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REVIEW

Establishment of tissue-resident immune populations in the fetus

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Abstract
The immune system establishes during the prenatal period from distinct waves of stem and progenitor cells and continuously adapts to the needs and challenges of early postnatal and adult life. Fetal immune development not only lays the foundation for postnatal immunity but establishes functional populations of tissue-resident immune cells that are instrumental for fetal immune responses amidst organ growth and maturation. This review aims to discuss current knowledge about the development and function of tissue-resident immune populations during fetal life, focusing on the brain, lung, and gastrointestinal tract as sites with distinct developmental trajectories. While recent progress using system-level approaches has shed light on the fetal immune landscape, further work is required to describe precise roles of prenatal immune populations and their migration and adaptation to respective organ environments. Defining points of prenatal susceptibility to environmental challenges will support the search for potential therapeutic targets to positively impact postnatal health.

Keywords Fetal immunity · Prenatal development · Embryogenesis · Immune ontogeny

Introduction

The development of the immune system during the prenatal period is layered in time and space to meet developmental needs and prepare the human fetus for postnatal life [1]. The identification of fetal immune cell populations specific to their tissue environment at a certain fetal age suggests that the immune system serves roles for organ growth and maturation [2–5]. Furthermore, strong evidence describes the persistence and self-maintenance of fetal-derived immune cell populations in most adult tissues, contributing to the resident immune compartments independent of adult long-term hematopoietic stem cells (HSC) [6, 7]. This exemplifies the potential for lasting impact of the prenatal period on postnatal immunity. Tissue residency is conventionally assigned to an immune population that possesses self-renewal capacity, expresses a tissue-specific transcriptional program, e.g., homing receptors, and undergoes little to no exchange with the circulating pool of immune cells [8, 9]. During embryogenesis, the spatiotemporal seeding of the immune system in the various organ systems can be considered the establishment of immune tissue residency. We here review the formation of tissue-resident immunity in the human fetus, supported by evidence from animal models, focusing on recent insights in prenatal gut, lung, and brain development.

Establishment of the fetal immune system in time and space

The immune system develops in spatiotemporarily highly coordinated waves of hematopoiesis [1, 10], described for mouse myeloid lineages as primitive, transient definitive, and definitive waves [11]. In humans, early hematopoietic cells (primitive wave) are detected in the extraembryonic yolk sac (YS) around 4 postconception weeks (PCW[1]).

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[1] PCW describes the fetal age since fertilization, which occurs about 2 weeks later than the start of the last menstrual cycle, which, in turn, is used to estimate gestational age (EGA) in weeks. Throughout this

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Primitive HSC-like and erythro-myeloid progenitors (EMPs) give rise to premacrophages, mast cells, natural killer (NK), and innate lymphoid cell (ILC) progenitors, while megakaryocytes and erythroid cells enable oxygen supply to the growing fetus [12–14]. Concomitantly, definitive HSC and multipotent progenitors (MPP) that are capable to give rise to all mature blood and immune cells for the life of the individual are generated in the intraembryonic hemogenic endothelium in the aorta-gonad-mesonephros (AGM) region [15–17] and other hemogenic regions, including the umbilical cord and placenta [18]. In human fetuses after 6 PCW, YS- and AGM-derived macrophages and mast cells can be detected among fetal peripheral tissues such as skin, brain, and kidney; some of them constitute lifelong self-maintained populations that are independent from repopulation by bone marrow (BM)–derived precursors, as shown in mice, while others such as in the gut will be gradually replaced by circulating precursors derived from definitive HSC [11, 12, 14, 15, 19]. Between 6–9 PCW, both YS- and AGM-derived progenitors and definitive HSC with long-term repopulation capacity will seed the fetal liver to commence definitive hematopoiesis with long-term reconstitution potential [20, 21]. The fetal liver is the major hematopoietic organ during fetal development, contributing during all developmental stages and supporting active erythro-myeloid hematopoiesis and HSC expansion [12, 22]. After establishment of the hematopoietic fetal liver, the fetal BM will be seeded by fetal liver–derived HSC at 11–12 PCW, establishing it as the niche for lifelong hematopoiesis after birth, including the quiescent state of adult HSC [23]. Interestingly, osteoclasts, stromal cells essential for hematopoietic niche homeostasis, are EMP-derived cells [24]. In contrast to YS- and AGM-derived macrophages and mast cells, differentiation of the neutrophil lineage, dendritic cells (DC), and monocytes are considered dependent on the fetal BM niche in humans [23, 25] and mice [26]. B cell lineage expansion is a major feature of second trimester fetal BM hematopoiesis [23]. T cell differentiation and maturation on the other hand occur mainly in the fetal thymus and start with the onset of fetal liver hematopoiesis (6–9 PCW) [27].

Tissue-resident immune ontogeny through the lens of fetal macrophage development

The establishment of tissue compartmentalization and lifelong self-sustained immune cell populations originating from embryonic precursors is best described for macrophages in developing mouse tissues [11, 13], which is accompanied by limited but readily increasing human evidence (reviewed in [28]). A nowadays outdated concept hypothesized that tissue-resident macrophages arise from blood monocytes differentiating in the BM, known as the "mononuclear phagocyte system" [29]. Methodological achievements like fate-mapping experiments revealed that certain cell types in certain tissues originate from different developmental pools of precursor cells of early and late embryonic, neonatal, and adult stages. The complex composition of immune cells of different origins is termed "layered immune system," where certain cell types have distinct origins ranging from entirely or partly fetal- to adult-derived precursors. This concept is for instance apparent in the establishment of tissue-resident macrophages, mast cells, γδ T cells, and ILCs [7, 14, 30–32].

In addition to the primitive and definitive waves of hematopoiesis, another wave of "transient definitive hematopoiesis" from multipotent progenitors (EMPs and lymphoid–myeloid progenitors (LMP)) gives rise to early lymphoid populations and tissue-resident macrophages via a monocytic precursor in mice [33]. Importantly, this wave is still independent of long-term HSC and includes YS EMPs, which seed the fetal liver, where they develop into multiple myeloid lineages including fetal monocytes until mouse embryonic day (E)16.5 [34]. These monocytes seed virtually all embryonic tissues except the brain after mouse midgestation and adapt their transcriptional program to their niche to become specialized tissue-resident macrophages [33]. Tissue-resident macrophages are, to varying levels, self-maintained in postnatal tissues, independently of definitive HSC and replenishment by blood monocytes in steady-state conditions [35, 36]. Prominent examples of these macrophages of strict embryonic origin that are independent of circulating macrophages include brain microglia [37], epidermal Langerhans cells [38], and alveolar macrophages [39]. Other populations can be gradually replaced by adult HSC–derived monocytes, including liver Kupffer cells, splenic, and intestinal macrophages [36, 40, 41].

In an attempt to explain the layered nature of tissue-resident macrophage populations across tissues and ages of an individual, Guilliams and Scott [42] proposed a niche model, hypothesizing that during organogenesis, tissue niches are created (e.g., in the brain) and become available for a limited number of premacrophages from the YS (first primitive wave), which in turn, upon engraftment, receive signals to adopt a tissue-specific phenotype. With continued development, more tissue niches become available for the second transient-definitive wave of embryonic precursors (fetal liver–derived monocytes) until the niche is full. Accordingly, the contribution of definitive HSC-derived monocytes to neonatal tissue composition would be attributable to continued organ maturation after birth. Replacement only occurs when spots in the niche become available. Differences in niche availability for replacement with circulating precursors

Footnote 1 (continued)

review, we report PCW and EGA verbatim from the source reports to avoid introducing inaccuracies.

(i.e., liver, spleen, and intestine available vs brain, skin, and lung being unavailable) might stem from absence or presence of self-renewal-promoting cues, respectively, which are likely to be highly tissue- and/or precursor-specific [42, 43].

