Allegati:

- **INTRODUCTION**
  - The immune system's first steps
- **REVIEW:**
  - Prenatal development of human immunity J.-E. Park et al. 604
  - Microbial–host molecular exchange and its functional consequences in early mammalian life S. C. Ganal-Vonarburg et al. 608
  - Contributions of maternal and fetal antiviral immunity in congenital disease L. J. Yockey et al. 612
  - Vaccination strategies to enhance immunity in neonates T. R. Kollmann et al.

A mother breastfeeds her newborn baby, transferring mucosal and systemic immune memory via her milk.

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THE IMMUNE SYSTEM'S FIRST STEPS

By **Seth Thomas Scanlon**

In the early 1950s, Peter Medwar and colleagues showed that transplant tolerance could be induced in adult mice by inoculating them in utero with cells from a donor strain. This elegant experiment revealed that the immune system in early life is functionally distinct and responsive to programming that persists into adulthood. Nearly 70 years later, the complexity and developmental trajectory of the fetal–neonatal immune system are much better understood. We have also begun to appreciate that the immune system in early life does not develop in isolation but is instead strongly influenced by maternal cells, commensal microbes, and pathogens.

This special issue surveys recent advances in the field of early life immunology. Review articles highlight distinctive features of human fetal immune system development elucidated by unbiased multi-omics analysis, the impact of commensal metabolites and xenobiotics on immunity before and after birth, how maternal and fetal immune components work together to combat viral infections during pregnancy (and what happens when these mechanisms fail), and the potential of vaccination approaches to boost fetal–maternal immunity and protect neonates against the pathogens that most frequently cause them harm.

These discoveries should inform future public health initiatives and draw renewed attention to the vulnerability of children in early life, laying the groundwork for vaccination strategies to target pathogens that cause congenital and neonatal infections as well as therapies to treat disorders such as stillbirth and prematurity. Evidence is also mounting that immune system programming that starts in early life may influence the risk of developing conditions such as allergic, autoimmune, reproductive, and neuropsychiatric disorders in later life, further underscoring the translational implications of this kind of research.

The immune system's first steps

Seth Thomas Scanlon

Science **368** (6491), 598-599.
DOI: 10.1126/science.abc3140

REVIEW

Prenatal development of human immunity

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The blood and immune systems develop in parallel during early prenatal life. Waves of hematopoiesis separated in anatomical space and time give rise to circulating and tissue-resident immune cells. Previous observations have relied on animal models, which differ from humans in both their developmental timeline and exposure to microorganisms. Decoding the composition of the human immune system is now tractable using single-cell multi-omics approaches. Large-scale single-cell genomics, imaging technologies, and the Human Cell Atlas initiative have together enabled a systems-level mapping of the developing human immune system and its emergent properties. Although the precise roles of specific immune cells during development require further investigation, the system as a whole displays malleable and responsive properties according to developmental need and environmental challenge.

Animal model systems have provided fundamental evidence that shapes our understanding of developmental hematopoiesis. Studies performed in mouse, zebrafish, and chicken have established that blood and immune system development occur across distinct anatomical sites (Fig. 1). The first blood cells are extraembryonic, developing in close association with endothelial cells of the yolk sac (*1*). Embryonic hematopoietic stem cells (HSCs), capable of repopulating an adult host in transplant assays, originate from the aorta gonad mesonephros (AGM) region (*2*). The fetal liver and bone marrow (BM) are subsequently seeded by both yolk sac–derived progenitors and AGM-derived HSCs (*3*). However, developmental timelines are not chronologically identical between species. For example, mouse fetal thymus is notably immature compared with human thymus, which supports complete naïve T cell differentiation in utero (*4*). Furthermore, some population-defining markers are poorly conserved, making it difficult to directly apply findings from animal studies to humans. Increasingly, the influence of maternofetal microbial exposure on the fetal immune development is recognized, and both commensal and pathogenic microbial repertoire differs among species (see accompanying reviews). Studies directed at human immune development have been hampered by tissue access and experimental limitations, but single-cell multi-omics technologies have expedited new findings. In this review, we discuss how these technologies have provided an unprecedented view of early life immunity. We describe key insights into how immune development is layered across time and space and explain how immune cells both prepare the fetus for antigen challenge and adopt noncanonical roles in development.

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From single cells to system-level development

The challenge of unraveling blood and immune system development in the prenatal human requires a high-performance tool kit. Single-cell RNA sequencing (scRNA-seq) has emerged as a powerful tool for the systematic understanding of the immune system, permitting an unbiased identification of cell state and the resolution of complex mixtures of cells (*5*). Droplet-based scRNA-seq methods, such as 10X, are now scalable to the extent that whole organs can be adequately sampled. For example, our group has profiled single cells from yolk sac and liver to reconstruct early hematopoiesis, from thymus to capture T cell development, and from skin and kidney to elucidate the seeding of peripheral organs (*4*, *6*). Computational techniques have permitted comparison of cell states across tissues and prediction of critical receptor ligand interactions that shape immune cell fate in specific tissues (*7*). Correlation with imaging techniques—for example, in situ transcriptomics—has allowed comprehensive characterization of tissue microenvironments (*4*, *7*–*9*). Developmental trajectories have been inferred within single tissues, as cells are captured at varying stages of differentiation and by integrating samples from a range of gestational ages. This tool kit is beginning to provide a comprehensive overview of early immune development. Meanwhile, considerable challenges remain in tracing the origin of specific immune cells to distinct waves of hematopoiesis. Advances in single-cell DNA sequencing combined with analytical techniques to track distinct clones may bring us closer to this goal.

The developing immune system in space and time

In this section, we follow human immune system development across space and time. We begin by discussing cell types as they first emerge in the yolk sac or fetal liver, before considering the thymus as a key site of T cell development. This cannot be an exhaustive description of immune composition because about 40 immune cell states have been identified in these tissues to date. Instead, we focus on how single-cell multi-omics approaches have advanced our understanding of the human fetal immune system (Fig. 2).

Yolk sac and AGM

An analysis of the human embryonic yolk sac demonstrates the presence of HSC-like progenitors, macrophages, mast cells (MCs), natural killer (NK) cell progenitors, and innate lymphoid cell (ILC) progenitors alongside megakaryocytes and erythroid cells from four postconception weeks (PCW) (*6*).

Macrophage origin has been intensively studied because tissue macrophages arise independently from HSCs and self-renew under homeostatic conditions in mouse models (*10*). Tissue-resident macrophages in the liver, lung, brain, and epidermis were shown by fate mapping to arise from yolk sac hematopoiesis through the erythromyeloid progenitor (*11*, *12*). Although the yolk sac contribution is retained in some tissues (e.g., the liver, brain, and epidermis), macrophages are gradually replaced by HSC-derived monocytes at other sites (e.g., the gut, lung, and heart). This process depends in part on how "open" the niche remains to circulating cells (*10*). In the mouse, the precise contributions of the first versus second waves of yolk sac hematopoiesis and whether the macrophages arise from a monocyte intermediate remain unresolved (*10*). In human fetal development, tissue-specific macrophages are observed from the earliest time points sampled (*6*, *13*, *14*). Single-cell dissection of human AGM revealed a distinct hemogenic endothelial population that gives rise to macrophages (*13*). By 6 PCW, the embryonic pancreas is laden with macrophages, microglia accompany the developing brain, and Hofbauer cells line the placenta (*7*, *14*, *15*). Identification of these cells in appreciable numbers before the onset of fetal liver hematopoiesis at 6 to 9 PCW lends support to yolk sac or AGM-derived macrophages seeding peripheral tissues. Attempts to use transcriptional similarity between yolk sac macrophages and fetal liver macrophages to parse tissue macrophage ontogeny are not sufficiently reliable owing to environmentally related gene expression after tissue residency. However, these profiles have allowed characterization of macrophage diversity essential for development, for example, erythroid island macrophages providing support for erythropoiesis

and Kupffer cells with prominent scavenging function in the fetal liver (*6*).

In parallel to macrophages, fate-mapping studies have demonstrated that tissue MCs arise from both yolk sac and HSC-derived precursors in mouse and that patterns of yolk sac MC retention are tissue specific (*16*, *17*). In human development, a clear MC signature is present in both the yolk sac and fetal liver (*6*). Connective tissue MCs in fetal skin and kidney are closely related to fetal liver MCs by single-cell gene expression profile (*6*). This early dedication by the embryo to MC production is puzzling. The best-characterized function of MCs is their participation in allergic responses on immunoglobulin E (IgE) binding via the high-affinity IgE receptor (*18*). Liver and yolk sac MCs appear ill prepared for this task, because neither expresses the IgE receptor alpha subunit gene (*FCER1A*) (*6*). Early MC production may occur to equip developing mucosal sites and connective tissues with resident immune cells or to provide a pool of pathogen-associated molecular pattern–responsive innate effectors. However, additional functions in supporting angiogenesis are predicted. In mice, embryonic skin MCs express genes involved in vascular and neural patterning (*6*, *16*). In adult mammals, MCs support both physiological and inflammatory angiogenesis (*18*). The role of MCs in prenatal vascular development warrants further investigation.

NK cells, ILC progenitors, and their common lymphoid progenitors can be identified from yolk sac and fetal liver single-cell transcriptome (*6*, *19*). In later stages, they are found as more diverse and differentiated cells in multiple fetal organs (*9*, *20*). In contrast to the maternal decidual NK cells whose role during pregnancy has been well characterized (*7*, *21*), our understanding of fetal NK cell function to date is limited. Although fetal NK cells are considered to be immature and hyporeactive compared with adult NK cells, they already possess killer activity (*22*, *23*). Moreover, fetal or infant NK cells resemble their adult counterparts at several levels, suggesting that they are poised to respond when the right stimuli, such as viral infections, are present (*23*). Concordantly, NK cells are abundant in infant intestines, are equipped with cytolytic granules, and display superior degranulation activity compared with adult intestinal NK cells (*20*). In addition to NK cells, other ILCs have been shown to be enriched in the fetus compared with infants (*24*). Among them, innate lymphoid tissue inducer (LTi) cells play a critical role in the formation of secondary lymphoid organs (*25*, *26*). By interacting with stromal cells, LTi cells induce positive feedback to recruit additional LTi cells as well as other immune cells, generating a lymphoid environment (*27*). Thus, innate lymphocytes develop very early in the human embryo and are involved in both tissue protection and remodeling.

This earliest wave of hematopoiesis in the yolk sac displays dedication to immune cells with structural and physiological roles alongside equipping the embryo with a basic repertoire of innate immune effectors. The precise roles of these cells in tissue development and the checkpoints that prevent damaging immune responses in utero require further investigation.

Liver and BM

Definitive HSCs can generate the full complement of erythroid, megakaryocyte, myeloid, and lymphoid cell lineages in fetal liver, but neutrophils remain absent until BM hematopoiesis is established (*28*).

In contrast to macrophages, monocytes and dendritic cells (DCs) are considered HSC-dependent populations. In the mouse, both are traceable to a clonogenic precursor in BM named the macrophage-DC progenitor (*29*). In human development, the first signs of

