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REVIEW

Maternal-Fetal Microchimerism: Impacts on Offspring's Immune Development and Transgenerational Immune Memory Transfer

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Summary

Maternal-fetal microchimerism is a fascinating phenomenon in which maternal cells migrate to the tissues of the offspring during both pregnancy and breastfeeding. These cells primarily consist of leukocytes and stem cells. Remarkably, these maternal cells possess functional potential in the offspring and play a significant role in shaping their immune system development. T lymphocytes, a cell population mainly found in various tissues of the offspring, have been identified as the major cell type derived from maternal microchimerism. These T lymphocytes not only exert effector functions but also influence the development of the offspring's T lymphocytes in the thymus and the maturation of B lymphocytes in the lymph nodes. Furthermore, the migration of maternal leukocytes also facilitates the transfer of immune memory across generations. Maternal microchimerism has also been observed to address immunodeficiencies in the offspring. This review article focuses on investigating the impact of maternal cells transported within maternal microchimerism on the immune system development of the offspring, as well as elucidating the effector functions of maternal cells that migrate through the placenta and breast milk to reach the offspring.

Key words

Microchimerism • Prenatal • Postnatal • Phenotype modulation • Development of the immune system • Transgenerational immune memory transfer

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Introduction

Microchimerism is a phenomenon characterized by the presence of a small population of cells with different genetic information within an individual. It is a physiological phenomenon that occurs typically in humans [1,2] and also in biomedical models such as mice [3,4] and rats [5] and is highly likely to occur in placental mammals in general. During pregnancy, there is a transfer of fetal cells into the mother's tissues (feto-maternal microchimerism), maternal cells into the fetal tissues (materno-fetal microchimerism), and even older siblings or other embryos present in the uterus into the fetal tissues (feto-fetal microchimerism) (Fig. 1) [6]. Microchimerism can also occur through medical procedures such as blood transfusion or organ transplantation [7]. Although microchimerism is now established as a proven phenomenon by solid experimental evidence, its functional effects have not been thoroughly explored in detail.

Materno-fetal microchimerism (MFMc), refers to the presence of cells carrying the mother's genetic information in the offspring's body acquired during prenatal development (*in utero*). Materno-newborn microchimerism (MNMc) reflects the acquisition of the mother's cells by the offspring after birth through breastfeeding. On one hand, maternal microchimerism (MMc, abbreviation used when MFMc and MNMc are taken together) is associated with benefits such as the contribution to the development of the offspring's immune system, compensation for genetic disorders, and representation of the function of the developing adaptive immune system in early life [8]. However, a higher

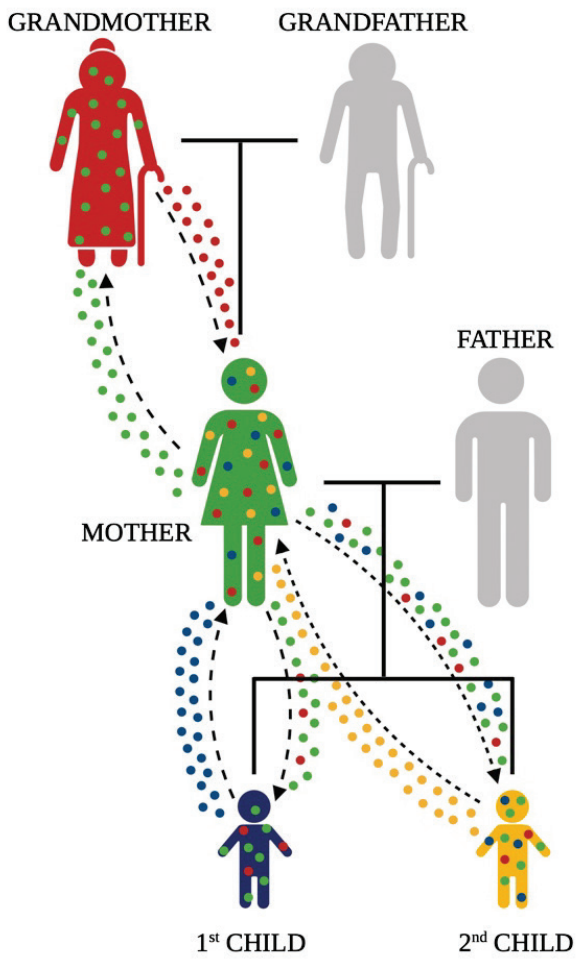

Fig. 1. Scheme of cell migrations in between the generations. During pregnancy, maternal cells migrate to the offspring's tissues and vice versa. These cells can persist in the individual for several decades. This leads to the migration of not only the mother's cells but also the grandmother's cells to the offspring in subsequent generations. Cell migration also occurs from older siblings to younger ones through the mother, who acquired these cells from previous pregnancies. Adapted from [6]. Created with BioRender.com.

frequency of MMc has also been linked to certain autoimmune diseases such as type 1 diabetes, myositis, neonatal lupus, systemic scleroderma, and biliary atresia [1]. The contribution of MMc to these benefits or disadvantages remains unclear.