During embryogenesis, tissue-resident macrophages contribute to differentiation, maturation, and remodeling of fetal organs, as described in detail for the brain below. Epidermal Langerhans cells are first observed in human fetal skin at 4–5 PCW [44]. In adult skin, they exhibit a specialized phenotype which includes the capacity to repopulate and present antigen, amidst supporting barrier function and repair, and contributing to extracellular matrix remodeling [32, 44–46]. While their developmental function remains incompletely understood, fetal Langerhans cells present a mature phenotype by 16 PCW [47, 48]. Prenatal skin macrophages have been suggested to support angiogenesis and chemotaxis [48]. In the fetal liver, fetal Kupffer cells are considered to adopt similar functions as their adult counterparts, coordinating erythropoiesis and iron recycling [49, 50], responding to bacterial compounds by secreting bona fide tissue-resident macrophage cytokines TNFα, IL-6, and IL-1β in early gestation humans [51, 52], and showing high proliferative capacity, as well as peroxidase activity in mice [53]. The tissue-resident macrophages of embryonic origin of the spleen, red pulp macrophages, are potent iron recyclers and removers of senescent erythrocytes from the neonatal circulation in mice [54, 55], a role they may assume during embryogenesis.

Fetal tissue-resident lymphoid cells

Fetal lymphoid cells partially derive from the wave of transient definitive hematopoiesis prior to definitive hematopoiesis and include NK cells [56], likely lymphoid tissue inducer cells (LTi) [30, 57], as well as marginal zone and B-1 B cells [58], and thymus-derived fetal γδ T cells [32, 59], where, for the latter two, a transient HSC precursor has been described in mice that disappears with adulthood [60]. Similar to myeloid cells, fetal lymphoid cells possess functions that support prenatal development. B-1 cells, considered part of the innate immune system, function in a T cell–independent manner by spontaneously secreting antibodies with limited specificity in peripheral tissues [61, 62], conferring the fetus with unspecific yet immediate immune response capacity. Fetal EMP-derived NK cells are highly potent cytotoxic cells, yet hyporesponsive to HLA^{neg} target cells compared to their adult HSC–derived counterparts [3, 56]. The early developmental origin of human ILC lineages has recently been mapped across fetal tissues during early gestation (8–12 PCW), establishing their varying proportions in and proliferative (i.e., differentiating) states per organ [63]. In a fate-mapping model in mice, prenatal ILC were gradually replaced postnatally, suggesting potential specific roles in their various prenatal tissue sites [30], which will require further investigation. Thymus-derived lineages of fetal T cells derive from fetus-exclusive HSC [64] and appear in the periphery with the start of the second trimester to seed empty niches in lymphoid and mucosal tissues [65–67], reviewed in [68]. In humans, T cells of all lineages are established in utero after gestational week 14 [69–71], possessing innate-like [72], protective [5, 73, 74], and tolerogenic [65, 66, 75] immune response capacity. This is in stark contrast to mice, who only develop T cells after birth [76]. Of note, circulating fetal T cells in the cord blood at term primarily show a naive phenotype and an impaired response towards alloantigens [77], while T cells in the early fetal intestine possess an effector memory phenotype with clonal diversity [5, 66, 74]. This human prenatal T cell landscape adequately exemplifies the compartmentalization of fetal immunity across tissues: systemic, circulating immune cells do not necessarily reflect tissue-resident phenotypes and functions of self-sustained immune cells that contribute to local organogenesis and immunity later in life.

We display current seminal evidence for the human fetal immune landscape and their functional attributes in Fig. 1, and summarize human and mouse studies of fetal immune subsets across tissues in Table 1. Overall, at least in mice, the contribution of definitive hematopoiesis in fetal liver, and later fetal BM, to prenatal tissue-resident immunity has been suggested to be smaller than the contribution from EMP and LMP, which are giving rise to the majority of cell lineages supporting fetal organ development. As proposed by Hoeffel and Ginhoux [33], the stemness of BM-HSC might be preserved until fetal progenitors are fully consumed and have established tissue residency. Furthermore, as proposed by many, including [6], fetal stem and progenitor cells that sense maternal perturbations (infection/inflammation/microbiome/metabolic state) might give rise to progenitors with altered persistence, differentiation potential, and cellular output. Such impact on progenitor populations might amplify into deviations in long-term immune homeostasis and immunity later in life. Consequently, improving fetal and neonatal health will depend on a thorough understanding of prenatal-specific immunity in a spatiotemporal manner.

Development and function of resident immunity in the fetus

Mucosal immune populations in the fetal gut

As the largest barrier organ in the human body, the intestine has to coordinate nutritional demands and immune symbiosis with the gut microbiome [78]. Especially after birth, the mucosal intestine gets bombarded by environmental, nutritional, and microbial exposure [79]. As a result, the intestine needs to balance innocuous responses to these exposures,