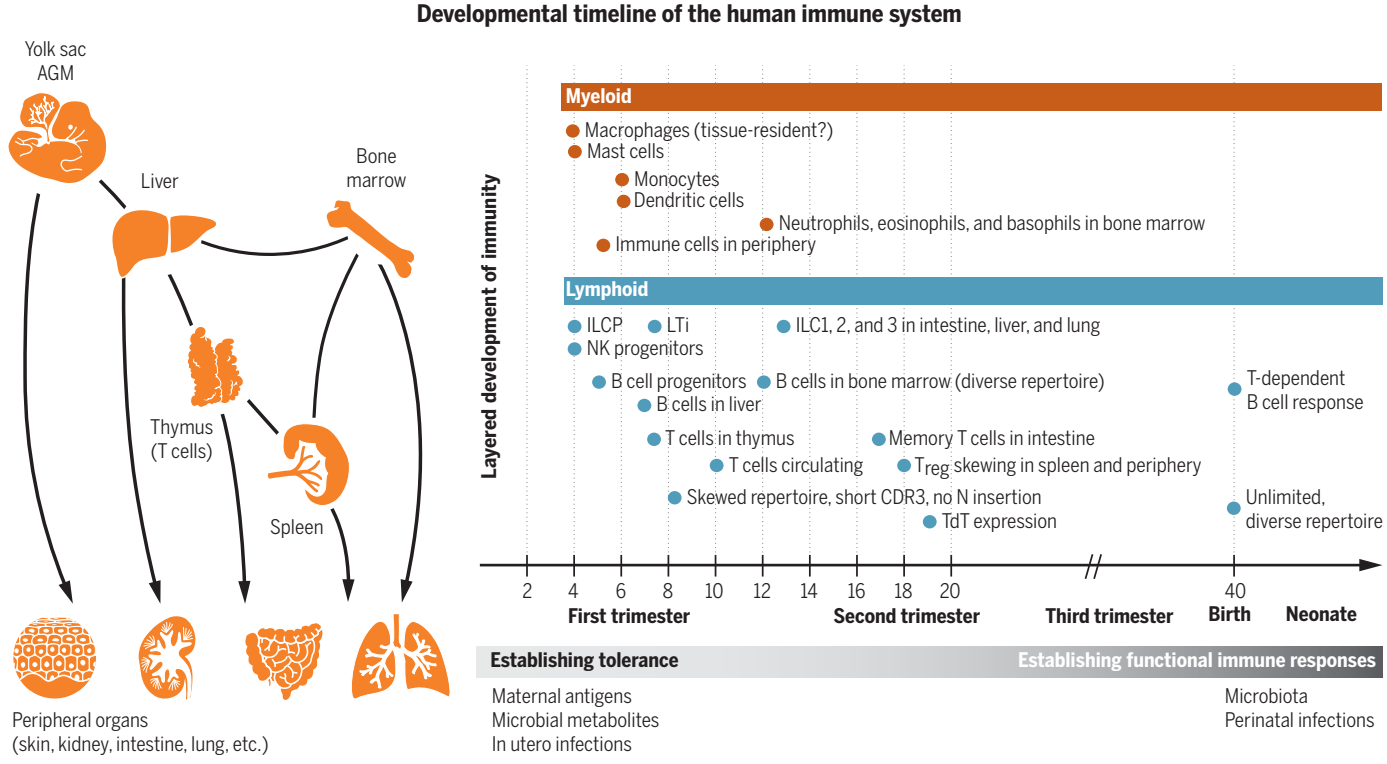

Fig. 1. Temporal and spatial development of the human immune system. The development of blood and immune systems during early human life occurs over several anatomical sites. The major site of hematopoiesis changes from the extraembryonic yolk sac to the intraembryonic AGM, liver, and BM. T cell differentiation and maturation are confined to the thymus. Immune cells seed other lymphoid or peripheral organs—including lymph nodes, skin, intestine, kidney, and lung—and adapt to the respective organ environment. Diverse immune cell types develop and mature at different gestational stages, which is necessary to establish tolerance and functional response based on developmental needs. This prepares the developing embryo and fetus for antigen exposure during pregnancy and after birth. ILCP, ILC precursor; CDR3, complementary-determining region 3; TdT, terminal deoxynucleotidyl transferase.

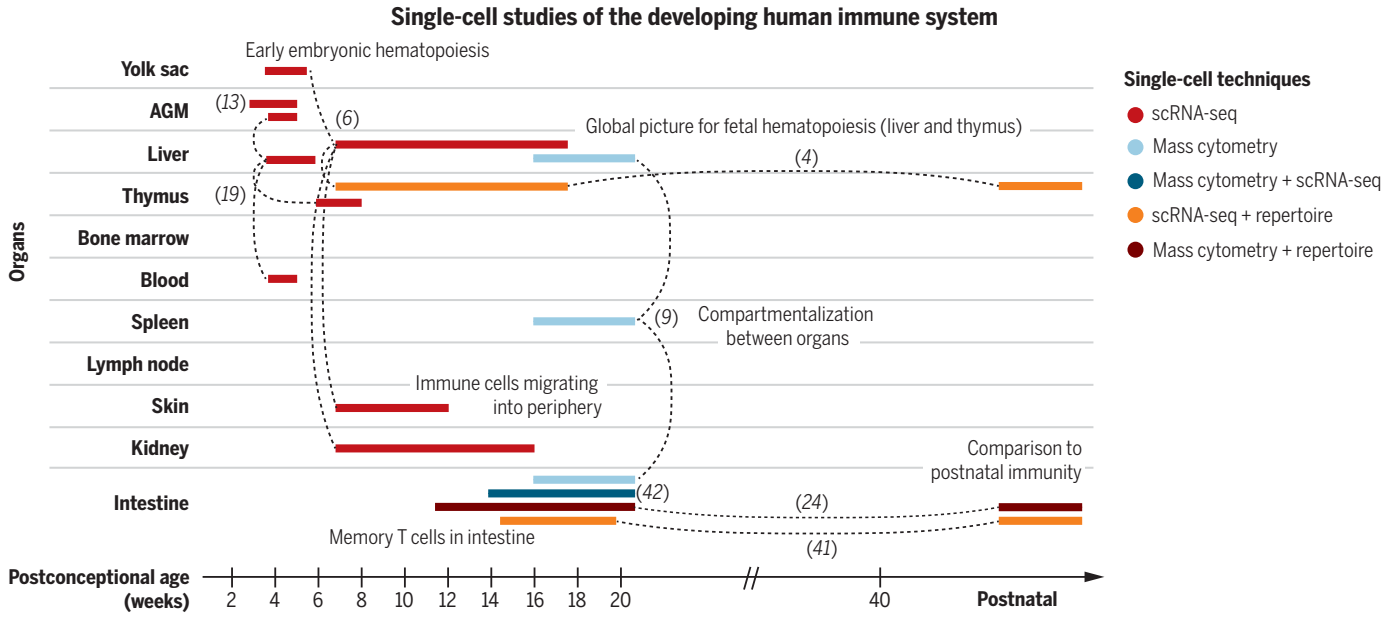

Fig. 2. Overview of single-cell studies detailing the developing human immune system. Diverse single-cell methods (depicted in color) have been applied to generate a comprehensive atlas of human immune system development. In many studies, multiple organs have been sampled together to investigate the migration, adaptation, and compartmentalization of immune cells. (Studies are indicated by the reference number, and dotted lines link the different organs sampled in each study.)

DC production are seen in the fetal liver from around 6 PCW (*6*). Conventional DC1, DC2, and plasmacytoid DCs are found in fetal tissues—including the lung, spleen, skin, and thymus—from 12 PCW and are relatively abundant compared with adult tissue DCs (*30*). Fetal DCs, like their adult counterparts, are capable of migrating, responding to Toll-like receptor ligation, and stimulating T cell proliferation and activation (*30*). Fetal DCs have the particular capacity to induce regulatory T cell differentiation, promote T cell interleukin-4 production, and inhibit T cell tumor necrosis factor–α (TNFα) production via arginase 2 (*30*). Thus, DCs play an important role in maintaining tolerance during fetal life.

The B cell lineage is first observed in the fetal liver from 7 PCW in the form of B cell precursors; mature B cells are present only after 9 PCW (*6*). This has been attributed partly to the change in HSC-intrinsic potential to generate B cells and the liver microenvironment support for B cell differentiation (*6*). At mid-gestation, the BM becomes the major source of B cells, and mature B cells are abundantly enriched in spleen (*31*). Although fetal B cells achieve diverse repertoire from early stages (*24*, *32*), the formation of germinal centers is attenuated until antigen exposure after birth, which is accompanied by active somatic hypermutation (*33*). Comparing intestinal B cells from second-trimester fetuses to infants with single-cell mass cytometry combined with B cell receptor repertoire analysis nicely demonstrated that fetal intestinal B cells are primarily follicular and transitional B cells, whereas plasma B cells are enriched in infants (*24*).

Another interesting aspect of B cell development that has been intensively studied in the mouse model is the tiered development of innate-like B-1 cells, which predominate in early gestation and are followed by conventional B-2 cells (*34*). However, the precise identity of human B-1–like cells has not yet been resolved (*35*). Future studies to generate a single-cell atlas of human fetal BM and spleen will provide a better view on human B cell ontology, highlighting organ-specific differences in the niche factors that support B cell differentiation.

"The clinical implications of fetal immune development and function reach far beyond life in utero."

Thymus and peripheral organs

The thymus provides an environment essential for T cell development. Early lymphoid progenitors originating from the fetal liver migrate into the thymus at 8 PCW, where they develop into naïve T cells (*36*).

Development and maturation of the thymus are mediated by an interplay between thymic stromal cells and immune compartments, which has been largely studied in mouse models. Comprehensive single-cell transcriptome profiling of cellular constituents of developing human thymus showed extensive communication between thymic epithelial cells, mesenchymal cells, early thymic progenitors, developing and mature T cells, and other immune cells (*4*, *19*). The proportion of each cell population also shows coordinated change across development, further proving the importance of harmony between multiple cell types for organ maturation (*4*).

Single-cell studies on fetal liver and thymus revealed detailed molecular signatures accounting for the transition from early thymic progenitors into naïve T cells (*4*, *6*, *19*). Hu and colleagues focused on the molecular profile of thymus-seeding progenitors (*19*). Our group extended this analysis toward later stages in development (*4*). Together, these findings revealed a continuous trajectory from early thymic progenitors developing into multiple mature T cell types.

Naïve T cells egress from the thymus and migrate into other tissues. Circulating T cells are observed at 10 to 11 PCW after functional thymic development (*37*). The absence or presence of microorganisms in the fetal environment remains a matter of debate (see accompanying reviews). Although a healthy pregnancy is most likely sterile, noninherited maternal alloantigens and microbial by-products may potentially activate the fetal immune system. To avoid damaging alloreactivity, the fetus needs to maintain tolerogenic immunity. Consequently, naïve T cells generated from the fetus are more likely to acquire a regulatory T cell fate compared with adult naïve T cells (*38*). Fetal regulatory T cells suppress the proliferation and cytokine secretion of other fetal T cells that are potentially self-reactive (*39*).

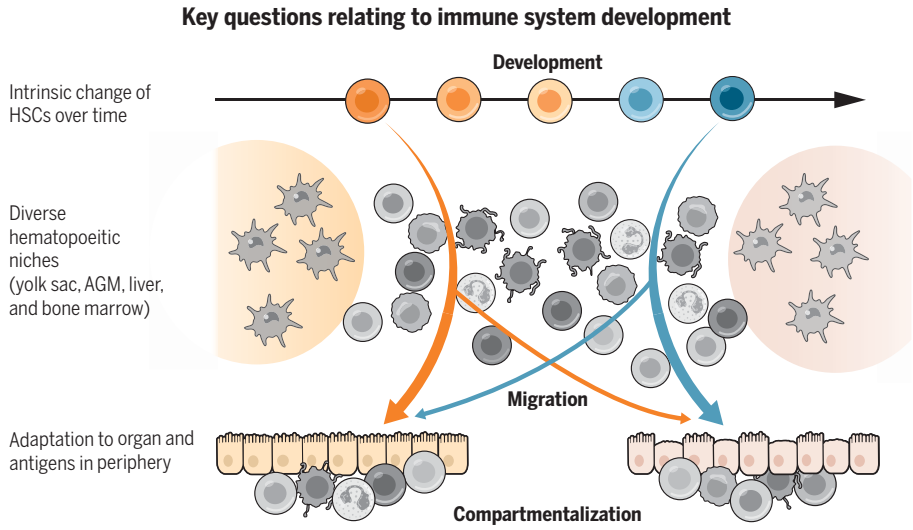

Fig. 3. Key questions to be addressed in future studies of immune system development. The diagram depicts pertinent questions relating to the developing immune system. How do the HSCs change in their potential throughout development? How do diverse hematopoietic niches differ from each other? What determines the migration of immune cells to the target organs, and how do they adapt to a new tissue environment? Single-cell profiling and spatial profiling techniques are now providing answers to these questions by assessing the immune system as a whole and identifying emergent properties of the collective.

Memory T cells have been identified in the fetal intestine, highlighting the potential of fetal T cells to respond to foreign antigens (*9*, *24*, *40*, *41*). Studies on intestinal $CD4^+$ T cells by single-cell techniques combined with repertoire sequencing identified the existence of memory T cell populations and regulatory T cells with the signature of clonal expansion, highlighting the balance between activation and suppression of adaptive immune response in the fetus (*24*, *42*). Intestinal $CD4^+$ T cells can also play a role in promoting development, as shown for the case of moderate TNFα expression (*41*). Thus, fetal adaptive immunity is substantially more mature than previously expected. Active areas of future research on the fetal immune system include the antigenic cues underlying fetal T cell activation and the roles they play in fetal development and protection.

Through this snapshot of fetal immune development across time and space, we note the emergence of both innate and adaptive immune cells with distinctive properties compared with their adult counterparts. Among the components missing from this overview are neutrophils. Current evidence suggests that around one-third of fetal BM cells are neutrophils or their precursors at 10 to 13 PCW, increasing to two-thirds at 21 PCW (*43*). Infants born prematurely or small for gestational age have lower circulating neutrophil counts, lower neutrophil reserve, and higher mortality from sepsis (*44*). Understanding how the fetal neutrophil compartment operates will provide insights into how early-life immune defense can be supported.

Conclusion

Multi-omics suspension and spatial-based technologies have provided ideal platforms to dissect and reconstruct the developing immune system (*4*, *6*, *9*, *13*, *19*, *24*, *41*, *42*). Many areas of uncertainty remain to be unraveled (Fig. 3). How do hematopoietic progenitors change throughout development? How do different tissue niches such as yolk sac, liver, BM, thymus, and spleen affect the progenitor populations and developing immune cells? How do immune cells migrate to and adapt in peripheral nonlymphoid tissues? How does the immune system communicate, learn, and form memory for future encounters?