Under physiological conditions, maternal cells can be found in various offspring tissues, both lymphatic and non-lymphatic. Typical organs of occurrence include the thymus, spleen, bone marrow, liver [4], heart, lungs, lymph nodes [9], and intestines (mainly in the intestinal mucosa and Peyer's patches) [10]. In some individuals, maternal cells have been detected also in the kidneys, pancreas, urinary bladder, skin [11], stomach [12], and even the brain [9].

The majority of microchimeric cells originate from the hematopoietic stem cell lineage, although cases of maternal mesenchymal stem cells in the offspring's tissues have also been detected [13]. In the fetal tissues, T lymphocytes, B lymphocytes, NK cells, monocytes/macrophages [2], and maternal granulocytes (neutrophils, eosinophils, and basophils) [14] have been identified. As granulocytes are typically short-lived cells (4-9 h) [15] and are rapidly cleared from circulation, their presence in the blood of young women suggests the possible presence of active hematopoietic stem and progenitor cells in the organism [14], indicating chimeric hematopoiesis. These maternal hematopoietic stem cells have been found in the offspring's tissues, specifically the c-kit$^+$ lineage of maternal stem cells in the bone marrow of most studied adult mice. Only 50 % of these mice contained MMc also in the peripheral blood leukocyte populations which differentiated from maternal stem cells in the bone marrow. Maternal leukocytes were not found in any individual that did not contain maternal stem cells in the bone marrow simultaneously, suggesting that these maternal hematopoietic stem cells may be essential for preserving MMc in effector leukocytes into adulthood [13].

As was previously mentioned, most maternal cells identified in the offspring's body are immune cells, suggesting that MMc may primarily be associated with the establishment of the immune system (and overall immune defence) in the offspring. It is believed that maternal cells not only initiate the development of the immune system *in utero* but also compensate for immune deficiencies in newborns, where the adaptive immune system is still developing [8]. Additionally, there is evidence that maternal cells can balance the immune deficiencies of the offspring into adulthood. For example, in mice deficient in interleukin 2 (IL-2), maternal cells producing IL-2 were found [16]. In mice deficient in B lymphocytes, maternal plasma cells secreting immunoglobulin G (IgG) were detected [17]. Quantification of MMc for specific immunologically relevant cell populations indicates that maternal cells are present in quantities that can have real impacts on immune effects [2].

The number of maternal microchimeric cells varies in individuals based on various factors, including significant differences among different tissues. For example, the numbers of maternal cells detected by flow cytometry ranged from 0 to 50 cells per 10^6 total fetal cells in the thymus, spleen, and liver of embryos. In the bone marrow, this number was even higher, reaching nearly 150 maternal cells per 10^6 fetal cells. There is also

considerable variability within individuals. In adults, MMc is usually detected in lower numbers than in fetal tissues [4]. In another study of MFMc the number of maternal cells was lower, averaging 3.7 maternal cells per 10^7 embryonic cells [18]. The variability of MMc is significant and depends also on the detection methods used. However, we can generally say that the number of microchimeric cells is very small, ranging from none to thousands of maternal cells per 10^7 fetal/offspring cells, depending on the particular organ. Nevertheless, even in such small quantities, microchimeric cells have observable effects, including on the individual's immune system. It could also enable the intergenerational transfer of the immunological memory.

The effect of MFMc depends on various factors, including the origin and type of microchimeric cells, the time elapsed since the establishment of microchimerism, and the individual's age. Materno-fetal histocompatibility, specifically polymorphisms of genes encoding major histocompatibility complexes (MHC I and MHC II), has the greatest influence on the impact of MMc in the host's body [1]. When maternal and fetal HLA-DQB1 and HLA-DRB1 are compatible from the fetus's perspective, especially when the maternal HLA-DBQ1*0301 allele is present, a higher frequency and representation of maternal cells in the offspring's tissues are detected. However, this type of tissue compatibility is not necessary for the occurrence of MMc. Generally, the absence of recognition of maternal MHC II HLA (human leukocyte antigen) alloantigen favors a higher incidence of maternal microchimerism in the individual [19]. The knowledge that we have about the establishment of MMc in outbred individuals is based on the studies of microchimerism in humans where only the presence, frequency, and phenotype of cells can be investigated. Studies concerning microchimerism in mice and other biomedical models are usually done in inbred individuals which can alter the results through higher probability, frequency, and longer preservation of microchimerism in individuals.