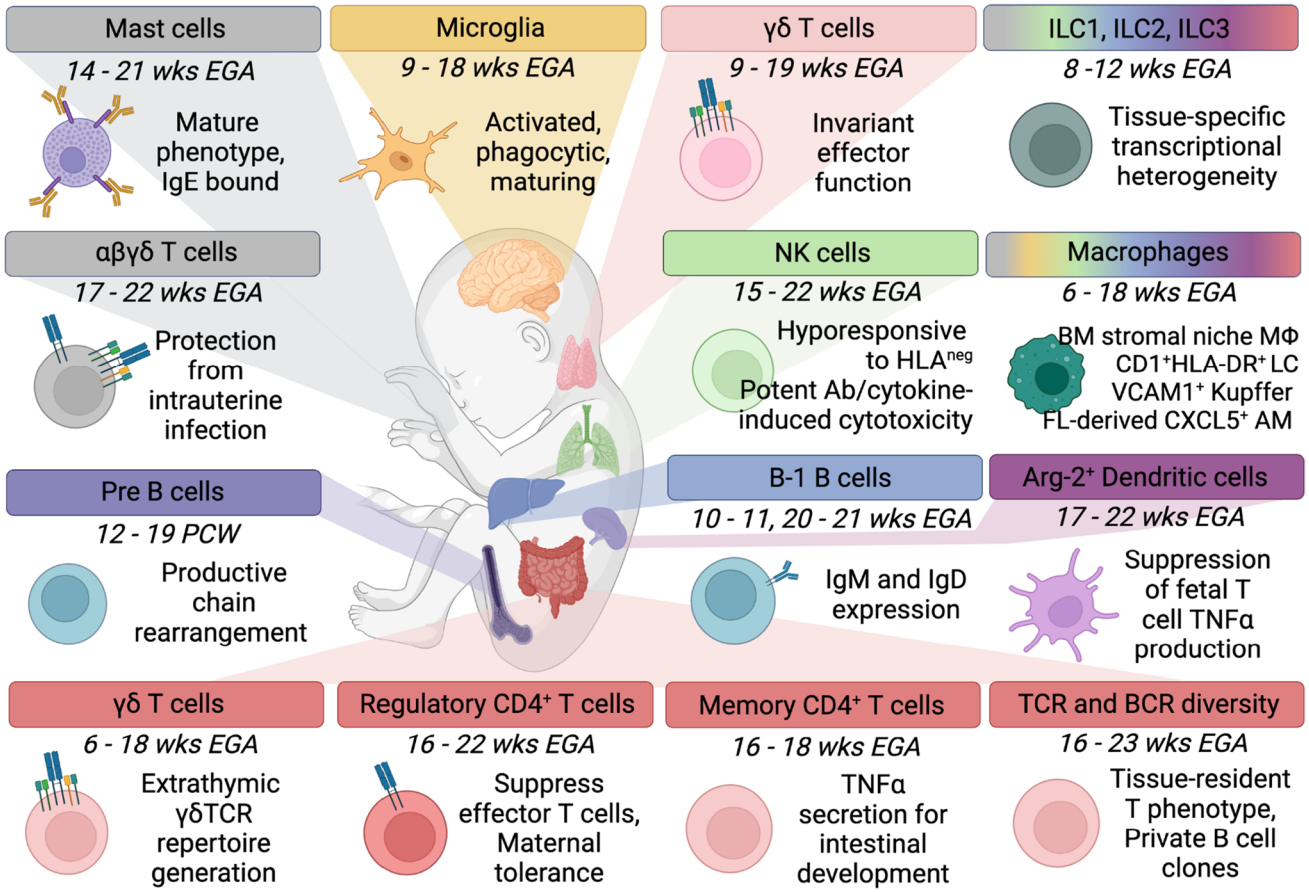

Fig. 1 Tissue-resident immune populations and functional features in human fetuses. While still incomplete, the current landscape of innate and adaptive immune populations residing in developing tissues during the first and second trimester indicates their role in organogenesis while providing protective/reactive *and* tolerogenic immunity. Representative seminal evidence is shown. Tissue-resident macrophages adopt specialized phenotypes supporting their niche development, including microglia in the central nervous system [2], Langerhans cells (LC) in skin [44], Kupffer cells in the liver [12], stromal macrophages (MΦ) in the bone marrow [23], and potentially red pulp [55] and alveolar macrophages (AM) [108, 117]. Similarly, innate lymphoid cells (ILC), bona fide tissue-resident cells, are distributed across tissues displaying site-specific phenotypes with as yet incompletely characterized function during fetal life [63]. Lung NK cells possess potent antibody (Ab)- and cytokine-induced cytotoxicity, yet are biased towards tolerating HLA^{neg} cells [3]. In the skin, mature mast cells are sensitized towards allergens via IgE [186], and differentiation of erythroid progenitors might supplement fetal liver erythropoiesis [12]. Innate-like B1 B cells are prominent in the fetal liver and bone marrow, concomitant with naive and memory B-2 B cells [61, 62], while B cell clonality in the gut is primarily private, and not shared between individuals [82]. B cell lineage expansion occurs in the second trimester bone marrow [23]. Spleen dendritic cells (DC) have antigen-presentation capacity, supporting tolerogenic T cell responses [110]. T cells have an innate-like, fast-response phenotype (invariant γδ T cells) in the thymus [59, 229], skin [73], and intestine [59], however, are strongly biased towards mediating (maternal) tolerance [66, 71, 75], yet show TCR diversity [67, 82] and effector memory [5, 67, 82] in the mucosal surface of the gut. Cord blood $CD71^{+}$ erythrocytes (not shown) are perinatal immunosuppressors, potentially derived from fetal liver erythroid precursors [12, 244, 245]. Created with BioRender.com

while at the same time provide protection against potential pathogenic exposure. Unfortunately, differences in intestinal development between humans and animal models — mice are born with an immature gastrointestinal mucosa [79] — highlight the hurdles and difficulties of studying human intestinal immune development. However, recent technological advances have made it possible to study the development of the mucosal intestine in precious human fetal tissue.

Several studies have emphasized that the human fetal intestine contains a distinct tissue-specific immune composition and signature [80] that consists of antigen presenting cells (APC), ILC subsets, T cells, γδ T cells, and B cells which can already be observed in human fetal intestine at 6 to 23 weeks of gestation [59, 63, 67, 78, 80–82]. A recent scRNAseq study performed in intestinal tissue from 17 human embryos, ranging from 8 to 22 weeks post conception, suggest that intestinal immune cells are rare prior to the first trimester [78]. However, an enrichment of myeloid cells could be observed before 10 PCW, whereas an influx of T cells, NK cells, ILC1, and ILC3 were observed at 12

Table 1 Immune populations resident in human and mouse fetal tissues. Color-coding of table mirrors tissue highlighted in Fig. 1. Information regarding non-human models is indicated in gray. *E12.5*, embryonic day 12.5; *YS*, yolk sac; *FL*, fetal liver; *EMP*, erythro-myeloid progenitor; *ILC*, innate lymphoid cell; *DC*, dendritic cell; *pDC*, plasmacytoid DC; *cDC*, conventional DC; *Treg*, regulatory T cell; *DN*, double negative; *DP*, double positive; *EGA*, estimated gestational age; *GI*, gastrointestinal tract; *PCW*, postconception week; *LTi*, lymphoid tissue inducer; *MAIT cell*, mucosal-associated invariant T cell; *mMΦ*, *pvMΦ*, *cpMΦ*, subdural meninges, perivascular space, and choroid plexus macrophages; *P*, postnatal day; *iNKT cell*, invariant natural killer T cell; *KIR*, killer immunoglobulin-like receptor; *wks*, weeks

Organ	Species	Gestational age	Immune cell	Phenotype/Function/Ontogenic process	Reference
Skin	Human	6-12 wks EGA, 18-30 wks EGA	Langerhans cell	$CD1a^+$, $CD207^+$, $HLA\text{-}DR^+$, $OKT\text{-}6^+$, and $ATPase^+$	[4, 44, 48, 246]
	Human	7 - 12 PCW	Megakaryocyte-erythroid-mast cell progenitor (MEMP), Mast cell, Megakaryocytes and mid/late erythroid cells	Erythroid progenitors can contribute to erythropoiesis and supplement fetal liver erythroid output	[12]
	Human	7-24 wks EGA	Macrophage, Monocyte, Neutrophil-myeloid progenitor, Monocyte-DC	$CD45^+CD206^+CD209^+$, Support angiogenesis and leukocyte seeding	[4, 12, 48, 233]
	Human	7-23 wks EGA	DC (pDC, cDC1, cDC2)	$HLA\text{-}DR^+CD1c^+$, $CD11c^+$	[4, 12, 48, 233]
	Human	7-12 PCW, 18-24 wks EGA	Mast cell	$CD45^+CD117^+$, Mature phenotype, IgE bound	[12, 48, 73, 217, 233]
	Human	7 - 12 PCW	Early lymphoid/T precursor		[12]
	Human	17-30 wks EGA	T cell	CD8 memory T cell, IFNγ production	[4, 73, 233, 246]
	Human	17-22 wks EGA	αβγδ T cell	Barrier function, Contribute to skin formation	[73]
	Human	18-24 wks EGA	Treg	$CD3^+FoxP3^+$, Treg accumulation coincides with hair follicle development	[4, 233]
	Human	7-12 PCW	ILC1, ILC2, ILC3, ILC precursor	$IL7R^+$, $RORC^+$ and KIT^+, Resemble adult ILC3	[12, 48, 63]
	Human	7-12 PCW	NK cell	Higher expression of GZMM and GZMK compared to adult	[12, 48]
	Human	7-12 PCW, 23 wks EGA	Immature B cell, (Pre pro) B cell	$CD19^+HLA\text{-}DRint\text{-}CD24^+$	[4, 12]
	Mouse	E12.5-17.5	YS- and FL-derived Langerhans cells	$CD11b^+F4/80^+CSF\text{-}1R^+CX3CR1^+$	[38]
	Mouse	E12-18	EMP-derived Monocyte	$CD11bhiF4/80loCD64{+}Ly6C^{+/-}$	[33]
	Mouse	E9.5, E12.5	Macrophage	$CD45^+$ $CD11b^{lo}F4/80^{hi}Ly6C^{neg}$, $CX3CR1^{high}$	[33, 247]
	Mouse	E14.5-16.5	YS-derived Mast Cell	$c\text{-}Kit^+Avidin^+$, Fetal-specific, Replaced postnatally by adult Mast Cells	[14]
	Mouse	E17.5	ILC2	$Lin^{neg}IL\text{-}7Ra^+Thy1^+ST2^+$	[30]
Kidney	Human	7-16 PCW	Macrophage, DC	Anti-inflammatory gene signatures	[248]
	Human	9-16 PCW	Monocyte, T cell, NK cell	Reduced cytotoxic signatures	[248]
	Human	12-16 PCW	B cell	No evidence of class-switching	[248]
	Human	7 - 12 PCW	(Pre pro) B cell, ILC precursor, Early lymphoid/T precursor, NK, Neutrophil-myeloid progenitor, pDC, DC1, DC2, Monocyte-DC, Monocyte, Macrophage, megakaryocyte-erythroid-mast cell progenitor (MEMP), Mast cell, Megakaryocytes	Single-cell transcriptome map, Lack of erythroid cells	[12]
	Mouse	E12-18	Monocyte	$CD11bhiF4/80loCD64{+}Ly6C^{+/-}$	[33]
	Mouse	E11-18	Macrophages	Promote tubular cell proliferation, branching, nephron formation; $CD45^+$ $CD11b^{lo}F4/80^{hi}Ly6C^{neg}$	[33, 249, 250]