Completion of the developing immune atlas by focusing on the organs and time points that are currently missing, extending the analyses for comparison with the adult immune cells, and system and cross-species comparisons will provide further knowledge about how the human immune system evolved and is established and sustained. The clinical implications of fetal immune development and function reach far beyond life in utero. Fetal-specific hematopoietic progenitor cells are now recognized as likely cells of origin for blood cancers, including Down syndrome–associated acute megakaryoblastic leukemia, juvenile myelomonocytic leukemia, and infant acute lymphoblastic leukemia. Early-onset primary immunodeficiencies with impaired response to pathogen challenge and/or autoimmunity may also be influenced by developmental cues and changes. In these settings, aberrant hematopoiesis also results in abnormal immune function. The biological insights from in-depth understanding of the developing immune system promise to revolutionize stem cell transplantation and tissue engineering for immunotherapy and regenerative medicine in the near future.

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ACKNOWLEDGMENTS

We thank J. Eliasova for graphical images. **Funding:** We acknowledge funding from the Wellcome Human Cell Atlas Strategic Science Support (WT211276/Z/18/Z). M.H. is funded by Wellcome (WT107931/Z/15/Z), the Lister Institute for Preventive Medicine, and the NIHR Newcastle Biomedical Research Centre. S.A.T. is funded by Wellcome (WT206194), ERC Consolidator and EU MRG-GRammar awards, and the Chan Zuckerberg Initiative (CZF2019-002445). B.G. is a Wellcome Investigator (206328/Z/17/Z) and is also supported by core funding from Wellcome and MRC to the Cambridge Stem Cell Institute. L.J. is funded by an NIHR Academic Clinical Lectureship. J.-E.P. is supported by an EMBO Advanced Fellowship (ALTF 623-2019). **Competing interests:** In the past 3 years, S.A.T. has consulted for Biogen, GenenTech, and Roche and is a member of the ForeSite Labs Scientific Advisory Board.

10.1126/science.aaz9330

Prenatal development of human immunity

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Science **368** (6491), 600-603.
DOI: 10.1126/science.aaz9330

REVIEW

Microbial–host molecular exchange and its functional consequences in early mammalian life

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Molecules from symbiotic microorganisms pervasively infiltrate almost every organ system of a mammalian host, marking the initiation of microbial–host mutualism in utero, long before the newborn acquires its own microbiota. Starting from in utero development, when maternal microbial molecules can penetrate the placental barrier, we follow the different phases of adaptation through the life events of birth, lactation, and weaning, as the young mammal adapts to the microbes that colonize its body surfaces. The vulnerability of early-life mammals is mitigated by maternal detoxification and excretion mechanisms, the protective effects of maternal milk, and modulation of neonatal receptor systems. Host adaptations to microbial exposure during specific developmental windows are critical to ensure organ function for development, growth, and immunity.

Living organisms are constrained by the thermodynamic boundaries of obtaining and using sufficient energy within the available environment to construct their cellular and noncellular biomass during mammalian fetal and neonatal development. The consumption of plant, animal, and other xenobiotic material that is partly metabolized by microorganisms in the maternal and neonatal gastrointestinal tract improves energy harvesting for growth but exposes the developing mammal to a wide range of chemicals. Although it is possible for mammals to live in the absence of a microbiota, analytic techniques using isotopically labeled intestinal bacteria have revealed that normally even systemic organ systems and potentially the fetus are promiscuously bathed by molecules synthesized either by microbes on mucous membranes, or by dietary intake, but which cannot be generated by host metabolism itself (*1*, *2*). Here, we examine the potential impact of exposure to microbial constituents and other xenobiotics in early life, from fetal development to the early postnatal period.

Maternal exposure to microbial metabolites and xenobiotics

Mice may be bred in aseptic "germ-free" conditions in isolators with normal fecundity, development, and life span provided that their food is fortified with micronutrients. Vitamin K, for example, is usually produced by bacteria and must be given as a dietary supplement to germ-free mice for normal blood clotting (*3*). The microbiota triggers extensive adaptations in every organ system (*3*), either through sensing of the live microbes at body surfaces or through signaling from microbial metabolites that reach host tissues. Colonized animals are less susceptible to opportunistic infections through the competitive protective effect of the microbiota. Maturation that drives the adaptive and innate immune systems allows scalable responses to later pathogen challenges (*4*). In addition to the functional adaptations that occur in mucous membranes exposed to the microbial biomass, there are also extensive long-range effects on the neural, cardiovascular, hepatic, endocrine, adipose, and skeletal systems.

Although many of these pervasive adaptations can be recovered by colonizing an adult germ-free animal, there are also precise developmental time windows that require microbial colonization for normal immune development and the assembly of a healthy microbiota (*5*). The question is whether such windows are exclusively postnatal, as the young mammal acquires its own microbiota, or whether the molecular diaspora from the mother's microbiota is also important for antenatal development.

Maternal nutrition is clearly an important factor for development in early life. The energy requirements of the fetus and neonate benefit from the mother's microbiota, which optimizes her overall nutritional state by providing vitamins and essential amino acids, detoxifying xenobiotics, and breaking down otherwise indigestible foods (*6*). Bacterial folate positively influences embryonic and fetal development, and short-chain fatty acid microbial metabolites are important to sustain maternal intestinal barrier integrity (*6*).

Effects of the microbiota are mainly studied by comparing germ-free and colonized mice. To understand the specific effects of a maternal microbiota alone, transitory colonization systems have been used, so the mother is only colonized for a short time in pregnancy but delivers germ-free pups (Fig. 1). This approach has been combined with nonradioactive isotope labeling of the transitory bacteria to trace the penetration of microbial molecules into the mother and her offspring. The model shows that maternal microbial metabolites drive the development of her pups' innate immune system and maturation of the intestinal epithelium (*7*). One of the receptors for the chemical signals involved is the aryl hydrocarbon receptor (AhR), which is essential for normal immune function (*8*).

There are poorly understood differences in how different microbes metabolize dietary xenobiotics, drugs, or environmental contaminants (*9*), because microbial genes are mainly annotated by inference from sequence homologies, or their function is unknown. There are also fundamental differences between how eukaryotes and prokaryotes handle xenobiotic (bio)chemicals. The mammalian host generally detoxifies xenobiotic metabolites through the addition of polar functional groups (-OH, epoxide, -SH, or -NH_2), followed by the addition of additional polar head groups (glucuronyl, acetyl, methyl, and sulfonyl), which make the resultant polar molecule susceptible to renal elimination. By contrast, microbial metabolism has evolved to break molecules for carbon sources and/or dispose of reducing equivalents using hydrolases, lyases, reductases, group transfer, or radical chemistry (*9*, *10*). An important unmet need is to understand how far exposure to natural xenobiotics, whether sourced directly from the diet or taken in artificially high amounts as food

Box 1. Is the developing fetus sterile?

The healthy fetus is enclosed in utero by the amniotic membrane and receives blood from the placenta. It has long been thought to be completely sterile, but this notion has been challenged by recent studies that reported a very small microbial biomass in placental tissue, cord blood, or meconium. However, methodological challenges, contradictory results, and our current immunological understanding cast doubts on the interpretation of these findings (*18*). The detection of very small amounts of bacterial DNA may be confounded by laboratory or reagent contaminants. Furthermore, viable bacteria are transiently found in the bloodstream of healthy neonates following minor trauma common during birth. Additionally, bacterial profiles described vary substantially, with taxa from the oral or the skin microbiota that are potential contaminants. Because placental tissue has no lumen, colonizing bacteria would be subject to immune elimination. Finally, cesarean section and sterile fostering of fully colonized experimental animals generate germ-free neonates. Pathogenic bacteria that successfully resist antimicrobial destruction are expected to provoke an inflammatory reaction predisposing to premature birth.

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supplements, may show idiosyncratic effects on the fetus or neonate after metabolism by the mother's intestinal microbes. This is ethically difficult, as animal models do not precisely mirror human development mechanisms or the human microbiota composition.

The maternal–fetal interface

Placentation and antenatal hematopoiesis

The basis for antenatal exposure of the developing fetus to circulating microbial metabolites and xenobiotics is the placental interface between the maternal and fetal bloodstream (*6*). Mice and humans have a hemochorial placenta, in which the fetal trophoblast invades the uterine tissue and the endothelium of the maternal blood vessels. A vascular labyrinth starts to form as the fetal umbilical arteries invade the maternal decidua on embryonic day 12.5 (E12.5) in mice (within the first trimester in humans), progressing to an efficient interface between the maternal and fetal vascular systems for respiratory gas exchange, nutrient transfer, excretion, and some detoxification. This provides only a limited barrier to molecular transfer, and non-ionized molecules of relative molecular mass $M_r < 500$ reach the fetal circulation by passive diffusion. Fetal organ systems and immunity are developing in parallel. In mice, primitive hematopoiesis begins in the yolk sac at E7, and hematopoietic stem cells are found in the fetal liver and thymus starting from E10.5 (*6*).

Placental and maternal handling of xenobiotics

The potential risks of the limited placental barrier became apparent through the thalidomide disaster, when a synthesized xenobiotic taken in early pregnancy caused nonhereditary phocomelia (grossly underdeveloped or missing limbs) in babies (*6*). To regulate key compound classes, the placenta has a series of transport proteins, which help to coordinate maternofetal nutrient, excretory, and xenobiotic exchange (*11*). Multidrug resistance protein 1 (MDR1, P-glycoprotein, ABCB1) is an adenosine 5′-triphosphate (ATP)–dependent transporter of xenobiotics present on trophoblast cells, hepatocytes, intestinal epithelial cells, and renal tubular cells. Overall, this transporter helps to protect the fetus from xenobiotics as shown by the occurrence of cleft palate deformities in globally MDR1-deficient mouse fetuses where the dam was administered a polycyclic antihelminth analog (*12*). Two additional transporters (ABCG5 and ABCG8) expressed in the placenta, liver, and intestine are known to limit the uptake of plant-derived sterols, which are themselves subject to microbiota metabolism. Female mice that are globally *Abcg5* deficient exhibit cardiomyopathy, thrombocytopenia, and infertility (*13*). How far selective placental expression of any of these transporters contributes to regulating fetal exposure to maternal microbial metabolites remains unclear.

Environmental toxins, such as dioxins and dioxin-like compounds, are metabolized through the cytochrome P450 superfamily of enzymes, such as Cyp1a1, which are induced through the AhR. High AhR expression is found in the placenta, the liver, and in mucous membranes. Although small amounts of AhR ligands derived from food and the microbiota are beneficial to fetal development and postnatal immune function, toxic amounts are restricted through overall AhR-dependent activation of Cyp1a1 in intestinal, hepatic, and placental tissues (*14*). Limiting fetal xenobiotic exposure is therefore generally a function of a triple layer consisting of maternal intestine, liver, and placenta. Failure to protect the fetus or neonate from exposure to maternal microbial metabolites, as well as other ingested chemicals, may have lasting developmental consequences and predispose these children to metabolic and immunological diseases.

The microbiota potentially affects the biochemical environment of the fetus and neonate in a variety of ways

Effects of microbiota metabolites can be categorized according to whether the chemicals are synthesized endogenously by the microbiota or whether they are secondary metabolites of compounds that are taken as food, pharmaceuticals, or as environmental contaminants. Each of these categories can alter the composition and biomass of the microbiota, as well as generate different portfolios of xenobiotic chemical exposures according to the metabolic capacity of various taxa present [reviewed in (*15*)]. The general effects of dysbiosis on adult (maternal) intestinal, metabolic, and immune functions have been considered in numerous reviews.

Endogenous microbial compounds

Many endogenous microbial compounds are recognized by innate pattern recognition receptors. Comparisons of adult germ-free and colonized mice indicate steady-state penetration of host tissues by Toll-like receptor (TLR) ligands [e.g., lipopolysaccharide (LPS) or flagellin] and NOD ligands, which have been linked to important maturation processes in the host immune system (*16*, *17*). Although we believe that the developing fetus and the placenta are sterile (*18*) (box 1), stable isotope–labeling studies show that there is rather promiscuous penetration of most classes of endogenous microbial compounds into the adult host, especially from bacteria in the lower small intestine (*2*), and these are likely to reach the placenta.

Central nervous system alterations resulting from antenatal LPS exposure have long-term behavioral consequences in rodent models.

