NPJ Vaccines* 2020; **5:** 47.

45 Harris VC, Armah G, Fuentes S, et al. Significant correlation between the infant gut microbiome and rotavirus vaccine response in rural Ghana. *J Infect Dis* 2017; **215:** 34–41.

46 Fix J, Chandrashekhar K, Perez J, et al. Association between gut microbiome composition and rotavirus vaccine response among Nicaraguan infants. *Am J Trop Med Hyg* 2020; **102:** 213–19.

47 Chilton PM, Hadel DM, To TT, Mitchell TC, Darveau RP. Adjuvant activity of naturally occurring monophosphoryl lipopolysaccharide preparations from mucosa-associated bacteria. *Infect Immun* 2013; **81:** 3317–25.

48 Liu H, Wang J, He T, et al. Butyrate: a double-edged sword for health? *Adv Nutr* 2018; **9:** 21–29.

49 Chénard T, Prévost K, Dubé J, Massé E. Immune system modulations by products of the gut microbiota. *Vaccines (Basel)* 2020; **8:** E461.

50 Delgoffe GM, Kole TP, Zheng Y, et al. The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity* 2009; **30:** 832–44.

51 Rohrbeck L, Adori M, Wang S, et al. GPR43 regulates marginal zone B-cell responses to foreign and endogenous antigens. *Immunol Cell Biol* 2021; **99:** 234–43.

52 Nakajima A, Nakatani A, Hasegawa S, et al. The short chain fatty acid receptor GPR43 regulates inflammatory signals in adipose tissue M2-type macrophages. *PLoS One* 2017; **12:** e0179696.

53 Wu W, Sun M, Chen F, et al. Microbiota metabolite short-chain fatty acid acetate promotes intestinal IgA response to microbiota which is mediated by GPR43. *Mucosal Immunol* 2017; **10:** 946–56.

54 Kim M, Qie Y, Park J, Kim CH. Gut microbial metabolites fuel host antibody responses. *Cell Host Microbe* 2016; **20:** 202–14.

55 Yang W, Xiao Y, Huang X, et al. Microbiota metabolite short-chain fatty acids facilitate mucosal adjuvant activity of cholera toxin through GPR43. *J Immunol* 2019; **203:** 282–92.

56 Bolognini D, Dedeo D, Milligan G. Metabolic and inflammatory functions of short-chain fatty acid receptors. *Curr Opin Endocr Metab Res* 2021; **16:** 1–9.

57 Püngel D, Treveil A, Dalby MJ, et al. *Bifidobacterium breve* UCC2003 exopolysaccharide modulates the early life microbiota by acting as a potential dietary substrate. *Nutrients* 2020; **12:** 948.

58 Stefan KL, Kim MV, Iwasaki A, Kasper DL. Commensal microbiota modulation of natural resistance to virus infection. *Cell* 2020; **183:** 1312–24.

59 Wu MH, Pan TM, Wu YJ, Chang SJ, Chang MS, Hu CY. Exopolysaccharide activities from probiotic *bifidobacterium*: immunomodulatory effects (on J774A.1 macrophages) and antimicrobial properties. *Int J Food Microbiol* 2010; **144:** 104–10.

60 Schiavi E, Gleinser M, Molloy E, et al. The surface-associated exopolysaccharide of *Bifidobacterium longum* 35624 plays an essential role in dampening host proinflammatory responses and repressing local T_H17 responses. *Appl Environ Microbiol* 2016; **82:** 7185–96.

61 Fanning S, Hall LJ, Cronin M, et al. *Bifidobacterial* surface-exopolysaccharide facilitates commensal-host interaction through immune modulation and pathogen protection. *Proc Natl Acad Sci USA* 2012; **109:** 2108–13.

62 Xiu L, Zhang H, Hu Z, et al. Immunostimulatory activity of exopolysaccharides from probiotic *Lactobacillus casei* WXD030 strain as a novel adjuvant in vitro and in vivo. *Food Agric Immunol* 2018; **29:** 1086–105.

63 Jiang L, Schinkel M, van Essen M, Schiffelers RM. Bacterial membrane vesicles as promising vaccine candidates. *Eur J Pharm Biopharm* 2019; **145:** 1–6.

64 Stentz R, Carvalho AL, Jones EJ, Carding SR. Fantastic voyage: the journey of intestinal microbiota-derived microvesicles through the body. *Biochem Soc Trans* 2018; **46:** 1021–27.

65 Aytar Çelik P, Derkuş B, Erdoğan K, et al. Bacterial membrane vesicle functions, laboratory methods, and applications. *Biotechnol Adv* 2022; **54:** 107869.

66 Cai W, Kesavan DK, Wan J, Abdelaziz MH, Su Z, Xu H. Bacterial outer membrane vesicles, a potential vaccine candidate in interactions with host cells based. *Diagn Pathol* 2018; **13:** 95.

67 Durant L, Stentz R, Noble A, et al. *Bacteroides thetaiotaomicron*-derived outer membrane vesicles promote regulatory dendritic cell responses in health but not in inflammatory bowel disease. *Microbiome* 2020; **8:** 88.

68 Li M, Zhou H, Yang C, et al. Bacterial outer membrane vesicles as a platform for biomedical applications: an update. *J Control Release* 2020; **323:** 253–68.

69 Leduc I, Connolly KL, Begum A, et al. The serogroup B meningococcal outer membrane vesicle-based vaccine 4CMenB induces cross-species protection against *Neisseria gonorrhoeae*. *PLoS Pathog* 2020; **16:** e1008602.

70 Carvalho AL, Miquel-Clopés A, Wegmann U, et al. Use of bioengineered human commensal gut bacteria-derived microvesicles for mucosal plague vaccine delivery and immunization. *Clin Exp Immunol* 2019; **196:** 287–304.

71 Lee DH, Kim SH, Kang W, et al. Adjuvant effect of bacterial outer membrane vesicles with penta-acylated lipopolysaccharide on antigen-specific T cell priming. *Vaccine* 2011; **29:** 8293–301.

72 Rezasoltani S, Yadegar A, Asadzadeh Aghdaei H, Reza Zali M. Modulatory effects of gut microbiome in cancer immunotherapy: a novel paradigm for blockade of immune checkpoint inhibitors. *Cancer Med* 2021; **10:** 1141–54.

73 Borriello SP, Hammes WP, Holzapfel W, et al. Safety of probiotics that contain *Lactobacilli* or *Bifidobacteria*. *Clin Infect Dis* 2003; **36:** 775–80.

74 Nishiyama K, Takaki T, Sugiyama M, et al. Extracellular vesicles produced by *Bifidobacterium longum* export mucin-binding proteins. *Appl Environ Microbiol* 2020; **86:** e01464-20.

75 WHO. Immunization Agenda 2030. Geneva: World Health Organization, 2020.