Table 1 (continued)

Spleen	Human	15-22 wks EGA, 17-23 wks EGA	NK cell	Various differentiation stages present (CD56, CD16, NKG2A), KIR$^+$ CD56$^+$ or NKG2A$^+$ or CD16$^+$, hyporesponsive cytotoxicity against HLA^{neg} target cells, strong cytokine-mediated cytotoxicity response	[3, 251]
	Human	19-22 wks EGA	CD5$^+$ B cell	Unconventional phenotype, unsupportive of Ig production	[252]
	Human	8-12 PCW	ILC1, ILC2, ILC3	ILC3 dominant population	[63]
	Human	12-24 wks EGA	DC	cDC1, cDC2, Responsive to TLR stimulation, Induce adult T cell proliferation, Arg-2$^+$ DC regulate fetal T cell TNFα production	[110]
	Human	16-23 wks EGA	iNKT cell	CD3$^+$TCR Vα23$^+$ CD1d-PBS57 tetramer$^+$, immature and less differentiated phenotype (CD4$^+$CD8^{neg}), high proliferative capacity, and hyporesponsive IFNγ response	[98]
Bone marrow	Human	12-19 PCW	Erythroid cells, Megakaryocyte, B cells, DC, Eosinophil/basophil/mast cell, Neutrophil, Monocyte, T/NK	Granulocyte emergence, DC subset diversification, B-lineage expansion, Mature T and NK cells, Erythropoiesis, Tissue-resident HSC/multipotent progenitors	[23]
	Human	15-22 wks EGA	NK cells	Various differentiation stages present (CD56, CD16, NKG2A), KIR$^+$	[3]
	Human	20-21 wks EGA	Memory B-2 and innate-like B-1 B cells	IgM and IgD expression	[62]
Thymus	Human	7-15 PCW	SP, DP, DN T cells, γδ T cells, ILC3, NK cells, T naive, Macrophages, DC1, DC2	Potentially tissue-resident GNG4$^+$ CD8αα$^+$T(I) colocalize with DC1, and non-activated DC in the perimedullary region; activated DC and Treg enriched in the center of the medulla, Additional fetal thymus-specific unconventional T cell subsets	[253]
	Human	8 - 12 PCW	ILC1, ILC2, ILC3	High proportion of putative ILC1	[63, 253]
	Human	15-21 wks EGA	Invariant γδ T cells	Programmed effector function, Generated independently of functional TCR expression; compared to gut γδ T cells: distinct, nonoverlapping TCRDV2R	[59, 229]
	Human	12-22 wks EGA	CD14$^+$ monocytes/macrophages, pDC, cDC1 and cDC2	/	[110]
	Mouse	E17.5-19.5	DN, DP thymocytes	Thymus homeostasis	[254]
	Mouse	E12.5-P0	Conventional T cells, non-conventional T cells, myeloid	/	[255]
Liver	Human	7 - 17 PCW	(Pre pro) B cell, ILC precursor, Early lymphoid/T precursor, NK, Neutrophil-myeloid progenitor, (p)DC precursor, DC1, DC2, Monocyte (precursor), Monocyte-macrophage, Kupffer cell, Erythroblastic island macrophages, megakaryocyte-erythroid-mast cell progenitor (MEMP), Mast cell, Megakaryocytes and early/mid/late erythroid cells	Single-cell transcriptome map by developmental stage (7-8, 9-11, 12-14, 17-17 PCW)	[12]
	Human	10-11; 20-21 wks EGA	Memory B-2 and innate-like B-1 B cells	IgM and IgD expression	[62]
	Human	15-22 wks EGA, 17-23 wks EGA	NK cells	Various differentiation stages present (CD56, CD16, NKG2A), KIR$^+$, hyporesponsive cytotoxicity against HLA^{neg} target cells, Strong cytokine-mediated cytotoxicity response	[3, 251]
	Human	6-24 wks EGA	NK cells	Cytoplasmic CD3 delta, epsilon$^+$, Lower cytotoxicity than cord blood or adult NK cells	[118]
	Human	8-12 PCW	ILC1, ILC2, ILC3	Lymphoid precursor (IL-3RA$^+$) derived	[63]
	Mouse	E6.5-10.5	Kupffer cells	/	[34]
Gut	Human	12-27 wks EGA	T cell	Extrathymic T cell differentiation	[88]

Table 1 (continued)

	Human	12-27 wks EGA	$CD4^+$ T cell	Memory phenotype ($CD45RA^{neg}CCR7^{neg}$), Clonal expansion (compartmentalization), $TNF\alpha^+$ Intestinal tissue damage by too high concentrations of $TNF\alpha^+$ CD4 T cells Involvement in preterm necrotizing enterocolitis	[5, 67, 74, 82, 94, 95]
	Human	8-23 wks EGA	$CD8^+$ T cell	Memory phenotype, Impaired cytotoxicity, Tolerance against forein antigen, Increased GI viral susceptibility at birth	[78, 82, 95, 96]
	Human	6-9 wks EGA	γδ T cell	/	[59]
	Human	18-23 wks EGA	MAIT cell	$CD3^+CD161hiTCR\ V\alpha7.2^+\ PLZF^+$, mature phenotype, innate-like antimicrobial responses	[99]
	Human	16-23 wks EGA	iNKT cell	$CD3^+TCR\ V\alpha23^+$ CD1d $tetramer^+$ (loaded with a-GalCer analog PBS57), mature phenotype and robust IFNγ responses	[98]
	Human	16-22 wks EGA	Treg	$CD4^+FoxP3^+CD25^+$, Detected in mesenteric lymph node, Support tolerance towards alloantigens	[66, 71]
	Human	6-22 PCW	LTi	Recruit immune cells to site of developing intestinal immunity	[57, 78, 89–91]
	Human	8-23 wks EGA, 8-12 PCW	ILC1, ILC2, ILC3	/	[63, 78, 82]
	Human	8-23 wks EGA	NK cell	/	[63, 78, 87]
	Human	15-22 wks EGA	NK cell	Various differentiation stages present (CD56, CD16, NKG2A), KIR^+, Detected in mesenteric lymph node, no cytotoxic response against HLAneg target cells	[3]
	Human	8-23 wks EGA	Immature B cell, Transitional B cell	Support lymphoid follicle and Peyer's Patch development	[82, 90]
	Mouse	E12.5	Macrophage	$CD45^+\ CD11b^{lo}F4/80^{hi}Ly6C^{neg}$	[33]
	Mouse	E12-18	Monocyte	$CD11bhiF4/80loCD64^+Ly6C^{+/-}$	[33]
	Mouse	E17.5	ILC2	$Lin^{neg}IL\text{-}7Ra^+Thy1^+ST2^+$	[30]
Lung	Human	8-16 PCW	Alveolar macrophages	/	[108]
	Human	8-12 PCW	ILC2, ILC3	ILC3: Immunity against in utero infection	[63, 109]
	Human	15-22 wks EGA	NK cell	Various differentiation stages present (CD56, CD16, NKG2A) that, KIR^+, hyporesponsive cytotoxicity against HLA^{neg} target cells, strong antibody-and cytokine-mediated responses	[3]
	Human	12-22 wks EGA	$CD14^+$ monocytes/macrophages, pDC, cDC1 and cDC2	/	[110]
	Human	18-23 wks EGA	MAIT cell	$V\alpha7.2^+\ CD161^{hi}\ CD8^+$	[99]
	Human	14-23 wks GA	T cells	memory CD4 and CD8 T cells, cytotoxic granzyme B^+Tbet^+ CD8 T cells, $PD1^+Ki67^+$ Tregs, and $CD161^+Vα7.2–CD4+TCR\alpha\beta^+$	[74, 95]
	Human	16-23 wks EGA	iNKT cell	$CD3^+TCR\ V\alpha23^+$ CD1d-PBS57 $tetramer^+$	[98]
	Mouse	E13-18	Alveolar macrophages	Maintaining physiological surfactant levels, lung morphogenesis	[114, 116, 256–258]
	Mouse	E19	ILC2	M2 polarization of AM, maintenance of lung homeostasis, epithelial cell plasticity, barrier surveillance	[30, 124, 259]
Brain	Human	9-18 wks EGA	Microglia	Activated, Transcriptionally heterogenous, Undergoing maturation, Phagocytosis, CNS (immune) surveillance	[2]
	Human	16-24 wks EGA	Astrocytes	Cytokine production (IL-1β, IL-6, TNFα)	[165]