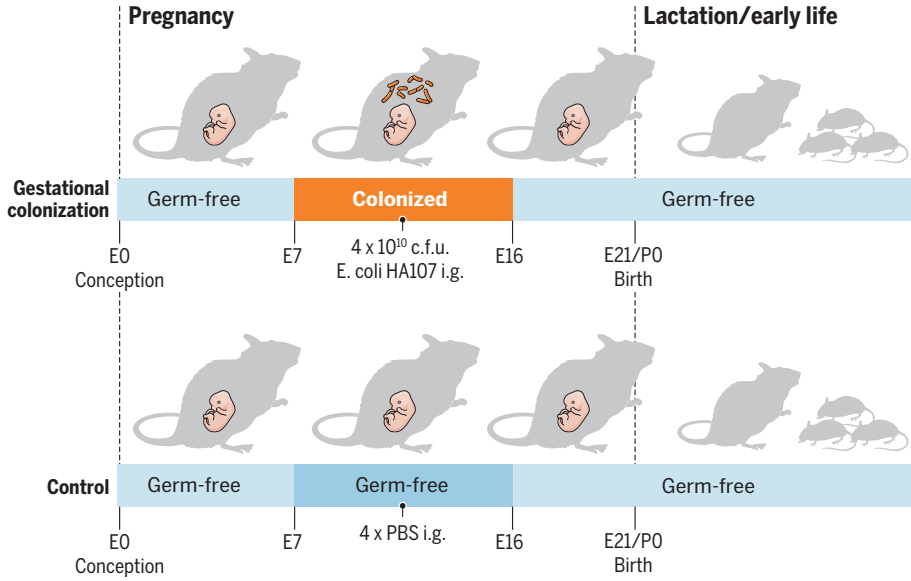

Fig. 1. Experimental model of reversible gestational colonization. The auxotrophic *Escherichia coli* HA107 strain, which is deficient in the synthesis of two bacterial-specific amino acids, meso-diaminopimelic acid and D-alanine, can be used to study the effect of the maternal microbiota on offspring development in the absence of an endogenous microbiota in the offspring. This strain is unable to replicate within the murine germ-free intestine where meso-diaminopimelic acid and D-alanine are absent. If germ-free pregnant dams are treated with HA107, they are transiently colonized but return to germ-free status before giving birth and will thus deliver germ-free pups. This experimental setup has been used to study the effect of maternal microbiota only during pregnancy on developmental processes in the offspring (*7*). i.g., intragastric; c.f.u., colony-forming units; PBS, phosphate-buffered saline.

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However, doses have been used that simulate intrauterine infections, leaving open the question of whether there are also effects from the steady-state penetration of LPS in the healthy pregnant female. Microglia are extensively branched with longer dendrites in adult germ-free mice, whereas microglial immune activation is greater in colonized animals and those treated with LPS during postnatal compared to fetal life (*19*). Therefore, the barriers of the intestine and the placenta coupled with early-life insensitivity to LPS signaling presumably provide protection against steady-state antenatal exposure. Damage to the intestinal barrier (through alcohol intake or parasitic infection) can increase LPS exposure at the maternal–fetal interface (*20*). LPS and glutamyl-diaminopimelic acid (binding TLR4 and NOD1, respectively) induce inflammation at the maternal–fetal interface and can be a risk factor for preterm birth (*21*).

Diet-derived xenobiotics that are metabolized by commensal microbiota

Extensive literature is available on various dietary components and how these can potentially be metabolized by the maternal microbiota possibly with effects on early-life development (table S1). These compounds are mainly plant-synthesized polycyclics (including flavones, isoflavones, and anthraquinones), terpenes, and polyols. Although there is little or no direct epidemiologic evidence either for or against relevant effects on a human fetus, some compounds are consumed in high amounts—for example, with the intention of influencing the gender of human babies.

In other cases, there is clearer evidence for clinically important effects. Retinoids in the maternal diet, whose availability in the intestine is directly regulated by microbial taxa, such as *Clostridia* (*22*), can affect the number of lymphoid tissue inducer cells in the fetus and thus development of secondary lymphoid organs in the offspring (*23*), as well as the induction of oral tolerance in models of allergy (*24*).

Microbes found in the large intestine, such as Bacteroides species, ferment dietary fibers to short-chain fatty acids (SCFAs). These contribute to host immune maturation, either by activating G protein–coupled receptors (GPCRs) on the surface of immune cells, or through inhibition of lysine deacetylases. In a murine model of asthma, feeding female pregnant mice a fiber-rich diet limited inflammatory airway responses and the development of asthma in their offspring through the production of SCFAs (*25*). Similarly, maternal dietary fiber fermentation during pregnancy and lactation can induce the differentiation of regulatory T cells in the offspring (*26*).

Bile acids have a distinctive position in the portfolio of maternal microbial metabolites that affect the fetus. Bile acid pools are generally increased through maternal hepatic synthesis in late pregnancy, and secondary bile acids are formed by microbial metabolism in the maternal gastrointestinal tract. In addition to lipid solubilization in the postnatal intestine, bile acids are known to exert metabolic and growth effects through the GPCR TGR5 and the farnesyl X nuclear receptor (FXR). The fetus is exposed to bile acids both from its own synthesis and from maternal transfer, although renal excretion depends on the mother. Solute transport proteins of the SLC21, SCL22, and ABCG2 classes are expressed in the placenta and may regulate fetal exposure to bile acid metabolites (*27*).

Nuclear receptors, including the AhR, FXR, pregnane X receptor, constitutive androstane receptor, and vitamin D receptor, can bind diet-derived ligands and are likely to detect different maternal microbial compound classes (*28*). They can direct transcriptional activity through epigenetic mechanisms (histone modifications or DNA methylation) and may be of special importance during in utero development, which constitutes the most active period for epigenetic DNA imprinting in a mammal's lifetime.

Birth and postnatal effects

Postnatal colonization with microbes and lactation

The neonate's body surfaces are colonized at birth, exposing the offspring to microbes and

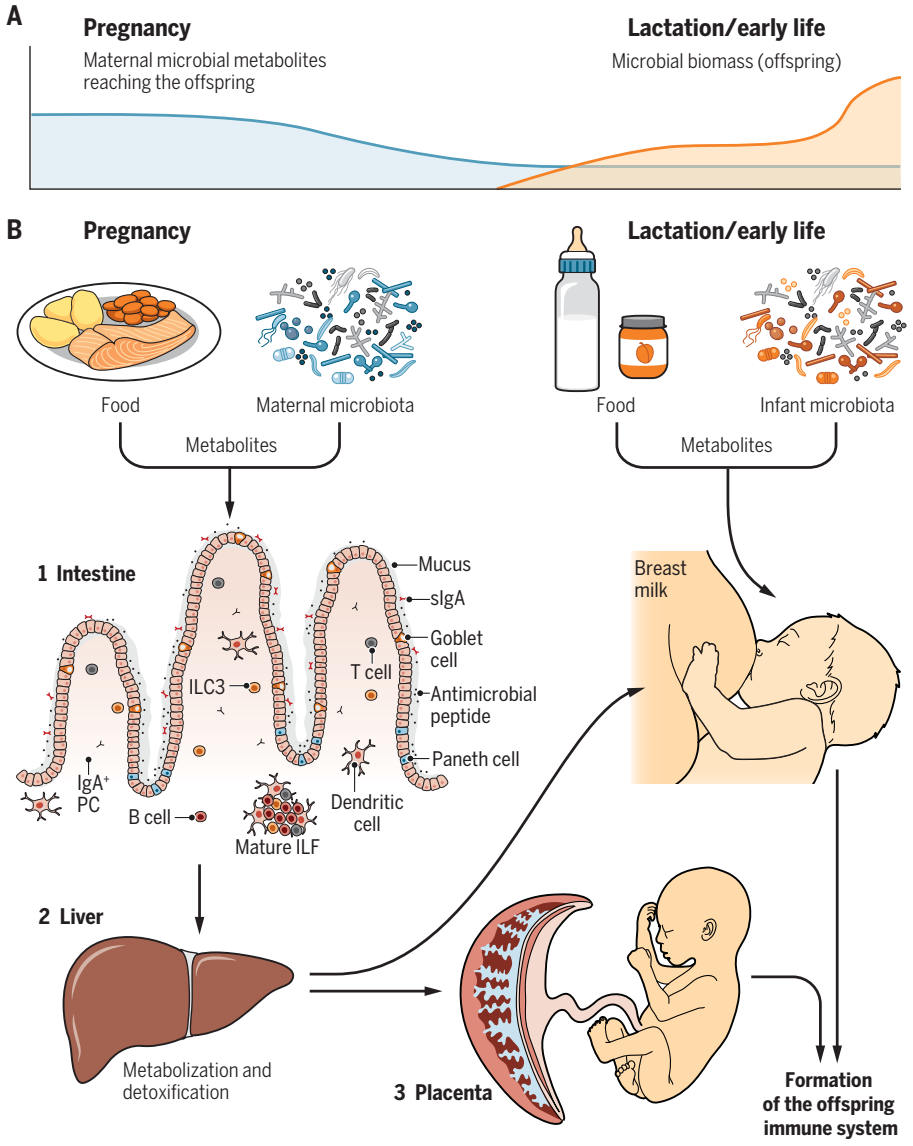

Fig. 2. Host barriers for exposure of the developing fetus and neonate to microbiota-derived metabolites. (**A**) Schematic view of the extent of exposure of the developing fetus or offspring to metabolites originating from or metabolized by the maternal microbiota in comparison to the biomass of microbes colonizing the offspring itself after birth. (**B**) A triple barrier consisting of the intestinal epithelial lining, the detoxifying liver, and the placental barrier partially protects the developing fetus from exposure to maternal bacterial and dietary metabolites. After birth, exposure to those metabolites continues through breast-feeding, further shaping the offspring's endogenous microbiota and immunity.

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their molecular diaspora without maternal intestinal or placental barriers. Maternal microbial molecules still reach the neonate through breast milk, although direct microbial exposure now becomes far more important.

Breast milk shapes the unstable early-life intestinal microbiota through secretory antibodies (*29*), milk oligosaccharides (*30*), or milk proteins, including lactalbumin and lactoferrin. Because the antibody repertoire of maternal milk is shaped by the mother's own microbiota and her previous exposure to pathogens, breast-feeding is an efficient way to transfer mucosal and systemic immune memory from mother to offspring. Once the offspring starts to consume solid food, these protective effects of milk disappear, leaving endogenous microbes to stimulate a "weaning reaction" in a critical developmental window in the young mammal (*31*).

Postnatal colonization and the innate immune system

As in adults, stromal and immune cells of the fetus and neonate express a series of innate immune receptors and antimicrobial effector molecules, allowing them to mount potent and protective immune responses upon infection. However, neonates are also susceptible to inappropriate inflammation upon encounter of microbial stimuli from commensal bacteria, as in the pathogenesis of necrotizing enterocolitis (NEC), a devastating immune-mediated disease in preterm neonates (*32*).

Different mechanisms modulate the innate immune system to control the fetal–postnatal transition. Most of these perinatal changes appear to be developmentally regulated and largely independent of the microbiota, probably reflecting the unreliable presence of microbial stimuli early after birth (*33*). Age-dependent differences exist for the expression of individual receptors and the anatomical distribution of antimicrobial peptides in mice and humans, but mechanistic insight into the functional role of the changes remains limited (*34*, *35*). For example, decreased prenatal expression of TLR4 by the human intestinal epithelium or repressed TLR4 signal transduction in neonatal murine epithelium may help to prevent inflammation upon early postnatal colonization (*36*–*38*). By contrast, enhanced expression of the flagellin receptor TLR5 by the murine neonatal epithelium contributes to the selection of a beneficial gut microbiota (*34*). Similarly, human blood monocytes undergo postnatal reprogramming through stimulation with the endogenous TLR4 ligands S100A8 and S100A9 to avoid hyperinflammation and promote immune homeostasis (*39*). Finally, maternally derived factors in amniotic fluid and breast milk modulate innate immune recognition and mucosal translocation of microbial stimuli to restrict their proinflammatory activity during the immediate postnatal period (*31*, *40*). For example, breast milk–derived secretory immunoglobulin A with affinity to enteric bacteria increases bacterial diversity and protects against the development of NEC in preterm neonates (*41*).

Thus, neonatal innate immunity is not simply less developed or immature. Rather, it is highly adapted and finely tuned to facilitate the fetal–postnatal transition of rapidly increasing microbial biomass and the development of long-term host microbial mutualism.

Postnatal colonization and adaptive immunity

The full maturation of the adaptive immune system occurs predominantly at weaning, when the young host is exposed to new antigens through higher intestinal microbial and food antigen loads. The intestinal mucosa acquires antigen-experienced T cells and activated plasma cells in a microbiota-dependent process (*3*). Antenatal B and T cell development in the fetal liver shifts to the bone marrow and thymus, respectively, and naïve B and T cells migrate into the secondary lymphoid tissues.

The extent to which the preweaning microbiota contributes to the trajectory of B and T cell repertoire development between early life and adulthood is not yet fully understood, although premature diversification appears to be a disadvantage for later immune responses that depend on natural antibodies and can potentially bias the adult B cell repertoire (*42*). Maternal milk immunoglobulins, themselves shaped by the composition of the maternal microbiota as well as neonatal T regulatory cells, delay the onset of secretory antibody production (*43*) and mucosal T helper cell maturation (*44*, *45*) in the offspring.

The process of development of some lymphocytes requires the presence of microbiota during a critical time window prior to weaning. Mice that are germ free until weaning have increased serum IgE concentrations and excessive intestinal mucosal natural killer T cells (*5*). Mucosal associated innate T (MAIT) cells, absent in germ-free mice, only efficiently seed tissues in response to microbiota-derived riboflavins in the first few weeks of life (*46*). Mucosal regulatory T cells require microbiota before weaning, limiting later susceptibility to colitis or allergic airway inflammation (*5*, *47*).