As mentioned before, the quantity of maternal cells in the offspring's tissues appears to be influenced primarily by maternal-fetal histocompatibility [20] and the suppressive response to Non-Inherited Maternal Antigens (NIMA) through regulatory T lymphocytes [9]. Exposure of the fetus to maternal cells during pregnancy leads to the development and accumulation of NIMA-specific regulatory T lymphocytes, inducing immunological tolerance to NIMA [21]. Additionally, it has been found that to maintain maternal microchimerism into adulthood, subsequent oral exposure to NIMA through breastfeeding by a NIMA-positive mother is necessary. This oral exposure plays a role in maintaining a greater quantity of NIMA-specific regulatory T lymphocytes compared to NIMA-specific effector T lymphocytes [9]. Maternal alloantigens cause the proliferation of regulatory T lymphocytes in fetal lymph nodes through a mechanism dependent on transforming growth factor β (TGF-β) [22]. The immediate postnatal period appears critical for establishing an optimal quantity of regulatory T lymphocytes relative to effector T lymphocytes. If a newborn mouse is nursed by a surrogate mother and is not exposed to NIMA antigens during breastfeeding, tolerance to cells expressing NIMA is lost, potentially leading to a complete microchimeric loss. Oral exposure to maternal MHC antigens in maternal milk can induce the generation of additional NIMA-specific regulatory T lymphocytes, which may be important for suppressing the proliferation of NIMA-specific effector T lymphocytes and preserving the microchimeric status. There is a correlation between MMc and NIMA-specific regulatory T lymphocytes, which are capable of suppressing delayed-type hypersensitivity immune reactions and lymphoproliferative responses of effector NIMA-specific T lymphocytes in adult mice [9]. Some regulatory T lymphocytes may differentiate into long-lived memory T lymphocytes, allowing long-term suppression of the response to NIMA antigens [22].

In this review article, we focused on materno-fetal/newborn microchimerism in relation to the offspring's immune system only. More emphasis is placed on the postnatal phase, particularly the influence of breast milk on the establishment and modulation of the offspring's immune system, since breastfeeding reduces the risk of gastrointestinal and respiratory infections and overall neonatal mortality, highlighting its significance [23]. Additionally, breastfeeding appears to be an important aspect in maintaining tolerance to maternal antigens and preserving maternal microchimerism into adulthood [9]. Another objective of this review article is to systematically characterize the populations of maternal cells transferred to the offspring during pregnancy and breastfeeding and assess their impact on the development of the offspring's immune system.

Current methods and approaches to detect microchimerism

Methods for investigating maternal-fetal

microchimerism naturally vary depending on the studied organism, with clear distinctions, particularly in the case of humans. Essentially, similar methodologies are employed for examining other types of microchimerism.

In humans, ethical constraints limit the study of microchimerism primarily to studies on blood, where the frequency of maternal microchimerism can be easily determined. To a lesser extent, microchimerism can also be examined from biopsy material. Research typically utilizes knowledge of HLA haplotypes [24] (determined serologically or through sequencing) for unequivocal identification of maternal microchimerism. Maternal cells can then be identified using polymerase chain reaction (PCR) [24,25] or fluorescent methods [16,25]. One such method is fluorescence in-situ hybridization (FISH), which can be employed to detect, for example, sex chromosomes, assuming the detection of maternal cells with XX sex chromosomes in the blood of a male offspring with XY sex chromosomes [26].

While the detection of microchimerism is considerably limited in humans, animal models, particularly mice, offer more possibilities. Moreover, due to the absence of restrictions on tissue size, it is possible to detect much smaller quantities of maternal cells within a larger population of offspring cells [8]. Detection can be based on differences in H-2 genes, including MHC class I glycoproteins. Alternatively, transgenic mouse models suitable for detecting even minor representations of microchimeric cell populations can be utilized. Embryos at the blastocyst stage can be transferred to surrogate mothers whose cells express a different H-2 haplotype [25] or a transgene, such as *LacZ* [27], luciferase (*Luc*) [28], or a variant of green fluorescent protein (GFP) [18]. Another approach involves mating mice differing in H-2 genes, the common leukocyte marker CD45 (CD45.1 and CD45.2) [4], or Thy1 antigen (Thy1.1 and Thy1.2) [25,29], or in the expression of a transgenic marker. This allows distinguishing between maternal and offspring cells without disrupting the physiological course of pregnancy. A similar approach can be used to determine maternal cells transferred during breastfeeding when newborn pups can be exchanged between mothers differing in such markers [10,29]. For detecting maternal cells, PCR can be employed, enabling the detection of maternal cells in entire individual organs in mice. Fluorescence microscopic methods and immunohistochemistry are also used for detection, providing information not only on the occurrence of maternal cells in offspring tissues but also on their morphology and localization [8]. However, these methods do not provide entirely quantitative information on the number of maternal cells in a given tissue, especially when based on sectional preparations typically sampling only a small part of the tissue. An optimal methodological combination could involve the presence of a fluorescent transgenic protein (e.g., GFP) in a heterozygous arrangement in the mother and crossing with a wild-type father, where half of the offspring will be wild-type. Subsequently, whole tissue clearing and analysis of GFP^+ microchimeric cells using light sheet fluorescence microscopy can be performed. Another method used for detecting microchimeric cells is flow cytometry [4,18], which, in combination with relevant antibody mixes, can additionally provide information on the phenotypic characteristics of maternal cells. Variants of single-cell OMICS analysis can also be used to examine, for example, the transcriptome or epigenetics of microchimeric cells [30]. For the proper phenotypization of the microchimeric cell populations, single-cell transcriptomics is a cutting-edge technology. It could be applied on the sorted cell populations (e.g. positive for fluorescent protein expression or relevant variant of the surface marker molecule) and in an unbiased manner provide ultimate information about the nature of the microchimeric cells including their tissue origin, differentiation and activation status or population heterogeneity. Recently study using single-cell RNA sequencing to study maternal microchimerism in more detail was conducted [31] indicating the potential of these new technologies for research in this field.