Table 1 (continued)

	Human	21-30 wks EGA	Astrocytes	Glia to astrocyte transformation	[168]
	Human	<36 weeks EGA	Periventricular area, meninges γδ T cells	Associated with injured white matter in preterm birth brain injury	[209]
	Mouse; Macaque	E12.5; E18.5-P14; E80	Microglia	Neurogenesis, Angiogenesis, Synaptic pruning, Synaptogenesis, Neuronal survival, Oligodendrogenesis, Neurogenesis	[156–161]
	Mouse	E9.5 - E16.5	Non-parenchymal (subdural meninges, perivascular space, and choroid plexus) macrophages	$CX3CR1^{+}$, Iba-1^{+}, mMΦ, pvMΦ, and cpMΦ share early embryonic precursor; mMΦ and pvMΦ: same longevity as parenchymal microglia (i.e., no input from circulation), cpMΦ: partially replenished	[142]
	Mouse	P6 - P30	Astrocytes	Synaptic pruning	[170, 171]
	Mouse	E16.5	Choroid plexus immune cells (Macrophages, Monocytes, Basophils, Neutrophils, B cells, Lymphocytes, DC, Mast cells)	Cyto- and chemokine production	[178]
	Mouse	E16.5	Choroid plexus and parenchymal DC	In microglial vicinity, response to inflammation	[172]
	Mouse	E16.5	Choroid plexus and parenchymal B-1 B cells	Innate-like characteristics, oligodendrogenesis	[174]
	Mouse	E19.5	Parenchymal, tissue-resident CD4 T cells	Microglia fetal-to-adult transition, Sense adult peripheral immune events	[173]
	Rat	E20 - P7	Mast cell	Sexual differentiation	[179]

PCW [78], coinciding with the development of gut-associated lymphatic tissue (GALT) [79, 83–85]. The basic intestinal immune landscape — proportions of major innate and adaptive immune cells — is established prior to the second trimester and is stable into infancy [82]. However, diversity within the major immune cell populations can be observed, with an enrichment of $CD11c^{+}$ APC, ILC, and $CD4^{+}$ T cells, and reduced plasma cells in the fetus compared to infant [82]. Interestingly, in the adult, maintenance of intestinal mucosal immunity occurs both through migration of circulating BM-derived cells and by differentiation from tissue-resident progenitor cells that were seeded during fetal development [86]. For instance, macrophages are replenished by circulating monocytes [41], while ILC subsets and their progenitors reside in the tissue itself [63, 81, 87]. Also T cell progenitors reside in the intestine itself, as intestinal T cell differentiation is independent of the thymus [59, 88]. In humans, GALT, including lamina propria, mesenteric lymph nodes (mLN), and Peyer's patches (PP), begins to develop 11–12 PCW, and at gestational week 19 the fetal intestines reach full structural maturity [79, 83–85, 89].

Several non-immune cells such as mesenchymal and epithelial cells are important for development of intestinal immunity [57, 78, 89–91]. It has been shown that interactions between mesenchymal lymphoid tissue organizing (mLTo), endothelial LTo (eLTo), and LTi cells — which belong to the ILC family — are critical for recruiting immune cells to the site of the developing intestinal immunity [57, 78, 89–91]. In addition, epithelial cells in the fetal intestine will produce chemerin to attract macrophages from the circulation into the fetal gut mucosa [92], while after birth IL-8 and TGFβ secreted by epithelial cells and mast cells will attract macrophages to the intestinal mucosa [93].

The general hypothesis that the fetal immune system is naive and will only mature after birth has been challenged by the observation that a relatively mature adaptive immune system is present in the fetal gut [82]. For instance, clonally expanded $CD4^{+}$ T cells with a memory phenotype (Tm) [5, 67, 74, 82, 88, 94, 95] show no overlap with regards to clonality in blood, and are present in the fetal intestine at 13–23 weeks of gestation [5, 67, 88, 94], highlighting compartmentalization of the fetal immune system. T cell receptor repertoire diversification and clonal distribution of intestinal T cells starts in utero and continues after birth [82]. These fetal $CD4^{+}$ Tm cells can be found in colocalization with APC in the fetal intestine and are able to produce pro-inflammatory cytokines upon stimulation [5, 67, 74]. In particular, intestinal $TNF\alpha^{+}CD4^{+}$ Tm cells are able to support intestinal stem cell growth and epithelial development [5]. Importantly, a too high concentration of TNFα-producing $CD4^{+}$ Tm cells mediates intestinal tissue damage, which is in line with the observation of increased frequencies of $TNF\alpha^{+}CD4^{+}$ Tm cells in the intestine of necrotizing enterocolitis (NEC)–affected preterm infants [5]. Overall, these data showed that TNFα-producing $CD4^{+}$ Tm cells are involved in tissue generation in the fetus, while preterm birth-induced activation can induce intestinal inflammation and damage in premature infants. $CD8^{+}$ T cells with a

memory phenotype [95, 96] can also be detected in the fetal intestine, with proportions increasing after birth. However, fetal and infant intestinal $CD8^+$ T cells showed reduced functional capacity compared to adult intestinal $CD8^+$ T cells [96], which is in contrast with results observed in mice [97]. This impaired cytotoxic $CD8^+$ T cell immunity suggests a tolerance support towards foreign antigens after birth, while at the same time it might contribute to the increased gastrointestinal viral susceptibility observed during early life. The expression of CD69 and CD103 on both CD4 and CD8 Tm cells suggests that these intestinal T cells are of the tissue-resident memory (TRM) phenotype [82]. Also γδ T cells [59], invariant NK T cells [98], and mucosal-associated invariant T (MAIT) cells with a mature phenotype and innate-like antimicrobial responses are present in human fetal intestine [99]. The presence of these TRM T cells suggests in utero exposure to foreign antigens, potentially mediated through swallowing of amniotic fluid by the fetus as early as 11 weeks of gestation (EGA) [100, 101]. Mishra et al. detected a low but consistent microbial presence in human fetal organs, including the gut, and with live fetal-isolated microbial strains capable of inducing memory T cell activation and proliferation in vitro [95]. Even though this study suggests that fetal microbiota are capable of educating fetal intestinal T cells, the presence of microbiota in utero is still very controversial [102–104], predominantly due to the lack of appropriate controls and methodological limitations. Nevertheless, it remains imperative to elucidate which agents are responsible for in utero maturation of intestinal T cells.

B cells, on the other hand, have a relatively immature phenotype in the fetal gut, and maturation will occur after birth [82, 90]. In the fetal intestine, an enrichment for transitional B cells can be observed, a subset that has recently migrated from the BM and/or liver, while an enrichment of IgM^+ plasma cells can be observed in the intestine of infants [82]. The in utero function of B cells is likely to support the development of lymphoid follicles and PP, as observed in mice [105]. After birth, the expansion of plasma cells will likely shape the selection of a healthy and beneficial intestinal microbiome.