Conclusions

In this Review, we have considered the impact of the microbiota on the early-life mammal. In fetal life, this comes mainly from penetration of molecules synthesized by maternal intestinal microbes or microbial metabolism of food substances (Fig. 2). Our knowledge of these effects at physiological levels of metabolite penetration remains very limited, and the epigenetic and signaling mechanisms involved have primarily been studied thus far in the context of toxicology. After birth, adaptations occur mainly to contain the impact of the rapidly increasing endogenous neonatal microbial biomass and its molecular diaspora. It is clear that there are age-dependent modulations of signaling to innate receptor ligands and that maternal antibodies can shield the neonatal immune system from premature repertoire diversification and shape the composition of the early-life microbiota.

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ACKNOWLEDGMENTS

Funding: This work was supported by funding from ERC (HHMM Neonates) to A.J.M., Collaborative Research Center CRC1382 to M.W.H., and Peter Hans Hofschneider Professorship to S.C.G.-V.
Competing interests: The authors have no competing interests.

SUPPLEMENTARY MATERIALS

science.sciencemag.org/content/368/6491/604/suppl/DC1
Table S1
References (*48*–*89*)

10.1126/science.aba0478

Microbial–host molecular exchange and its functional consequences in early mammalian life

Stephanie C. Ganal-Vonarburg, Mathias W. Hornef and Andrew J. Macpherson

Science **368** (6491), 604-607.
DOI: 10.1126/science.aba0478

REVIEW

Contributions of maternal and fetal antiviral immunity in congenital disease

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Viral infections during pregnancy can have devastating consequences on pregnancy outcomes, fetal development, and maternal health. In this review, we examine fetal and maternal immune defense mechanisms that mediate resistance against viral infections and discuss the range of syndromes that ensue when such mechanisms fail, from fetal developmental defects to establishment of chronic infection. Further, we highlight the role of maternal immune activation, or uncontrolled inflammation triggered by viral infections during pregnancy, and its potential downstream pathological effects, including tissue damage and fetal demise. Insights into the respective contributions of direct viral toxicity versus fetal and maternal immune responses that underlie the pathogenesis of congenital disease will guide future treatment strategies.

Antiviral immune responses must be carefully balanced to maximize elimination of the pathogen and minimize damage to the host. Infections during pregnancy pose a distinct threat to both the mother and fetus, as the need to defend against the pathogens may conflict with mechanisms that maintain tolerance to the developing allogeneic fetus or disrupt normal developmental programs. Consequently, multiple defense pathways in the mother and fetus have evolved to protect against these threats. Despite these pathways, an expanding collection of "TORCH" pathogens (Box 1) are able to overcome these barriers and cause congenital infections. Common disease manifestations associated with classic TORCH syndrome include microcephaly, hearing loss, ocular abnormalities, hepatosplenomegaly, placental insufficiency, and fetal loss (*1*). The burden of congenital infection remains high: Cytomegalovirus (CMV), for example, infects up to 1 to 5% of infants worldwide and is the leading cause of long-term pediatric disabilities (*2*, *3*). In addition, maternal viral infection, even in the absence of transmission, can result in long-term consequences for the newborn, including abnormal neuropsychiatric development in the case of influenza and abnormal immune system development in the case of HIV-1 (*4*, *5*). Infection during pregnancy can also lead to more severe disease or prolonged infection for the mother (*6*).

In this review, we cover resistance mechanisms used by the fetus and maternal–fetal interface that prevent viral infections. We also examine how, when these mechanisms fail, viral infection and antiviral responses can lead to disruption of placental and fetal development and consequent congenital diseases. Finally, we highlight some gaps in the field toward the development of effective prevention and therapeutics for congenital infections.

Box 1. TORCH pathogens comprise *Toxoplasma gondii*, "other," rubella virus, CMV, and herpes simplex virus (HSV) (*7*).

The "other" category includes syphilis, parvovirus B19, Coxsackievirus, varicella zoster virus (VZV), HIV, Zika virus (ZIKV), hepatitis B virus (HBV), and hepatitis E virus (HEV). Other pathogens with the potential to cause pregnancy complications and perinatal infection include chikungunya virus (CHIKV), human papillomavirus (HPV), Ebola virus, gonorrhea, chlamydia, and group B streptococcus.

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Routes and timing of viral infection during pregnancy

In this section, we briefly discuss some of the consequences of viral infection during pregnancy and some of the changes that occur during pregnancy that may dictate susceptibility to infection. The timing of viral infection during pregnancy influences disease outcome in the fetus, potentially because of viral tropism, fetal developmental stages, and dynamic immune changes during pregnancy (Fig. 1).

There are several routes through which viruses may reach the fetus (*7*, *8*). A virus may reach fetal blood vessels in the villous tree from maternal blood to floating villi or from the maternal decidua basalis to anchoring villi. Viruses may also reach the fetus through the amniotic sac from the parietal decidua or the cervix (Fig. 1A, arrows). Maternal blood does not interface with the chorionic villi until the beginning of the second trimester, so the virus likely reaches the fetus from the anchoring villi or parietal decidua before then (*8*).

Early pregnancy

During the first trimester (0 to 13 weeks), placental development and organogenesis occur, making the fetus particularly susceptible to severe disease. Among viral infections during early pregnancy associated with severe outcomes, HSV, HPV, and CMV may affect placental development, leading to spontaneous pregnancy loss or preterm birth (*9*–*11*) (Fig. 1).

Pregnancy is accompanied by dynamic changes in the immune cell composition and cytokine expression at the maternal–fetal interface. During the first trimester, implantation and placenta development is accompanied by increased inflammatory cytokine expression and accumulation of immune cells at the decidua (*6*). In the second trimester (14 to 26 weeks), the maternal–fetal interface is dominated by a more "anti-inflammatory" phenotype. This includes a shift toward M2-like macrophages, decidual natural killer (NK) cells, and regulatory T cells that may be important for maintaining tolerance and normal fetal growth (*6*). Given these tightly regulated immune changes that occur during pregnancy, dysregulation of these responses in the setting of an antiviral immune response may affect normal placental and fetal development.

In addition to affecting the placenta and maternal–fetal interface, several viruses are capable of crossing the placental barrier and reaching the fetus; the most common include VZV, rubella, CMV, HSV, and ZIKV (Fig. 1). Fetal growth restriction, which can be mediated by both placental and fetal factors, is another common outcome and is associated with parvovirus B19, CMV, HBV, VZV, ZIKV, and HIV-1 (*1*). Some viruses, such as VZV and rubella, affect many different organ systems. However, the most common systems affected during early pregnancy infections are the central nervous and hematologic systems, which continue to develop through the second trimester (Fig. 1) (*1*).

Late pregnancy and peripartum infection

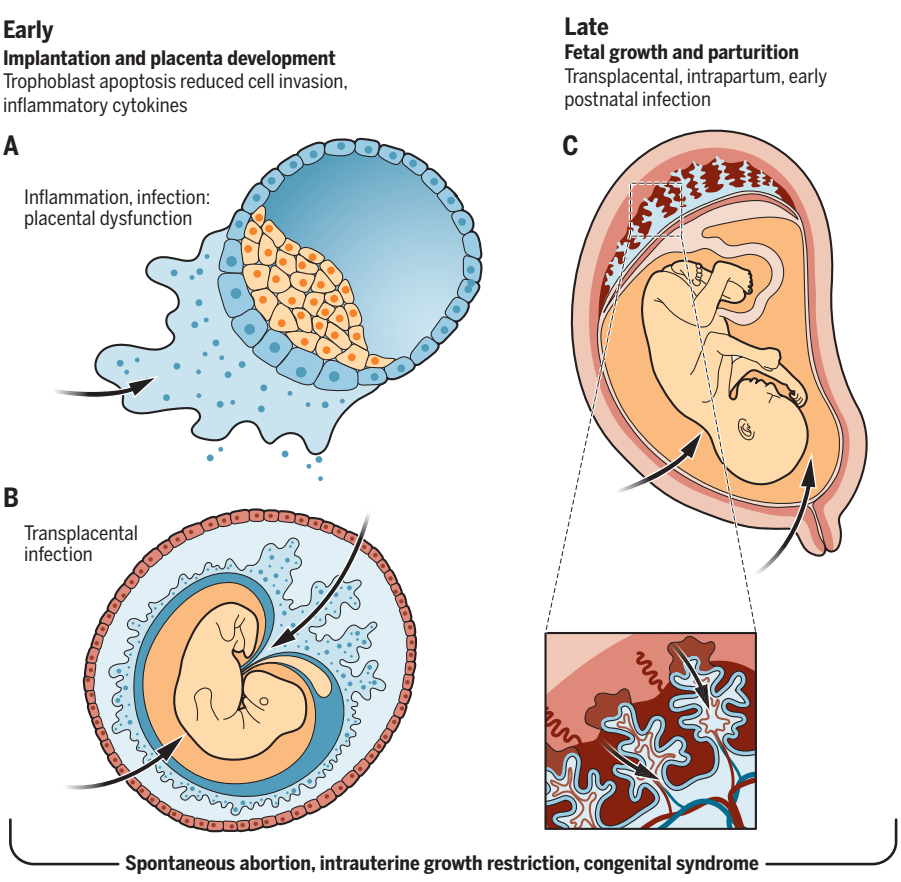
Infections by certain viruses during the third trimester (weeks 27 to 40) and peripartum period can lead to high rates of neonatal mortality or lifelong infections (Fig. 1). Primary maternal infections with HSV or CMV are correlated with a high risk of vertical transmission, particularly during late pregnancy or perinatal periods, when infection may occur through ingestion or aspiration of cervicovaginal secretions during delivery or breastfeeding (*9*–*11*). Infections with VZV, Ebola, Lassa, and HEV increase maternal and fetal mortality during late pregnancy; VZV and HEV infections are linked to ~30% infant mortality, with maternal mortality reaching 20% in HEV infections (*3*, *12*). Vertical infection with CHIKV occurs at delivery in about half of pregnancies with viremia, resulting in newborn encephalitis in 50% of cases (*13*)

(Fig. 1). Additionally, transmission of HBV and HCV, which can occur during all three trimesters, can lead to chronic infection of the infant (*1*). The vertical transmission rates of HIV-1, which typically occurs in the intrapartum and postpartum period, often also result in chronic infection, although antiretroviral therapy has substantially reduced transmission rates (*14*). Nevertheless, even uninfected HIV-exposed infants can suffer delayed growth, increased susceptibility to infections, and increased mortality (*4*). This heightened susceptibility to infection is accompanied by altered immune profiles of the infant, including decreased T cell counts, increases in activated T cells, and decreased neutrophil numbers. The mechanisms underlying these altered newborn immune responses are not well understood but may be caused by increased maternal inflammation or increased risk of maternal infection (*4*). Thus, chronic maternal viral infections can affect the fetus even in the absence of infection.

As new viruses emerge, determining their effects on pregnancy and the best management for pregnant patients is an important concern. A recent example is coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2). Members of the coronavirus family, including the SARS-CoV and the Middle East respiratory syndrome (MERS-CoV) coronaviruses, are associated with severe pneumonia and can pose serious health risks to pregnant women and cause neonatal complications (*15*). There are limited data about clinical complications and vertical transmission of pregnant woman with COVID-19, although major symptoms overlap with those observed in nonpregnant individuals; these include fever, cough, lymphopenia, coagulopathy, and pulmonary infiltrate. Available studies mostly include women who developed COVID-19 in late pregnancy and thus viral RNA could not be detected in the neonatal throat swabs or breast milk (*16*). In addition, the coagulopathy seen during SARS-CoV2 infection may contribute to hypertensive complications including preeclampsia in pregnant patients with COVID-19. Given the limited availability of studies from earlier pregnancy stages and the small sample size of available studies, further analyses are urgently needed for a better understanding of maternal–fetal transmission of SARS-CoV-2 and its role in pregnancy complications.

Antiviral resistance mechanisms during pregnancy

The placenta and maternal–fetal interfaces are equipped with specialized mechanisms to protect the fetus from infection. The observation that most viral infections of the mother are not transmitted to the fetus supports their effectiveness in most cases (*7*). The innate and



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VIRUS	TIMING OF INFECTION	MAJOR ROUTE OF INFECTION	SEQUELAE OF VIRAL INFECTION DURING PREGNANCY
HSV-2	A B C	Intrapartum	Microcephaly and intracranial calcifications/ eye defects/ miscarriage
HPV	A	in utero (ascending)	Miscarriage
CMV	A B C	In utero (transplacental)	Microcephaly and intracranial calcifications/learning disabilities/ abnormal long-term neurodevelopment/auditory defects
Varicella	B C	In utero (transplacental)	Microcephaly and intracranial calcifications/seizures, psychomotor defects/eye defects/ increased maternal and neonatal mortality
Rubella	B	In utero (transplacental)	Microcephaly and intracranial calcifications/learning disabilities/ abnormal long-term neurodevelopment/eye defects/auditory defects/hematologic and cardiac defects/miscarriage and stillbirth
Zika	A B C	In utero (transplacental)	Microcephaly and intracranial calcifications/learning disabilities/ abnormal long-term neurodevelopment/eye defects/miscarriage
Ebola	A B C	In utero (transplacental)	Increased maternal mortality/ miscarriage and stillbirth
HIV	B C	Intrapartum	Learning disabilities/abnormal long-term neurodevelopment/ chronic infection of fetus/ neonate
HCV	B C	Intrapartum	Chronic infection of fetus/neonate
HEV	C	In utero (transplacental)	Increased maternal mortality/ miscarriage, stillbirth, and neonatal mortality
Lassa	B C	In utero (transplacental)	Increased maternal mortality/miscarriage, stillbirth, and neonatal death
CHIKV	C	Intrapartum	Encephalitis and hematologic and cardiac defects
Parvovirus B19	A B C	In utero (transplacental)	Learning disabilities/abnormal long-term neurodevelopment/hematologic and cardiac defects (including hydrops fetalis)/ miscarriage

Fig. 1. Overview of congenital viral infections by routes and timing of infection. (**A** and **B**) Early pregnancy. (**C**) Late pregnancy. Arrows indicate potential routes of viral infection. (**D**) Table showing the routes and timing of infection and congenital disease by different viruses. The most common timing of infection is highlighted in blue.