Impact of maternal-fetal microchimerism on offspring's immune system development

The concept of microchimerism provides an intriguing possibility for the mother to assist in the development of the still-immature immune system of the offspring through maternally derived cells. The development of an individual's immune system occurs throughout their life. Similar to other bodily structures, the ontogenesis of the immune system follows an abbreviated phylogenesis. Initially, the innate (evolutionarily older) part of the immune system develops, primarily involved in the initial response to foreign molecules. Over time, the adaptive part of the immune system also evolves, requiring interaction with foreign antigens and a complex process of "educating" lymphocytes through central and peripheral tolerance mechanisms. This ensures that their responses are

directed mainly against foreign antigens, generally maintaining tolerance to self-antigens.

Due to the need for prolonged interaction with the surrounding environment for optimal immune system development, an individual in early life requires specific protection. Given the close interactions between the offspring and the maternal organism, both prenatally *in utero* and postnatally through lactation, it is conceivable that the maternal organism may contribute to the offspring's protection. But could the intimate interaction between the offspring and the mother also influence the development of the offspring's immune system?

The answer to this question lies in a range of experimental data indicating that various bioactive molecules, immune complexes, antibodies, and cytokines are transferred from the mother to the offspring both *in utero* and during lactation. These substances not only help protect the offspring from infections but also aid in establishing the proper direction of immune system development and the establishment of physiological gut microbiota [32]. Could the mother contribute in ways other than the intergenerational transfer of molecules?

Maternal-fetal microchimerism causes alteration of immunophenotypes

In the early 1990s, a mouse knock-out model for IL-2 (IL-2 KO) was developed. IL-2 is a crucial growth and differentiation factor not only for T lymphocytes but also for thymocyte differentiation and the maintenance of normal thymus function [16]. IL-2 plays a role in the development of regulatory T lymphocytes in the thymus, their proliferation, regulation and maintenance in peripheral tissues. It also affects effector T lymphocytes, their differentiation and fate decision. IL-2 can promote Th1 or Th2 response in activated $CD4^+$ T lymphocytes and suppress Th17 and Tfh responses. Finally, IL-2 is also important for the effector function of $CD8^+$ T lymphocytes as it can influence those effector activities by inducing the expression of some proinflammatory cytokines (like interferon γ (IFN-γ) and tumor necrosis factor α (TNFα)). Because of such a complex role in the control of both the differentiation and homeostasis of T lymphocytes [33], it was expected that IL-2 KO mice would exhibit a dramatic immunological phenotype [16]. However, it was surprising to find that IL-2 KO mice were essentially immunologically normal, with normal representation of cell populations in the thymus and peripheral T cell subset composition [34]. Re-evaluation of this model by Wrenshall *et al.* revealed the presence of maternal cells producing IL-2 in the thymus, spleen, and lymph nodes of IL-2 KO mice. These cells were transferred during pregnancy through the placenta from mothers heterozygous for IL-2 and were able to influence the expected phenotypic expression of IL-2 KO mice. The maternal origin of these cells was confirmed by an experiment in which the mother mouse had an IL-2 promoter fused with GFP, and these GFP^+ cells were subsequently detected in the thymus and spleen of the offspring (Fig. 2). This finding led to the hypothesis that the transport of cytokines, growth factors, and cytokine-producing cells during pregnancy, and potentially during lactation, can modify the phenotype of mouse models and complicate the interpretation of acquired data [16]. For example, transforming growth factor β1 (TGF-β1) is transported from heterozygous mothers to TGF-β1-deficient mice during pregnancy and lactation, allowing normal embryonic development without lethal cardiovascular abnormalities [35]. During pregnancy, there is also migration of maternal B cell lineage cells into the offspring deficient in B lymphocytes. Maternal IgG-secreting cells were found in the spleen and bone marrow of 3 out of 11 offspring deficient in B lymphocytes. Those IgG-secreting cells persisted in the offspring until the 24^{th} week after birth [17].