Mucosal immune populations in the fetal lung

Lung-resident immune cells are key to mount immunity towards airborne pathogens to which the airways and interstitium are permanently exposed [106]. Mature DC and alveolar macrophages (AM) are among the first cells to encounter invading microbes [106]. Although the fetus is not exposed to airborne pathogens and the lung continues to mature postnatally into the first years of life [107], immune cells and precursors already seed the fetal lung, establishing immunity to upcoming lifelong challenges. A distinct immune cell repertoire can be detected in the human fetal lung as early as 8 weeks post conception [3, 63, 74, 95, 98, 99, 108–110], coinciding with the start of the pseudoglandular stage of lung development (i.e., differentiation of epithelial cells, formation of conduction airway and terminal bronchioles, formation of pulmonary blood vessels) [107].

AM are the most abundant immune cell type in the adult lung, maintaining lung homeostasis through clearance of debris, invading pathogens, and pulmonary surfactant (reviewed in [111–113]). Macrophages scattered in the fetal lung have been detected around 13–17 PCW in humans [108, 110], and at E13.5 in mice [114]. Currently, little is known about the origin and phenotype of human fetal AM. In mice, however, lung development occurs in parallel with seeding of fetal liver-monocyte derived AM, a self-replenishing population whose maturation is dependent on GM-CSF [39, 115] and maintained until adulthood [36]. The role of fetal liver–derived monocytes in the establishment of AM in the murine fetus was confirmed in a fate-mapping approach [33, 34, 116]. Different reservoirs of fetal monocytes, i.e., BM-derived monocytes, YS-derived macrophages, and fetal-liver monocytes, were tested for their ability to serve as AM precursors. Fetal liver–derived monocytes showed superior GM-CSF responsiveness based on their proliferation-associated gene expression yielding in higher proliferative capacity in vitro [116], showed upregulation of bona fide macrophage differentiation markers such as CD64 and MerTK upon seeding of future resident tissue in vivo, and outcompeted YS macrophages and BM monocytes in colonizing an empty AM niche [33]. In addition, using humanized mouse models, it has been confirmed that human fetal-liver AM precursors are able to seed the lung, giving rise to AM [117].

Next to macrophages, also DC and NK cells will be one of the first immune cells to encounter invading pathogens in the newborn and adult lung. Although lung-resident DC are well characterized in the adult lung, information on DC in the developing fetal lung is limited. However, gene array and flow cytometry analyses have revealed insights into the presence of human fetal lung DC at gestational week 12–22 [110], including plasmacytoid DC (pDC), conventional (c) DC1, and cDC2, showing tissue-specific phenotypes. Data from human fetal lungs obtained at gestational week 15–22 showed that NK cells were present in the mesenchyme of the developing lung tissue [3]. NK cell differentiation and killer immunoglobulin-like receptors (KIR) acquisition starts early, likely before 15 weeks of gestation in the developing fetal lung, with fetal lung NK cells being the most differentiated in comparison to fetal liver, BM, lymph node, and spleen NK cells [3]. This suggests that NK cell differentiation occurs within the lung itself. While fetal lung NK cells are less differentiated compared to circulating adult NK cells, their high expression of multiple KIR and intracellular expression of perforin and granzyme B suggests that

they may be functional [3]. Indeed, in agreement with fetal liver NK cells [118], fetal lung NK cells show degranulation and cytotoxic activity against HLA^{neg} target cells, albeit to a lower extent than their adult peripheral counterparts [3]. In contrast, fetal lung NK cells were highly responsive to cytokine stimulation and antibody-dependent killing of target cells [3]. Interestingly, upon IL-12 stimulation, the percentage of fetal lung NK cells that express IFNγ are higher than adult peripheral NK cells [3]. In conclusion, although fetal lung NK cells are hyporesponsive to cells lacking HLA class I, they possess a strong cytokine- and antibody-mediated immune response capacity, suggesting a balance between fetal-maternal tolerance while still maintaining the ability to respond to infection in utero.

Characterizing ILC populations across human fetal tissues revealed a transcriptomic map of diverse subpopulations with site-specific expression profiles in 8–12 PCW fetuses [63]. ILC1, ILC2, and ILC3 subsets can be found in the fetal lung, with ILC3 being the predominant ILC subset [63]. During murine fetal growth, ILC are involved in the development of secondary lymphoid organs (spleen, lymph nodes, and PP) [119–121], while in non-lymphoid tissues, such as the intestine and lung, mature ILC are considered to establish immunity during colonization and after birth (reviewed in [122, 123]). Increasing frequencies of a CD103-expressing subset of ILC3, indicating intraepithelial origin, have been identified in the human fetal lung with increasing gestational age (8–20 weeks EGA) [109]. At 20 weeks EGA, these $CD103^+$ ILC3 will make up 15% of ILC3 in the fetal lung [109]. These cells can also be detected in the amniotic fluid at 8–20 weeks EGA, likely through migration from the fetal lung or fetal intestine, where they are believed to mount immunity against in utero infections [109]. Results from lineage tracing and parabiosis experiments in mice have shown that mature ILC2, identified by markers such as Lin^{neg} $IL7Ra^+$ $Thy1^+$ $ST2^+$, are present in the lung at E17.5 [30]. An extensive proliferation of ILC2 occurs in the murine lung at the time of birth, which is linked to the first breath [124]. This first breath leads to a mechanical stimulus that induces IL-33 secretion by type 2 alveolar epithelial cells, which is able to activate a variety of cells in the lung such as regulatory T cells (Treg), DC, mast cells, NK cells, AM, and ILC2 [124]. Due to the activation by IL-33, ILC2 of newborn mice start to secrete IL-13, which in turn drives AM polarization towards an M2 phenotype [124]. Two months after birth, only 5–10% of tissue-resident ILC2 are embryonically derived, suggesting a gradual exchange of the tissue-resident pool by circulating ILC [30].

Recent reports show that adaptive immune cells present in the second trimester human fetal lung (13–23 weeks EGA) include memory CD4 and CD8 T cells, cytotoxic granzyme B^+ $Tbet^+$ CD8 T cells, $PD1^+$ $Ki67^+$ Tregs, and $CD161^+$ Vα7.2–$CD4^+$ TCR-αβ + T cells [74, 95]. Furthermore, the second trimester human lung harbors a population of innate-like $V\alpha7.2^+$ $CD161^{hi}$ $CD8^+$ MAIT cells, with IFNγ and IL-22-producing capacity [99], and invariant NKT cells (CD1d-PBS57 $tetramer^+$ $CD3^+$ $TCRV\alpha24^+$) [98]. Beyond their detection and phenotype-based functional implications, the role of T cells for prenatal lung development and immunity remains to be investigated.

Although not immune cells per se, clinical studies in humans suggest that mesenchymal stromal cells (MSC) possess immune-modulatory properties making them a potential target for cell therapy in idiopathic pulmonary fibrosis (IPF) [125] and acute respiratory distress syndrome (ARDS) [126]. Fetal lung-resident MSCs show lung-specific properties that are distinct from BM-derived and adult lung-resident MSC, and play a role during lung development [127]. They produce higher amounts of macrophage migration inhibitory factor (MIF) when compared to adult lung-resident MSC, a chemokine which hinders random macrophage migration and supports lung development by promoting growth of pulmonary arterial smooth muscle cells [128]. Moreover, MIF is expressed tenfold higher in newborns compared to children and adults, underpinning its involvement in lung development [128, 129].

Parenchymal and border-associated immune populations in the fetal central nervous system

Neurodevelopment of the human central nervous system (CNS) follows spatially and temporally highly orchestrated patterns (reviewed in [130]). Knowledge about the development of tissue-resident immunity in the brain during the fetal period remains incomplete. Even in the adult CNS, steady-state immune surveillance of brain surfaces was only recently characterized more comprehensively [131–134]. In the parenchyma, homeostatic tissue residency is dominated by microglia, the primary brain macrophage population, while central and systemic inflammation generates a small population of resident T cells [135–137]. Immune and non-immune roles of microglia in brain development have been extensively described in physiological and pathological conditions ([138], reviewed in [139, 140]).