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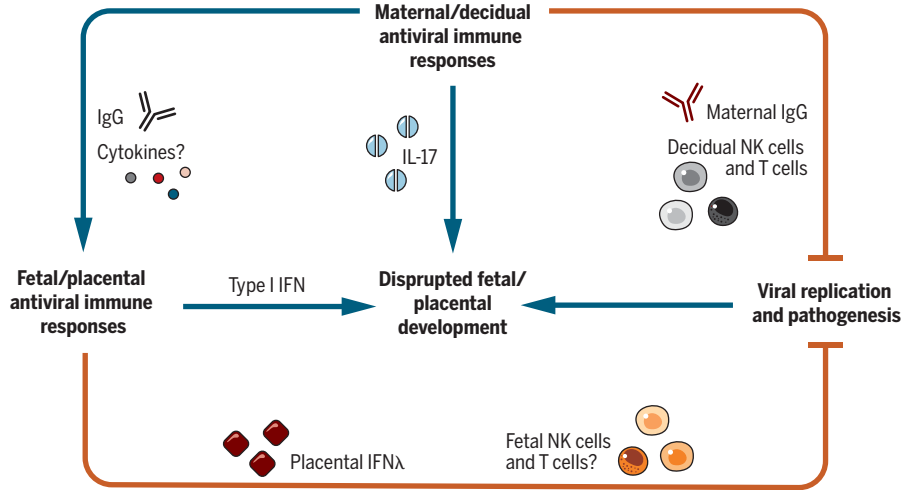

Fig. 2. Potential contributors to viral infection–mediated abnormal fetal development. Shown is a conceptual model of the potential contributions of maternal and fetal immune responses, as well as direct viral effects on disrupted fetal development. Maternal and fetal immune responses are also important for limiting viral replication. Specific examples are shown.

adaptive immune responses at the maternal–fetal interface and within the fetus need to limit infection without interfering with fetal development (Fig. 2). Thus, they provide some examples of specific antiviral mechanisms.

At the interface between the maternal blood and fetal blood of the chorionic villi, the syncytiotrophoblasts form a formidable barrier to infection (Fig. 3). These cells create a physical barrier through cell–cell fusion and constitutive expression of type III interferons (IFNs), exosomes, and antimicrobial peptides, which confer antiviral resistance in a paracrine manner (*8*, *17*). In the chorionic villi, fetal macrophages or Hofbauer cells undergo robust proliferation during viral infection and other nonviral villitis. Many viruses, including CMV, ZIKV, HSV, and Coxsackie virus, have been detected within Hofbauer cells (*18*). It is not clear whether Hofbauer cells limit viral spread or primarily serve as a site for viral replication. Maternal immunoglobulin G (IgG) also accumulates in the villous core, providing another potential barrier to infection (*19*). At the interface of the uterus and placenta, the decidua or maternal-derived lining of the uterus, is composed of 40% immune cells, including NK cells, macrophages, and T cells (*20*). Although these decidual immune cells generally have a less inflammatory phenotype than their counterparts in the blood, there is evidence for antiviral activity of decidual NK cells (dNKs) and CD8 T cells against HIV-1 (*21*) and CMV (*22*, *23*). For example, ex vivo experiments have shown that dNKs produce IFN-γ to limit HIV replication in decidual macrophages, and dNKs can produce perforin and induce cytotoxicity in human CMV (HCMV)–infected fibroblasts (*21*, *23*). These viral infections also alter the cytokine profiles of dNK cells, potentially affecting their baseline functions in mediating normal placental development.

If a virus surpasses the defenses of the decidua and placenta to reach the fetus, the fetus has the potential to develop innate and adaptive antiviral responses. Potential antiviral effector cells develop early during pregnancy (see accompanying reviews in this issue). For example, fetal NK cells are found in the liver as early as 6 weeks of gestation and show evidence of cytotoxicity as early as 9 weeks (*24*). CMV-specific T cells and IgM responses are present in CMV-infected fetal blood, indicating the ability to develop an antigen-specific response (*24*). CMV-specific $CD8^+$ T cells from infected newborns and fetuses can induce perforin-dependent lysis of infected cells, and they also produce granzyme and antiviral cytokines including IFN-γ and tumor necrosis factor-alpha (TNF-α) (*25*). Further functional evidence is needed to better understand the effectiveness of fetal immune responses in controlling viral replication or contributing to congenital disease in vivo. Variable fetal immune responses could be one reason why only a certain percentage of infected fetuses develop symptoms (*7*). Further, protection from intrauterine infection would help to explain why these immune responses develop so early in fetal development.

Mechanisms of virus- versus immune-mediated fetal damage

Even in the absence of transplacental transmission, viral infections can affect fetal development because of inflammatory responses in the placenta or infection-induced systemic changes in the pregnant mother, including metabolic alterations. In this section, we will focus on some of the molecular mechanisms by which viruses and immune responses to viruses affect placental and fetal development (Fig. 2).

Direct virus infection and damage

Direct virus damage, induced by killing or altered function of the infected cell by the virus, leads to severe complications during pregnancy. Infection of trophoblast cells by HSV or CMV can result in apoptosis and reduced cell invasion (*9*–*11*). HPV can also infect the extravillous trophoblast, causing abnormal placental pathology, spontaneous pregnancy loss, or preterm birth (*26*). Zika NS1 alters the surface glycosaminoglycans of placental endothelial cells, leading to increased vascular permeability and potential placental dysfunction (*27*). Such direct viral toxicity could contribute to placental abnormalities, as well as preterm birth, intrauterine growth restriction, and spontaneous miscarriage.

VZV, rubella, CMV, HSV, and ZIKV are all capable of infecting fetal neurons and neuronal precursors. In newborns, neuronal HSV infection results in apoptosis and neurologic damage (*28*). CMV-infected fetal brains present with white matter abnormalities, brain local necrosis, and hemorrhage. Neurological damage includes brain calcifications, microcephaly, and occipital horn anomalies (*2*). ZIKV can directly infect cortical neuron progenitors, as well as mature neurons and glial cells, resulting in cell death, neuroinflammation, and cortical thinning in postnatal brains (*29*, *30*). In newborn mouse models, CHIKV encephalopathy was associated with brain swelling and with the presence of virus in the cerebrospinal fluid, although it remains undetermined whether this was direct virus toxicity or inflammation-induced damage (*31*, *32*).

Maternal and fetal innate immune activation

Epidemiological studies have linked viral infection during pregnancy to an increased risk of psychiatric disorders in the adult offspring, including schizophrenia and autism spectrum disorders (ASDs), as well as neurological symptoms such as cerebral palsy (*5*, *33*). Infection-induced inflammation per se can cause disorders in the newborn, including ASD. The current widely used maternal immune activation (MIA) murine model is based on administration of a double-stranded RNA viral mimic, polyinosinic:polycytidylic acid (poly I:C), around midgestation in mice, which leads to behavioral symptoms resembling schizophrenia, depression, and ASD (*34*, *35*). One of the mechanisms of MIA-induced brain damage is a direct action of interleukin-17 (IL-17) from maternal T helper 17 (T_H17) cells on developing fetal cortical neurons (*36*). IL-17 receptor alpha signaling in a population of developing neurons of the somatosensory cortex (S1DZ neurons) results in increased

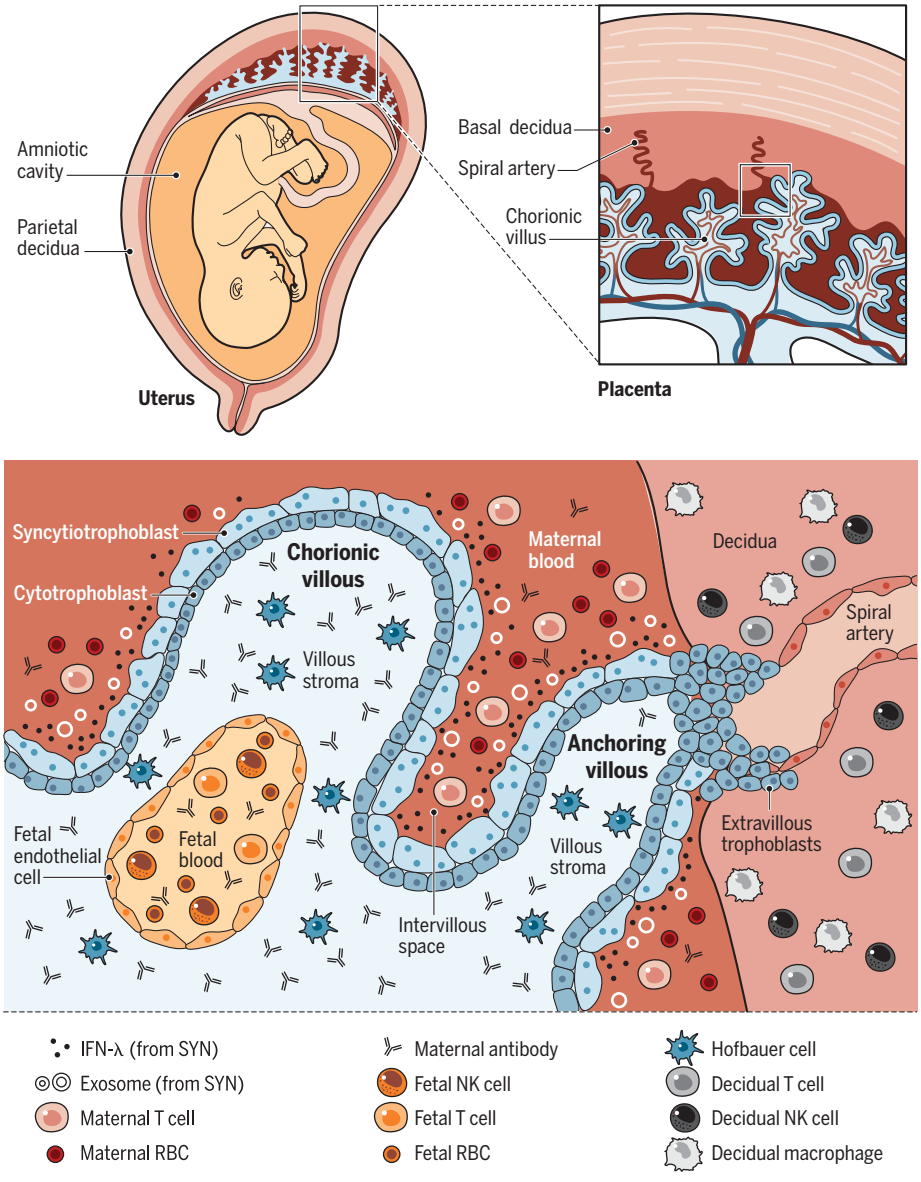

Fig. 3. Layers of defenses at maternal–fetal interfaces. Shown is an illustration of antiviral defenses at potential routes of infection: the chorionic villus, which is the interface between the maternal blood and fetal blood, and the anchoring villus, which is a connection between the decidua and villus.

neuronal activity and altered behavior (*37*). In addition to IL-17, mouse models of herpesvirus infection (MHV-68) showed that even in the absence of direct fetal infection, fetal inflammatory cytokines including IL-1β, IFN-γ, and TNF-α are elevated (*38*). Increased expression of proinflammatory cytokines are also observed in the human placenta after infection with CMV and other viral infections (*39*). The exact role of these cytokines in fetal brain development remains unknown.

Mouse models of ZIKV infection provide an excellent example of a host antiviral response inducing the termination of pregnancy. Type I IFN receptor signaling in the fetus, but not the mother, interferes with placental development, resulting in decreased fetal weight and fetal resorption that are independent of viral burden (*40*). The cellular mechanism by which the type I IFN response affects placental development has recently been elucidated. IFN-stimulated IFN-induced transmembrane (IFITM) molecules, which normally interfere with the viral fusion process, block fusion of cytotrophoblast to form multinucleated syncytiotrophoblasts, the cells of the placenta that interface with maternal blood (*41*, *42*). This cell fusion process is mediated by coopted retroviral envelope proteins called syncytins, which are necessary for placentation. This IFN-mediated disruption of pregnancy may be an evolutionarily adaptive "quality control" mechanism to minimize maternal investment early in a potentially unsuccessful pregnancy. However, it could also be an incidental consequence of antiviral responses interfering with the dependence on viral proteins for placentation.