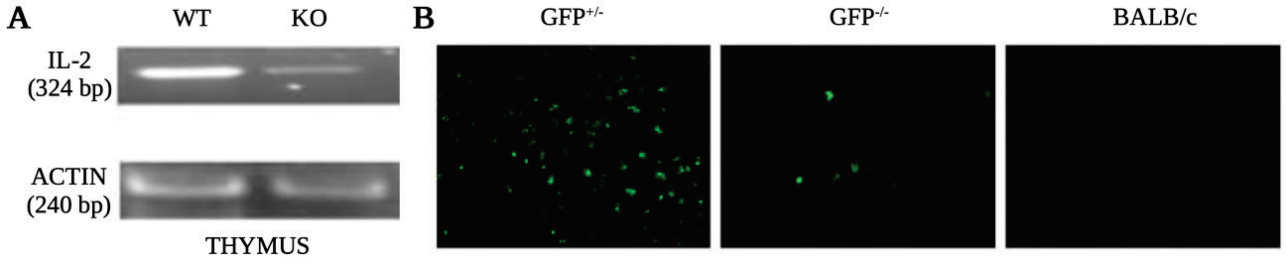

Fig. 2. Detection of maternal IL-2-expressing cells in IL-2 KO mice. (**A**) Using PCR, the IL-2 gene DNA was detected in the thymus of wild-type (WT) mice and IL-2 KO mice. (**B**) GFP^+ cells producing IL-2 were detected in thymus tissue sections from a $GFP^{+/-}$ mother (left), $GFP^{-/-}$ offspring born to a $GFP^{+/-}$ mother (middle), and a BALB/c individual used as a negative control (right). Adapted from [16]. Created with BioRender.com.

Prenatal period – pregnancy

Although the transfer of maternal cells to the fetus during pregnancy is widely accepted, most studies on materno-fetal microchimerism (MMc) focus on infants or adults, where maternal cells contribute to MMc development also through breastfeeding and therefore MFMc and MNMc cannot be distinguished.

Maternal immune cell migration to the fetus occurs through transendothelial migration *via* the placenta [3] or directly through umbilical cord blood into the fetal bloodstream. Berry *et al.* identified these cells in 16 out of 30 human fetuses during the second and third trimesters [19]. Umbilical cord blood is used for hematopoietic stem cell transplantation, and studies have examined the presence of maternal cells that could potentially cause graft-versus-host disease (GVHD) or graft-versus-leukemia (GVL) reactions [26,36,37]. Maternal cells could be for example detected in umbilical cord blood in 7 out of 49 samples using FISH. Some of these cells are $CD8^+$ cytotoxic T lymphocytes, and a small percentage of maternal $CD34^+$ hematopoietic stem cells was also present [26]. Another study identified various types of maternal immune cells in 27 out of 51 samples of human umbilical cord blood, with memory T lymphocytes being the most abundant. The functional potential of these leukocytes transferred from mother to offspring implies their impact on the child's immune system. The varying proportions of different cell types raise the question of whether this is a passive transport of cells from the mother's blood to the umbilical cord blood or an active migration of preferred functional cell types [36]. Subsequent studies confirmed the presence of maternal naive T lymphocytes, B lymphocytes, NK cells, myeloid cell populations, and hematopoietic stem cells in umbilical cord blood samples [37]. Maternal cells from the grandmother of the child were also found in umbilical cord blood, although their occurrence was three times lower than that of maternal cells [24].

In mice, the first maternal cells cross the placenta on the 7^{th} gestational day. These maternal cells are macrophages which settle in the mesodermal layer after gastrulation. These maternal macrophages display a matured phenotype and they could possibly participate in the “cleaning” of the embryo from pre-teratogenic cells, apoptotic bodies, and other cellular and tissue debris when the embryo does not yet have its own phagocytic cells. Maternal macrophages are replaced by embryonic macrophage precursors on the 9^{th} gestational day [38,39]. Maternal cells have also been detected in mouse embryos from the 12^{th} to the 19^{th} gestational day [18].

Immune cells cross the placenta into the lymph nodes of human fetuses, where they induce the development of regulatory T lymphocytes and suppress the immune reaction against maternal antigens. In addition to suppressing the reaction against non-inherited maternal antigens, this process may establish tolerance towards other foreign and self-antigens present during development *in utero* and influence the development of the offspring's immune system [22]. The spleen is another organ where maternal cells settle during pregnancy. However, maternal cells were not detected in the thymus or liver of newborn mice, suggesting that these cells might be predominantly mature lymphocytes [25].

Using a method based on quantitative PCR for detecting the luciferase (*Luc*) transgene, maternal cells were detected in the newborn heart, lungs, kidneys, liver, small intestine, spleen, and brain [28]. Among the cell types identified in the bone marrow of a 19-day-old mouse fetus were T lymphocytes, myeloid cells, B lymphocytes, and dendritic cells. The relative distribution of these cell types in the fetus differs from their distribution in the mother's peripheral blood, suggesting selective migration of maternal cells, particularly in the case of T lymphocytes. Maternal cells were found to contribute to the differentiation of fetal hematopoietic stem cells *in vitro*, with a preference for differentiation into leukocytes, specifically monocytes [4].