Microglia colonize the brain early in development. Their origin has been revealed in studies in rodents, showing that microglia are derived from primitive and YS EMP–derived precursors who establish themselves in the neuroectoderm by E9.5 [37, 141–143]. Rodent infiltrated microglia locate around subcortical regions (hippocampus) and corpus callosum on E13.5, then expand and migrate into distant brain regions for final residency, proliferating beyond birth following a precisely timed transcriptional program [144]. From rodent studies, it is known that their numbers contract in postnatal week 3 and subsequently stabilize to adult levels after week 4 [145, 146]. Microglial colonization coincides

with rodent brain maturation, and so does their morphology. Developmental, immature microglia are amoeboid (large and round) in shape, resembling activated adult microglia, and are dramatically different from the thin, ramified morphology of adult microglia at steady state, a morphology they assume in parallel with increasing maturation of individual brain regions after birth [147]. In humans, microglia are spotted in close mesenchymal vicinity to neural tissue by 4.5 weeks gestation, and in the neural tissue 1 week later, responding to chemokine signals [148–152]. Recently, human fetal microglia have been transcriptionally characterized on the single-cell level ranging from 9 to 18 weeks EGA, displaying tremendous heterogeneity, and undergoing dynamic processes of maturation to acquire a homeostatic profile, with overlapping and distinct features compared to adult microglia [2].

Microglia are macrophages and, as such, actively engaged in phagocytosis of cellular debris and opsonized synapses, apoptotic clearance, and induction of apoptosis [153–155]. In addition, microglia support a variety of non-immune developmental processes of the macaque and rodent fetal brain including neurogenesis [156] and angiogenesis [157]. In mouse late gestation and early neonatal periods, they contribute to complement-mediated synaptic pruning [158], synaptogenesis [159], survival of neurons [160], oligodendrogenesis, and myelination [161]. This variety of microglia functions depends on time and localization. As one type among tissue-resident macrophages derived from the first primitive and YS EMP–derived premacrophages, microglia are distinct from their counterparts residing in peritoneum, liver, or lung, due to the specific microenvironment in the CNS that primes their functional capacity [34, 43, 162]. Importantly, coinciding with maturation of the blood–brain-barrier (BBB) on E13 in mice [163], the brain niche is considered inaccessible for further precursor seeding after E14.5, thus rendering the microglial population derived from fetal origin, and requiring in situ repopulation capacity for the remainder of the individual's lifespan [42]. In human embryos, the BBB is considered mature around gestational week 8 [163]. Investigating developmental spatiotemporal patterning of human microglia and their progenitors is crucial in understanding both developmental and mature microglia diversity.

The developing brain parenchymal niche is furthermore colonized by astrocytes, a neural stem cell-derived, yet highly immunocompetent cell type. Their interaction with microglia and other infiltrating immune cell subsets makes them an important component of brain immunity, as they respond to and secrete inflammatory mediators (reviewed in [164]). For example, during the human second trimester, astrocytes produce TNFα and IL-6 upon IL-1β stimulation, a cytokine produced by LPS-responsive fetal microglia [165]. This promotes synaptic transmission and affects synaptic scaling [166]. Human astrocytes start transforming from glial cells after 12 weeks EGA [167–169]. Similar to microglia, astrocytes have phagocytic activity and engage in synaptic pruning [170], while simultaneously promoting microglial pruning during postnatal development [171].

Conventional immune cell subsets are found at very low frequencies in the unchallenged developing brain parenchyma [172–174]. In the adult brain in steady state, the majority of tissue-resident immune cells are non-parenchymal immune subsets residing in the organ border such as the dura mater, subdural meninges, and choroid plexus, and on the abluminal side of the BBB, to surveille and influence mature CNS function [131–133, 138, 175]. These brain-border-associated immune subsets in the choroid plexus include both adaptive and innate immune cells and show a phenotype distinct from microglia [138] and circulating immune cells, indicating their selective enrichment [134]. The choroid plexus is an epithelial bilayer that forms the blood-cerebrospinal fluid (CSF) barrier (BCSFB), that, in addition to immune cells, hosts mesenchymal, and neuronal cells. Its role for establishment of the fetal brain immune landscape across time and space remains scarcely described, while its involvement in mouse brain immune surveillance and embryonic neuroinflammation has been documented [176, 177].

A number of recent studies are starting to build a narrative of parenchymal and brain border-associated immune cell presence establishing in utero. The fetal immune composition in the choroid plexus on E16.5 in mice resembles both the adult and aging brain, with regard to gene expression patterns in B and T cells, macrophages, monocytes, basophils, DC, neutrophils, and mast cells, while unique embryonic features were identified as well [178]. For example, border-associated macrophages in the embryonic choroid plexus express CD206 and are MHC class II^{low}, compared to their adult $CD206^{low}$ and MHC class II^{high} (i.e., CD74) counterparts. They are also enriched for the expression of a set of markers (e.g., Lyve1) assigned to adult subdural-specific macrophages [162, 178]. Perivascular, meningeal, and choroid plexus non-parenchymal macrophages, previously assumed to be short-lived and continuously replaced by adult BM-derived circulating cells, were determined to share their embryonic origin with YS-derived parenchymal microglia (wave of transient definitive hematopoiesis) [142], indicating that these precursors are present during fetal brain development. Bulloch et al. describe another myeloid subset, $CD11c^{+}$ DC, in the choroid plexus, but also in the murine brain parenchyma on E16.5, co-localizing with microglia. In adult mice, these same cells responded to injury with microglia-like morphology [172]. At the same fetal age, Tanabe and Yamashita isolated T and B cells from the mouse brain, finding abundant innate-like B-1

cells expressing high levels of IgM [174]. These cells localize in the choroid plexus and meninges and, rarely, in the cortical parenchyma on postnatal day 1. They followed CXCR5-dependent recruitment in response to CXCL13 secreted from the choroid plexus, and promoted the proliferation of oligodendrocyte precursor cells (OPC) in vitro. This B-1 population was highly transient, reaching its peak frequency 1 day after birth, and disappearing by postnatal day 10. A small but functionally significant $CD4^+$ T cell population with a brain-resident phenotype is found in the adult human and mouse brain at steady state, with a subset localizing in non-vascular, non-meningeal, parenchymal spaces [173]. Of note, these brain-resident $CD4^+$ T cells, although rare in general, were particularly frequent in the fetal mouse brain right before delivery, when they were supporting the early-life transition of microglia from a fetal to mature transcriptional program, ultimately enabling proper synaptic pruning [173]. Anecdotal evidence for the involvement of non-microglial immune cells in organogenesis describes the estradiol-dependent involvement of mast cells in the development of sexual differentiation in the preoptic area in the rat [179].

Evidence for immune memory in the fetus

To complete the porous picture on local fetal immunity in humans with the generally better described system-level perspective, we will highlight evidence for fetal priming of immune function in humans and rodents, based on the mechanisms of innate trained immunity and adaptive antigen-specific memory.

The developing, circulating fetal immune system senses the maternal circulating environment at the fetal-maternal interface. The fetus is exposed to maternal antigen [66], maternal-derived pathogen-associated molecular patterns (PAMPs), DNA and other products from the mother's commensal microbiota [180–183], maternal antibodies (including passive immunity) [184–186], cytokines [180], and to vertical transfer of pathogens or their products, i.e., transmission of infection [187, 188], as well as vertical transfer of intact maternal cells (220). These maternal microchimeric cells seed across fetal organs to become part of the tissue-resident population with as yet incompletely described consequences for the fetus and neonate [189, 190]. These exposures may be categorized as inherent maternal support and education, and therefore be considered beneficial for fetal immune development. However, the fetus sensing maternal (immune) perturbations, as described in the concept of the developmental origins of health and disease, or "Barker hypothesis," may have lifelong detrimental consequences for offspring's immune health (reviewed in [6, 7]), as highlighted in Box 1 for prenatal immune activation of tissue-resident immune populations.