The toxic impacts of type I IFNs in the developing fetus have been previously implicated in Aicardi–Goutières (AGS) syndrome, a genetic disease that leads to type I IFN overexpression caused by impaired nucleic acid metabolism (*43*). AGS patients suffer clinical symptoms very similar to congenital infections, which is known as pseudo-TORCH syndrome. Thus, AGS serves as a proof of principle for the toxic effects of dysregulated fetal immune responses on human development.

Current therapies and future directions

For women who have been exposed to a viral infection during pregnancy, current therapies focus on limiting viral replication. For example, hyperimmunoglobulin is recommended after exposure to VZV (*3*), and antiretroviral therapy is effective at preventing HIV transmission from mother to fetus (*1*). For many other viruses, this strategy is limited by the general lack of effective antiviral therapies. A better mechanistic understanding of how viral infection disrupts fetal development will be crucial to designing future therapies that limit congenital diseases. In cases where immune responses mediate damage to the fetus, insights into molecular pathways that lead to viral control versus pathology will guide more targeted therapies against the latter. Some ongoing questions include: (i) During maternal infection with TORCH pathogens, what factors (maternal, fetal, and viral) determine whether a fetus will be infected and develop long-term sequelae? (ii) What role do the immune cells at the maternal–fetal interface, including Hofbauer cells and decidual immune cells, play in limiting viral spread, propagating viral infection (in the mother and fetus), and exacerbating disease? How do viral infections affect the normal developmental functions of these immune cells? (iii) Do fetal immune responses effectively control viral replication? Evolutionarily, how have in utero viral infections shaped fetal immune development both to limit immunopathology and control infection? (iv) Beyond IFNs, how do fetal antiviral immune responses mediate some of the diseases associated with congenital viral infection? (v) In addition to antibodies (see accompanying reviews in this issue), how do maternal immune responses reach and influence fetal development and viral control? (vi) How will emerging viral infections affect the short- and long-term health of both mother and fetus?

Given the distinct cell types at the maternal–fetal interface and the dynamic nature of fetal development, exploring viral–maternal–fetal tripartite interactions has the potential to reveal unexpected insights into developmental biology, immunology, and virology. In particular,

viral infections could be a functional probe with which to better understand immunological changes during pregnancy and fetal development. Principles learned while studying viral infections during pregnancy, such as the impacts on placental development and brain development, could then be applied to gaining a better understanding of the pathogenesis of other common nonviral pregnancy complications and congenital diseases.

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ACKNOWLEDGMENTS

Funding: This work was supported by the National Institutes of Health (grants AI054359, R01EB000487, R01AI127429, and R21AI131284 to A.I.). A.I. is an Investigator of the Howard Hughes Medical Institute. C.L. is a Pew Latin American Fellow. **Competing interests:** The authors declare no competing interests.

10.1126/science.aaz1960

Contributions of maternal and fetal antiviral immunity in congenital disease

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Science **368** (6491), 608-612.
DOI: 10.1126/science.aaz1960

REVIEW

Vaccination strategies to enhance immunity in neonates

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Neonates are particularly susceptible to infection. This vulnerability occurs despite their responsiveness to most vaccines. However, current vaccines do not target the pathogens responsible for most of the severe neonatal infections, and the time it takes to induce protective pathogen-specific immunity after vaccination limits protection in the first days to weeks of life. Alternative strategies include using vaccines to broadly stimulate neonatal immunity in a pathogen-agnostic fashion or vaccinating women during pregnancy to induce protective antibodies that are vertically transferred to offspring within their window of vulnerability. Protection may be further improved by integrating these approaches, namely vaccinating the neonate under the cover of vertically transferred maternal immunity. The rationale for and knowledge gaps related to each of these alternatives are discussed.

Infectious morbidity and mortality are highest in the first weeks after birth (*1*, *2*). This vulnerability is not unexpected, given the predominantly naïve phenotype of neonatal immune cells and distinctive immunological challenges at birth, which require discrimination between not only innocuous self-antigens and noninherited maternal antigens but also the wide assortment of foreign antigens associated with primary commensal colonization (*3*, *4*). Susceptibility to severe infection likely reflects a combination of these physiological constraints.

Vaccination remains one of the most cost-effective ways of preventing infection. Vaccines against poliomyelitis, hepatitis B, tuberculosis, tetanus, pertussis, diphtheria, *Haemophilus influenzae* type b (Hib), rotavirus, and measles are administered to millions of infants, preventing an estimated 2.5 million deaths each year (*5*). Although vaccination has clearly benefited older infants and children, it has been considerably less effective in the first month of life (*1*, *2*). The World Health Organization recommends vaccination against tuberculosis, hepatitis B, and polio as soon as possible after birth (<24 hours) to accelerate priming of protective immune components. Likewise, maternal vaccination protects against infection by certain pathogens through vertically transferred immunity (*6*). However, emerging evidence shows that neonatal infections in lower- and middle-income regions are caused by a diversity of pathogens (Fig. 1). A recent meta-analysis identified *Staphylococcus aureus*, *Klebsiella*, and *Escherichia coli* spp. as the dominant causes of bacteremia and sepsis in neonates (infants younger than 28 days) in sub-Saharan Africa (*7*). *Ureaplasma* spp. and Group B *Streptococcus* were most frequently identified among cases of suspected early onset sepsis (infants 3 days or younger) in South Africa (*8*), whereas respiratory syncytial virus (RSV) and *Ureaplasma* spp. were the most commonly identified pathogens in cases of possible serious bacterial infection in infants younger than 60 days in Southeast Asia (*9*). Notably, none of these pathogens are covered by vaccines currently in clinical use (Fig. 1). Furthermore, the inciting pathogen was not identified in >70% of cases of clinically suspected infection, despite the use of cutting-edge diagnostic approaches (*8*, *9*). Although some of these undiagnosed cases may not be bona fide infections, the proportion of causative pathogens missed by vaccination is still likely to be greater than currently appreciated. Thus, alternative strategies to enhance early life immunity against a wide variety of pathogens are needed. We summarize the principles underpinning vaccination of neonates and their mothers, including increasingly recognized pathogen-agnostic benefits, which highlight the need to consider the mother–newborn dyad as one immunological unit to optimally enhance early life immunity.

Pathogen-specific immunity after neonatal vaccination

The neonate is often inappropriately considered "immature" and therefore presumed unable to respond to vaccination. Dampened antibody responses to T cell–independent polysaccharide antigens of encapsulated bacterial pathogens, including Hib and pneumococcus, until 2 years of age correlate with reduced marginal-zone B cells. Nonetheless, the conjugation to protein carriers activates T cells, resulting in robust protective antibody responses even in neonates (*10*). Similarly, diphtheria–tetanus–whole cell pertussis and some acellular pertussis vaccine formulations have been described to elicit reduced responses in neonates compared with older infants (*11*). However, monovalent acellular pertussis vaccines administered to neonates induce strong primary responses and do not induce tolerance to vaccine boosters (*12*). Compared with older infants, neonates are just as, if not more, responsive to vaccines currently included in neonatal immunization programs, namely bacillus Calmette-Guérin (BCG) vaccine, oral polio vaccine (OPV), and hepatitis B vaccine (*13*, *14*). The serological response of the neonate is also robust in response to other vaccines not currently licensed for neonatal administration, for example, those targeting rotavirus, diphtheria, and tetanus (*10*).

Even live vaccines have an outstanding safety record in neonates. Disseminated BCG infection is exceptionally rare (<1 per one million vaccine recipients) and almost exclusively occurs in infants with underlying immune deficiency (*15*). Vaccine-associated polio primarily occurs in underimmunized populations, which facilitate person-to-person spread, persistence, and eventual reversion into a more virulent phenotype. Vaccine-associated polio is expected to further decline with reformulation of trivalent to bivalent OPV (*16*). Furthermore, evidence of similar rates of infection by non–vaccine-targeted pathogens in older children regardless of prior cumulative vaccine exposure argues against the misconception that vaccines may overload and weaken the immune system (*17*). Thus, neonates are exceedingly capable of responding robustly and safely to most vaccines.

Given that neonates are capable of robust vaccine responses, why have current vaccination programs not lead to mortality reductions in neonates that are comparable to those in older infants and children? First, current vaccines administered to neonates do not specifically target the pathogens that cause severe infection in the first weeks of life (Fig. 1). Although tuberculosis, hepatitis B, and polio can be acquired within the first weeks after birth, these infections clinically manifest mostly outside of the neonatal period. For pathogens that do cause severe infection in the first weeks after birth, such as RSV, *Ureaplasma*, and several other bacteria, vaccines are either unavailable or have not yet been tested in neonates. Second, priming a protective adaptive immune response in predominantly naïve neonatal cells often takes weeks (*18*), whereas infections can cause morbidity and mortality within the first few days after birth (*1*, *2*) (Fig. 2A). This discordance between when infections occur and the time it takes to prime protective pathogen-specific immunity makes strategies aimed at inducing protective neonatal adaptive immune components challenging. To more effectively protect against infections manifesting in the neonatal period, alternative strategies, such as boosting resistance through non–pathogen-specific (i.e., pathogen-agnostic) approaches

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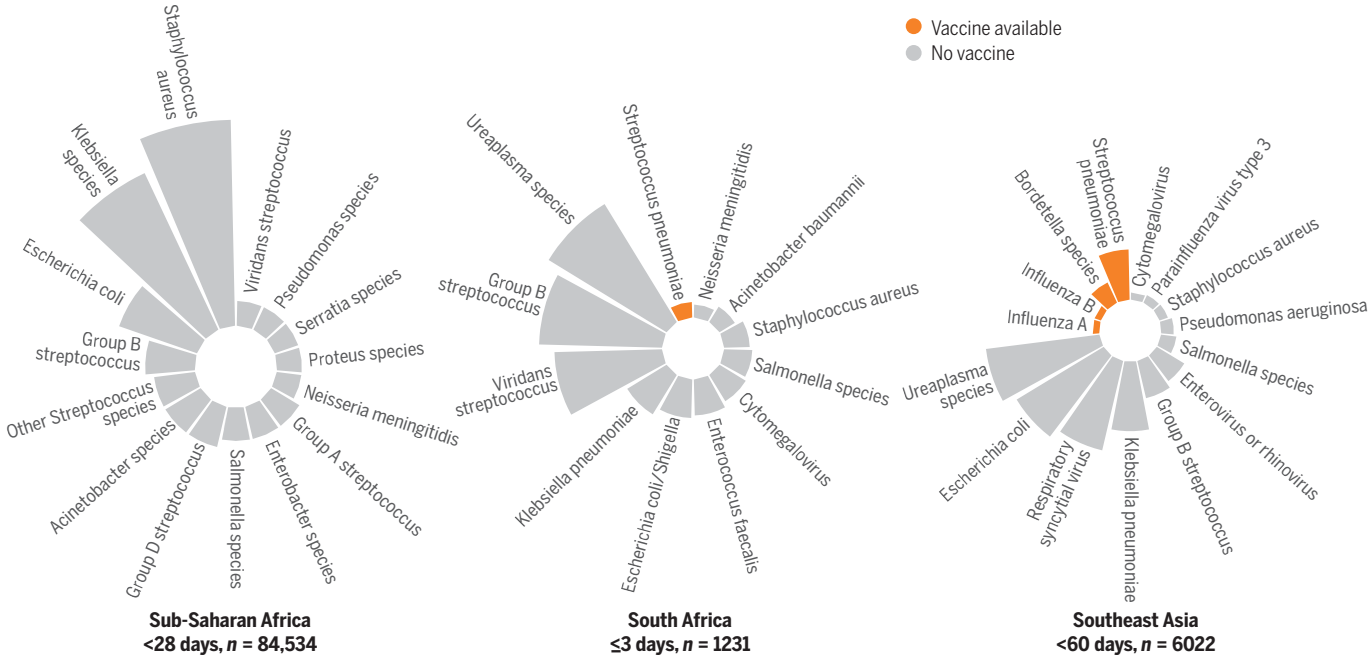

Fig. 1. Current maternal and neonatal vaccines do not cover most pathogens associated with severe infection in early life. Relative proportion of each pathogen, represented by the size of each segment, identified in recent studies of suspected sepsis in early life (*7*–*9*). The top pathogens from each study are shown, covering >90% of cases in which a pathogen was identified. Age range (in days) and number of neonates are shown for each study.

and/or promoting transfer of pathogen-specific maternal immunity, must be considered.

Pathogen-agnostic protection after neonatal vaccination

Accumulating evidence shows that live vaccines can broadly enhance host resilience against infection beyond their specific pathogen target (*19*, *20*). A recent meta-analysis encompassing >6000 low–birth weight neonates attributed an additional 38% reduction in neonatal mortality to BCG vaccine administered at birth, beyond protection against tuberculosis (*21*). A separate study including >7000 neonates showed a 40% reduction in mortality when OPV was administered with BCG vaccine within the first 2 days of life (*22*). These pathogen-agnostic protective effects appear to be fast-acting, because substantial reduction in overall neonatal mortality can be identified within the first 3 days after BCG vaccine administration (*21*), in contrast to the weeks required to achieve pathogen-specific immunity. Enhanced serological responsiveness to other vaccines in neonates administered BCG vaccine at birth further highlights the broad immunostimulatory effects of BCG vaccination (*23*).