Piotrowski and Croy focused on detecting MFMc in immunodeficient embryos carried by surrogate immunocompetent mothers. Maternal cells were identified in all cases of these embryos, with 86 % found in the thymus, 63 % in the liver, and 49 % in the spleen. Additionally, maternal cells were detected in the bone marrow, lungs, and heart using immunohistochemistry [27]. Compared to immunocompetent individuals, immunodeficient individuals had a slightly higher level of MFMc, suggesting a higher likelihood of maternal cell presence. In immunocompetent mice immediately after birth, maternal cells were detected in 63 % of cases in the lungs and heart, 56 % in the kidneys, 44 % in the liver, 38 % in the small intestine, and 19 % in the spleen and brain, but only in 85 % of examined pups [28]. However, these values are not directly comparable due to the different sensitivity of the detection methods used: flow cytometry for immunodeficient individuals and quantitative PCR to detect cells with the *Luc* transgene for immunocompetent individuals.

Postnatal period – breastfeeding

Breastfeeding has a positive impact on individual health and reduces neonatal mortality. Breast milk provides newborns with not only nutrients but also various immune components, including cytokines, antibodies, and immune cells [40]. Initially, it was believed that the presence of a large quantity of maternal antibodies in milk was the main cause of its positive effect on strengthening the newborn's immune system. However, it appears that immune cells migrating from the mother's tissues through milk to the offspring's tissues also play a significant role in the development of the immune system, and their effects may persist into adulthood [41]. The abundance of these cells in the newborn tissues is extremely low and is highly challenging to identify them, especially using microscopy (Fig. 3).

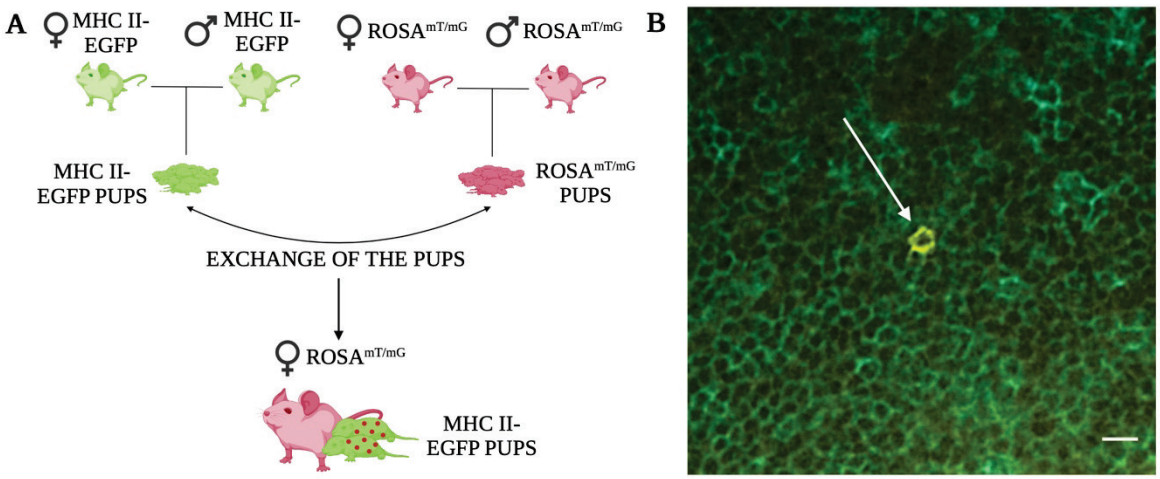

Fig. 3. Detection of maternal cells in the offspring's Peyer's patches using confocal microscopy. (**A**) Pups were exchanged between MHC II-EGFP knock-in mouse [42] (used to precisely identify newborn Peyer's patches due to strong EGFP signal of the resident APCs) [43] and ROSAmT/mG mouse [44] mothers. The presence of tdTomato$^+$ maternal cells were examined in the MHC II-EGFP knock-in mice offsprings. (**B**) tdTomato$^+$ maternal cell detected in Peyer's patch of 5-day-old MHC II-EGFP knock-in mouse offspring (confocal microscope ZEISS LSM880, 25× objective, scale bar: 20 μm).

The composition of breast milk varies among species, individuals, and throughout lactation [45]. The mother's diet [46], the health of both the mother and the child [47], and the changing needs of the child during development also influence milk composition [48]. The mother's nutritional diet directly affects the development of the offspring's immune system, and an imbalanced diet can increase the risk of neonatal infections [49]. These significant variations in composition complicate the detailed characterization of cellular populations in milk.

The number of cells in human milk ranges from thousands to millions per milliliter. Higher cell counts in colostrum compared to mature milk can be attributed to the dilution of cells in the larger volume of mature milk [50]. It can be stated with certainty that the offspring receives millions of cells daily through milk, which can potentially influence its development and protection against infections.