The fetal innate immune system might be trained by these transplacental exposures, leading to persistent transcriptional and epigenetic changes in innate cells and their capacity to respond to secondary challenges unrelated to the primary trigger/pathogen, a concept termed "trained immunity" in adult immune responses [222]. For instance, in utero exposure to hepatitis B virus (HBV) leads to a more mature monocyte phenotype and function in cord blood of uninfected neonates, most likely mediated by changes in cytokine environment, and affects T cell polarization, indicative of the concomitant effect on the adaptive arm [223]. Similar observations of altered immune capacity were made for placental malaria [224] and human immunodeficiency virus (HIV) [225]. In mice, the BM-lung immune axis was trained following maternal microbial exposure in utero, leading to protection from inflammatory airway disease via enhanced survival and proliferation of the lung DC population which was programmed in its BM-resident precursor [226]. Whether pathogen-nonspecific immunity in uninfected fetuses and neonates resulting from maternal inflammation/infection exists and is inherently beneficial for offspring's health warrants further research [227, 228].

Adaptive fetal immune memory in the periphery is observed after gestational week 14, following thymic emigration and seeding of peripheral organs which equips the human fetus with the complete repertoire of T cell subsets [69–71]. The fetal environment prioritizes unique phenotypes of T cells, including a strong bias towards immune tolerance and the preferential differentiation of naive T cells into Tregs [65]. Thymic [229] and circulating [72] γδ T cells with fetus-specific phenotypes serve fast-acting functions, in addition to innate-like features of $CD4^+$ and $CD8^+$ T cells [230, 231], which, in mouse $CD8^+$ T cells, are retained until adulthood [97]. Despite a relative sparsity in foreign antigen load, the fetal immune system recognizes transferred non-inherited maternal antigens by inducing alloantigen-specific Tregs [66, 232] and shows the potential to establish resident CD4 and CD8 memory (with unknown specificity) in the gut and skin in utero [5, 74, 96, 233]. Fetal $CD8^+$ T cell immunity is functional in its antigen recognition, even if less potent (i.e., decreased cytotoxicity) compared to adult cells [231]. Pathogen-specific $CD8^+$ T cell responses are detectable in cord blood, including to cytomegalovirus (CMV) [234], HIV [235], malaria [236], and HBV [237]. Even in the absence of the pathogen itself, the fetal T cell compartment senses foreign antigens, as maternal influenza vaccination is associated with potent antigen-specific T cells in cord blood [238].

While the mechanisms of priming beneficial vs. detrimental trained and antigen-specific memory remain to be

Box 1 Prenatal immune activation of tissue-resident populations and developmental disorders later in life

Evidence for postnatal consequences resulting from prenatal disturbances of resident immune populations are emerging for gut and lung, and have been extensively described for the brain. In the gut, transient prenatal maternal infection at E10.5 caused an increase in intestinal Th17 cells in the adult offspring that, while enhancing protection against gut infection, also increased susceptibility to colitis [180]. In addition, fetal exposure to chorioamnionitis has been associated with dysregulation of fetal gut immunity, intestinal inflammation, and the development of necrotizing enterocolitis weeks after birth [191, 192]. While the exposure of the lung to infections during the neonatal and adult phase is known to lead to chronic lung diseases, less is known about the consequences of inflammatory exposure on fetal lung immune development. In utero, the fetal lung is constantly exposed to the amniotic fluid and pregnancy complications like chorioamnionitis can lead to an inflammatory milieu within the amniotic fluid, resulting in increased leukocyte infiltration, surfactant production, decreased alveolarization (reviewed in [193, 194]), maturation of monocytes to alveolar macrophages, and both the induction as well as the paralysis of inflammatory responses in the fetal lung [195]. In the brain, an association exists between immune activation during embryogenesis, and neurodevelopmental disorders later in life, such as autism spectrum disorder, schizophrenia, and obsessive compulsive disorder [196–198]. Maternal stress/anxiety, nutrition, or infection (e.g., maternal immune activation (MIA)) can program the developing neural structures with long-term sequelae for behavior, cognition, and brain immunity [199–202]. For instance, early-life viral infection is considered to be associated with the adult onset of schizophrenia [197, 203, 204]. Aberrant microglia priming during development as a mechanistic link between immune activation and developmental disorders has been reviewed in [139, 196].

Briefly, mediators of prenatal immune activation and impaired neurodevelopment could be the vertical transmission of an infection, maternal antibodies that react with fetal neural tissue [205], microbiome alterations [206], or the transfer of an inflammatory milieu of maternal cytokines, PAMPs, or viral particles that in turn activate the fetal immune system itself [6, 207]. While glial-derived cytokines are critically contributing to normal brain development, elevated levels of systemic and central IL-1β, IL-6, and TNFα have been shown in experimental models to affect neural development and impair learning and memory, as well as behavior in rodents [208–211], via altered microglial and astrocyte functions, including microglial activation [139, 165, 212]. Increased permeability of the BBB and BCSFB in response to inflammation allows for infiltration of circulating and resident immune cells, and other immune mediators such as cytokines and antibodies into the brain parenchyma [177, 213–215].

In the adult mouse brain, microglia and border-associated macrophage populations show a highly reactive state in response to a neuroinflammatory microenvironment [138], which is supported by parenchymal infiltration of a non-resident BM-derived monocyte population to the site of brain injury [216]. In addition, CNS tissue-resident T cells establish memory after local and systemic infection [135, 137], and can sense microbiome alterations and antibiotic treatment in the periphery [173].

In the perinatal human brain, neuroinflammation and brain injury are common in infants born prematurely (reviewed in [217, 218]). Astrocytes and microglia secrete cyto- and chemokines causing leukocyte infiltration [139]. In humans and mice, accumulated γδ T cells have been found to fuel fetal brain inflammation via IFNγ and non-canonical (i.e., not IL-17) signals [219–221]. Peripheral microbial composition (i.e., expanded proportion of *Klebsiella* in the gut) correlates with inflammation and is predictive of the severity of brain injury in extremely preterm infants [220], emphasizing a gut-immune-brain axis with likely prenatal origin that links neuro- with systemic development.

investigated, targeted maternal and neonatal immunization strategies to support the development of fetal adaptive and innate immunity could be of immediate (postnatal) and lifelong benefit (reviewed in [228]).

Conclusion: opportunities for pathophysiological discoveries and therapeutic windows

Our knowledge on the capacity of the human fetus to mount tissue-resident immune responses remains incomplete and restricted to samples from early pregnancy. More evidence of innate and adaptive perinatal immunity exists on a systemic level (often cord blood studies), which can only be a snapshot of a spatially complex system and requires complementing data from animal models to complete the picture of organ-specific prenatal tissue residency. Differences between tissue and systemic immunity in the neonate do not allow us to understand (patho)biology within tissues using circulating cells in cord blood; yet, its accessibility makes it a useful source for biomarker discovery.

The dynamic perinatal period is susceptible to external influence. Deviations in programming of fetal immune precursors can have lifelong consequences for the health of the entire organism. This presents opportunities for interventions to support healthy developmental trajectories. Recent technical advances allow the in-depth, high-throughput assessment of a much smaller sample volume to generate high-dimensional maps of spatiotemporal dynamics of organ development in humans. Furthermore, to spur our understanding of human development, human gestational samples, complemented by stem cell-derived organoid models [239–243], instead of rodent, sheep, or non-human primate models with limited overlap in their gestational physiology, are needed to reveal functional overlap and separation of systemic vs. compartmentalized tissue-resident immunity, and identify windows of susceptibility and therapeutic opportunity.

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Declarations

Conflict of interest The authors declare no competing interests.

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