Mechanisms by which live vaccines confer pathogen-agnostic protective effects have not been established, but they likely include cross-reactive T cells (e.g., heterologous immunity) or activation of innate immune components (e.g., trained immunity) (*19*, *20*). Another unresolved question is whether pathogen-agnostic protective effects primed by live vaccines are restricted to the neonatal period. Analysis of >15,000 children in rural Guinea-Bissau showed that mortality reductions associated with BCG vaccine scarring were limited to children vaccinated within the first 4 weeks of life, with the most pronounced effect observed among those vaccinated within the first week of life (*24*). Although a distinctive window of opportunity in the neonatal period could be inferred from these data, this pathogen-agnostic protection has also been shown for older infants administered other live vaccines (*25*, *26*). An expanded window of plasticity for pathogen-agnostic immunity is supported by similar reductions in childhood mortality associated with live attenuated measles vaccine administered after 4 months of age (*27*). Given that pathogen-agnostic approaches have the potential to confer broad and fast protection to the neonate—bypassing each of the drawbacks associated with current pathogen-specific strategies for neonatal immunization—establishing protective mechanisms is an important next step.

Pathogen-specific immunity after vaccinating mothers

Multiple adaptations occur during pregnancy to accommodate growth and avert rejection of the semiallogeneic fetus. These tolerogenic adaptations are likely anatomically confined and/or restricted to cells with fetal specificity, because the response to vaccines administered during pregnancy is largely comparable to that of nonpregnant women (*28*). Vertically transferred maternal antibodies protect offspring in the early postnatal period (*6*). An important distinction between vaccination of mothers and neonatal immunization is the transient nature of the protective benefits conferred by non-self-renewing antibodies that functionally persist in infants only for several months, thereby deferring infection until the consequences are less severe (Fig. 2B).

Vaccination during pregnancy has already been shown to be effective for several important pathogens. For example, tetanus vaccination of pregnant women reduces neonatal mortality from tetanus by >90% (*29*). Protection of infants against respiratory illness and confirmed influenza infection ranges from 30 to 60% when mothers are vaccinated during pregnancy (*30*). Protective efficacy against pertussis in the first 2 to 3 months of life is ~90% after maternal vaccination (*31*). In light of these considerable benefits, developing vaccines for pregnant women that target other neonatal pathogens should be prioritized.

Maternal antibodies transferred across the placenta are almost exclusively immunoglobulin G (IgG), the levels of which exponentially increase in fetal tissues during the final weeks of gestation. Transfer is coordinated by binding to Fc receptors expressed by trophoblasts, macrophages, and endothelial cells, with preferential transfer of some isotypes (*32*). The accelerated transfer of maternal antibodies in later gestation means that immunity primed by maternal vaccination is drastically different for preterm infants. IgG levels are also reduced among small-for-gestational-age infants, as well as infants born to mothers with chronic infections, such as HIV or placental malaria (*33*). Maternal IgA and IgG antibodies are also transferred through

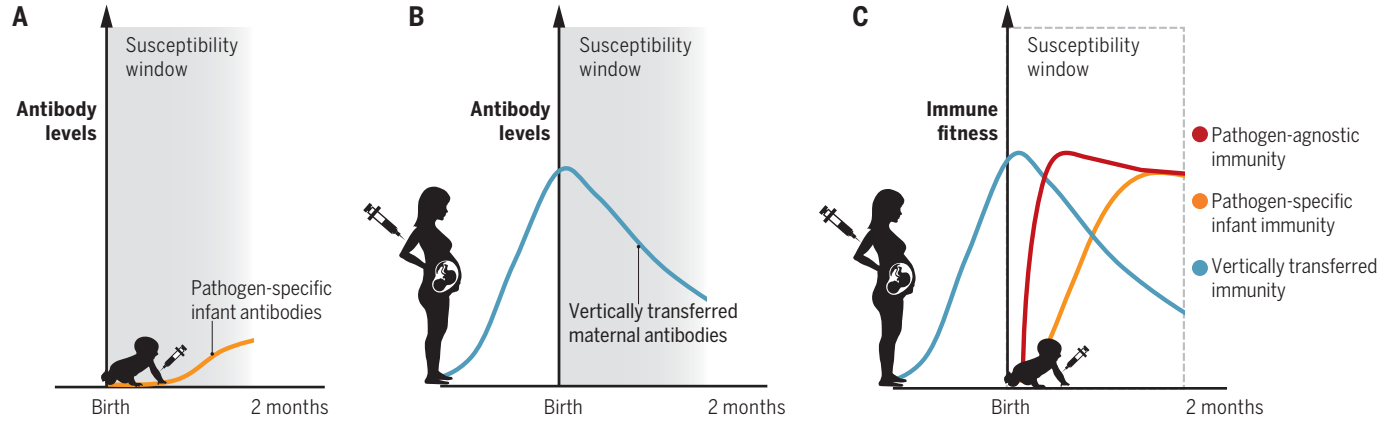

Fig. 2. Tempo of immunity provided by vaccination at birth compared with vaccination during pregnancy in relation to the neonatal window of infection susceptibility. (**A**) Antibody levels primed by neonatal vaccination increase with delayed tempo, which offers suboptimal protection during the early life window of susceptibility. (**B**) Maternal immunization provides high levels of pathogen-specific antibodies at birth and is an effective strategy for narrowing the window of susceptibility to specific pathogens. (**C**) Combining maternal vaccination with pathogen-agnostic and pathogen-specific benefits of neonatal vaccination may contribute equally to optimal neonatal immune fitness and efficiently close the early life window of susceptibility.

breastfeeding, and increased levels of both isotypes can be detected in breastmilk after vaccination during pregnancy (*28*). Optimal protection of neonates will require establishing the molecular determinants of antibodies transferred through breastmilk and whether they functionally complement placentally transferred antibodies.

Although vaccination during pregnancy raises concerns regarding safety, the vaccines currently administered to pregnant women have excellent safety profiles. There is no evidence of increased pregnancy complications with inactivated vaccines adjuvanted with alum or oil-based emulsions (*34*). Live attenuated vaccines, however, are currently not recommended during pregnancy. Nonetheless, analysis after their inadvertent administration suggests that they are safe. Rubella virus vaccine administration to >3500 pregnant women with documented serological susceptibility did not cause congenital rubella syndrome, and only one case of asymptomatic virus shedding was reported (*35*). Administration of OPV or yellow fever vaccine in outbreak settings did not cause increased rates of growth retardation, congenital anomalies, or pregnancy complications in women vaccinated during pregnancy (*34*). One potential exception is smallpox vaccination; But even in this case, the largest meta-analysis (including >12,000 pregnant women) showed only marginally increased (relative risk: 1.3) incidence of congenital defects, with a similar incidence of other complications, including spontaneous abortion, stillbirth, and preterm birth (*36*). Thus, most live vaccines appear to be safe during pregnancy.

Linking the mother–newborn dyad

Chronic maternal infection with a variety of pathogens can affect infant health independently from pathogen transmission (*37*), along with the tempo and quality of immune development (*38*). HIV-exposed but uninfected infants have reduced levels of maternal antibodies and show increased susceptibility to severe infection by unrelated pathogens compared with infants not exposed to HIV (*39*). Cord blood cells from neonates born to mothers with chronic hepatitis B virus infection produce increased antimicrobial cytokines after stimulation with various bacterial pathogens (*40*). These phenotypic changes in neonatal immune cells may reflect stimulation by antigens transferred in utero, with evidence of both activating and tolerogenic impacts on fetal immune components (*41*, *42*). Maternal programming of neonatal immunity also persists after birth by way of cells, cytokines, and antibodies acquired through breastfeeding (*43*) and by maternal cells that establish microchimerism (*44*). Thus, immune fitness, defined as resistance to severe infection, is dominantly influenced by maternal immunological experience.

Vertical transfer of maternal antibodies is teleologically conserved, and enriched for glycosylated antibodies that promote antimicrobial activity in neonates (*6*, *32*, *33*, *45*). Vertically transferred immunity can also dominantly influence the response of offspring to vaccination. High-titer maternal antibodies have often been associated with diminished primary antibody response of infants to vaccines (*46*, *47*). A classical study prompted by increased symptomatic measles infection among children immunized before their first birthday showed a muted serological response in children with high-titer pre-vaccination antibodies and increased responsiveness in children with reduced pre-vaccine titers (*48*). Interference of infant serological response is also observed for other live and inactivated vaccines, although the reduction magnitude is variable between studies and individual vaccines (*33*, *49*, *50*).

Interference by preexisting antibodies is not specific to infants and instead likely reflects control of excessive antibody production classically described in adults (*51*). Masking of immunodominant epitopes, regulation of B cell activation and germinal center maturation, and B cell inhibition through FcγRIIB cross-linking are potential mechanisms (*52*, *53*). The priming of memory B cells is much less sensitive to the presence of high titers of preexisting antibodies, because infant responses to vaccine boosters are consistently preserved with primary vaccination under the cover of high titers of maternal antibodies (*54*–*56*). T cell priming also appears to be intact, because the presence of antibodies affects neither proliferation nor effector cytokine production (*57*, *58*). Thus, interference is generally restricted to the primary serological response of offspring to vaccination. However, the clinical implications remain uncertain, because memory B and T cell responses primed by vaccination of neonates under the cover of maternal immunity remain intact.

Vaccination during early infancy under the cover of maternal immunity may in fact prime responses that are more protective, especially considering the aforementioned pathogen-agnostic protective benefits of live vaccines. A 78% reduction in mortality was shown for infants administered live attenuated measles vaccine at 4.5 months of age in the presence of maternal measles antibodies at the time of vaccination (*27*). The reduction of infant mortality associated with BCG vaccination in the neonatal period is further enhanced among infants born to mothers with prior BCG priming (*59*). A more balanced response by vertically transferred innate and adaptive maternal factors including antibodies, cytokines, cells, or metabolites likely explains these enhanced protective benefits. Considering this potential to enhance antimicrobial host defense, further narrowing the window of neonatal susceptibility against a wide range of pathogens will likely require

stimulating pathogen-agnostic and pathogen-specific immunity by neonatal immunization under the cover of maternal immunity (Fig. 2C).

Outlook

Neonatal infection is a complex, multifaceted problem with many critical dimensions yet to be defined. The pathogens associated with neonatal infections in low-to-middle income areas have only recently been systemically evaluated using modern diagnostic tools (*8*, *9*) (Fig. 1). The wide range of identified bacteria and viruses with varying virulence, combined with the large fraction of cases where a specific pathogen was not identified, suggests that complex immunological perturbations in the neonatal period drive clinical sepsis. Future diagnostic and treatment strategies will need to go beyond current approaches, which are narrowly focused on specific inciting pathogens. Likewise, designing vaccines that target the mother–newborn dyad implies knowledge of how mother and child are immunologically linked. However, current knowledge of how human pregnancy is sustained remains rudimentary. The necessity for specific molecules and immune cell subsets in maintaining maternal–fetal tolerance has almost exclusively been established using preclinical pregnancy models (rodents), which do not recapitulate the more prolonged gestational length and in utero accumulation of fetal adaptive immune components observed in humans (*60*).

Despite our present ignorance, vaccines that prime pathogen-specific immunity in the maternal–fetal dyad clearly work. We are on the brink of eradicating poliomyelitis with vaccines administered to neonates. Eliminating neonatal tetanus is also within reach by way of maternal vaccination. Boosted pathogen-agnostic immunity primed by live vaccines also shows promise, with nearly 40% reductions in overall infant mortality (*21*, *22*, *52*). These successes clearly highlight the protective potential of neonatal and maternal immune components. Enhanced protection will likely require previously unexplored strategies that combine vaccination of mothers and their newborns to simultaneously stimulate pathogen-agnostic and pathogen-specific immunity (Fig. 2C). Physicians are instructed to first "do no harm." This instills a reflexive reluctance to deviate from the status quo. Unfortunately, the current status quo is that nearly half of under-age-5 mortality occurs in neonates, and a large fraction of these deaths are due to infection. Perhaps actively excluding pregnant mothers and newborns from vaccine research is inadvertently causing even more harm. The priority should be to protect these vulnerable populations through research, not from it.

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ACKNOWLEDGMENTS

We thank C. B. Wilson for his inspirational mentoring and pioneering contributions defining the field of early life immunology. We are indebted to contributions by numerous investigators in this field and apologize that many important references were unable to be discussed due to space constraints. **Funding:** T.R.K. is supported by NIH/NIAID U19AI118608 and Telethon Kids and Perth Children's Hospital Foundation. A.M. is research director at the Fonds de la Recherche Scientifique, F.R.S.-FNRS, Belgium. S.S.W. is supported through NIH grants DP1AI131080, R01AI120202, R01AI124657, and U01AI144673; the HHMI Faculty Scholar's Program; the Burroughs Wellcome Fund; and the March of Dimes Foundation Ohio Collaborative. **Competing interests:** The authors have no competing interests to declare.

10.1126/science.aaz9447

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Science **368** (6491), 612-615.
DOI: 10.1126/science.aaz9447