Milk contains cells in different stages of differentiation, ranging from stem cells with pluripotent potential to fully differentiated cells. The largest cellular population, accounting for up to 98 % of non-immune milk cells, consists of epithelial cells [40]. These cells could enter milk through their release from the mammary gland epithelium during breastfeeding, because of stress response associated with milk synthesis and secretion or pressure generated during suckling [50]. Other cell populations include stem cells and immune cells, and their relative representation varies greatly among individuals and throughout lactation, but in most cases, it ranges from 0 % to 2 % in mature milk of healthy individuals [47,51].

Most studies focus on the occurrence of immune cells in milk due to their potential impact on the offspring's health. Most of these studies have been conducted using human milk, thanks to its easy accessibility in large quantities. Another reason is its direct relevance to the research of human infections, such as breastfeeding by HIV-1 positive mothers [52,53]. However, milk components can also be obtained from

mouse models, either directly from the mammary gland of mouse mothers [54,55] or from the stomach of mouse pups [10], albeit in much smaller quantities. This offers the advantage of possible experimental arrangements, such as monitoring the transfer of cells from milk to the tissues of the offspring by allowing wild type (WT) pups to be breastfed by transgenic mouse mothers, followed by the detection of transgenic cells in the offspring's organs.

The largest representation of leukocytes in milk consists of neutrophils (40-60 %) and macrophages (30-47 %), followed by lymphocytes (5-9 %) [45]. Among lymphocytes, the highest proportion in milk is of $CD3^+$ T lymphocytes (83±11 %), followed by $CD19^+$ B lymphocytes (6±4 %). A small population of $CD16^+$ NK cells has also been identified in human breast milk [56]. However, the relative representation of maternal cell populations differs in the offspring, with T lymphocytes accounting for 80 %, of which 75 % are $CD8^+$ T lymphocytes [10]. This difference may be due to the lifespan of these cells. While T lymphocytes are relatively long-lived cells and may contribute to the offspring's long-term immunity in various tissues, granulocytes are short-lived cells and are more likely to participate in the immune response directly in the intestinal mucosa.

In the following paragraphs, individual cell types will be described in detail in the context of MNMc.

Neutrophils

Neutrophils are the most abundant cell population in milk [45], but there is a lack of direct studies focusing on the effect of maternal neutrophils in offspring. This may be due to their short lifespan, making it challenging to study their potential functions in the tissues of the offspring. However, current views on the duration of neutrophil effector activity in tissues are changing, with studies suggesting their survival for up to a week [57]. Understanding the behavior of the neutrophil population in newborns can help comprehend the potential function of maternal neutrophils in offspring.

Newborns generally have a lower number of neutrophils, which express fewer adhesion molecules [58] and exhibit limited targeted migration towards chemotactic agents [59]. They also have lower expression levels of certain Toll-like receptors (TLRs) involved in recognizing pathogen-associated molecular patterns (PAMPs). Consequently, newborn neutrophils have limited functionality, including impaired phagocytosis, degranulation, and oxidative burst [58]. They are also unable to perform neutrophil extracellular traps (NETs), which hampers their ability to kill bacteria [60].

The limited functions of neutrophils in newborns can negatively affect the offspring's defence against pathogens. Since many pathogens reside on mucosal surfaces, including the gastrointestinal tract, maternal neutrophils received through milk could act as the first line of defence against pathogen entry into the offspring's tissues, thereby compensating for the inadequate neutrophil functions in newborns through breastfeeding.

Monocytes and macrophages

Macrophages constitute the second largest population of cells in milk [45]. These cells survive in the offspring's digestive tract for a limited time and can subsequently internalize into the intestinal mucosa or participate in immunological functions. Hughes *et al.* detected labelled macrophages in a mucosal tissue of duodenum and a single macrophage in the spleen of a mouse pup [61], but due to the detection of a single cell, interpreting this observation conclusively is challenging. Also this experiment is non-physiological as the neonatal mice were fed by a high amount of labelled macrophages administered from the peritoneal cavity of mice [61]. As only a few macrophages were identified in this setup, it is controversial if this could also happen in physiological breastfeeding. Moreover, considering the relatively short "life span" of monocyte derived macrophages, it is unlikely that they would travel from the offspring's digestive tract and rather contribute to the local immune response in the intestinal mucosa.

Monocytes travel from peripheral blood to tissues, where they can differentiate into tissue macrophages. They may play a role not only in innate immunity but also in inducing antigen-specific T cell responses by functioning as antigen-presenting cells. Milk macrophages likely also develop from monocytes, which differentiate into macrophages in the mammary gland and then travel to milk through the epithelium [62]. Therefore, they could have similar functions to tissue macrophages.

Monocytes can generally be divided into two subsets: pro-inflammatory short-lived $CD16^-$ $CD14^+$ monocytes with low expression of CX3CR1 which migrate from blood to inflamed tissues and anti-inflammatory $CD16^+$ monocytes with longer life and high expression of CX3CR1 which migrate to non-inflamed tissues depending on the chemokine CX3CL1 [63]. While in the peripheral blood there is a higher representation of

pro-inflammatory than anti-inflammatory monocytes, the opposite is true for macrophages profiles in maternal milk. Additionally, the total number of macrophages in breast milk was higher if the infant had an acute respiratory infection than if the infant was healthy. However the frequency of pro-inflammatory $CD16^-$ macrophages was lower in maternal milk from mothers who had breastfed infected children [64]. The prevalence of anti-inflammatory macrophage profiles in milk is likely part of their homeostatic tolerogenic role, preventing the development of immunopathological conditions [65]. In milk, a population of macrophages expressing HLA-DR can be found [66], which can serve as antigen-presenting cells and contribute to initiating an adaptive immune response to present antigens, thereby participating in the local immune response in the intestinal mucosa and providing basic defence against invading pathogens. Thanks to the ability of antigen presentation, maternal macrophages could also play a role in the development of offspring's immune system [61]. In addition to the classical monocyte/macrophage lineage markers like CD14 and CD11b, milk macrophages express molecules involved in T lymphocyte stimulation, such as CD40 and CD86 and some may also express the glycoprotein CD83. All these molecules are normally expressed by antigen-presenting cells. Subpopulations of milk macrophages are capable of expressing DC-SIGN [62], a C-type lectin specific to dendritic cells, which, among other functions, mediates interaction with the HIV virus [67]. Thus, macrophages could increase the risk of HIV transmission from mother to child [62].

Milk macrophages generally exhibit high phagocytic activity and engulf certain milk components [62], which can lead to their accumulation inside cells associated with the formation of lipid inclusion bodies. This results in changes in macrophage morphology, with the presence of numerous vacuoles causing a distorted shape [45]. A unique ability of milk macrophages is the spontaneous production of granulocyte-macrophage colony-stimulating factor (GM-CSF) [62]. Since GM-CSF and IL-4 are required for monocyte/ macrophage differentiation into dendritic cells [68], milk macrophages stimulated only by IL-4 differentiate into dendritic cells capable of stimulating T lymphocytes and thereby regulating the T cell immune response. This ability to produce GM-CSF seems to be acquired through the phagocytosis of milk components (or possibly some yet undescribed local factor), as monocytes from peripheral blood stimulated by milk have secondarily acquired the ability to produce GM-CSF [62].

During infection in a breastfeeding infant, changes in milk composition have been observed [47]. If the child suffers from a respiratory disease, the total number of leukocytes, including macrophages, and the frequency of anti-inflammatory macrophages increase in milk. Additionally, during infection, levels of cytokines such as IL-6 and IL-8 also increase in milk [64]. During breastfeeding, microorganisms present in the saliva of infected children return back to the milk gland with a small amount of milk which causes local inflammation in the mother's milk gland [47]. Higher concentration of IL-8 in milk may induce adhesion and diapedesis of maternal leukocytes into gut-associated lymphoid tissue of the offspring [64] so higher numbers of maternal leukocytes can be transferred to offspring during their infection.

T lymphocytes

Both major types of T lymphocytes, $CD4^+$ and $CD8^+$, are present in breast milk. However, their ratio in milk differs from that in peripheral blood, with a higher representation of $CD8^+$ T lymphocytes. Additionally, γδ T lymphocytes [56], as well as mucosal-associated invariant T cells (MAIT) and innate lymphoid cells (ILC), which are functional and phenotypic counterparts of T lymphocytes, are also found in small numbers in milk [69]. The presence of these cell types in milk may be associated with their abundance in the intestinal immune system, suggesting their migration from the gut to mammary glands through the gut-mammary gland axis [52]. Another subset of T lymphocytes present in milk are $CD127^-$ $CD25^{++}$ $Foxp3^+$ regulatory T cells [70].

The majority of T lymphocytes identified in breast milk are activated ($CD45RO^+$ $HLA\text{-}DR^+$) [52,56,70], while naive T lymphocytes ($CD45RA^+$) are less prevalent. The overall percentage of T lymphocytes in milk is higher than in adult peripheral blood but does not significantly differ from the representation in maternal peripheral blood shortly after delivery. Maternal memory T lymphocytes have the capacity to compensate for the lack of newborn's activated T lymphocytes until the complete development of the infant's adaptive immune system [56]. $CD8^+$ T lymphocytes of the infant lack the ability to produce cytotoxic and inflammatory mediators, and maternal cells may compensate for this functional immaturity, protecting the newborn from infections until the maturation of their own adaptive immune system [10].

The reason for the high representation of memory T lymphocytes in milk remains unclear. One possible explanation is that T lymphocyte activation occurs in the mammary gland due to the presence of cytokines such as tumor necrosis factor α (TNF-α) [71] and interleukin 1β (IL-1β) [72] in milk, which stimulate T lymphocyte mitotic activity and activation [73]. On the other hand, activated T lymphocytes and other immune cells present in milk themselves could express these cytokines, suggesting that their high concentration in milk may be a result of the expression of these stimulatory molecules by T lymphocytes and other cells in maternal milk [56]. Furthermore, the higher presence of antigen-specific $CD8^+$ T lymphocytes in milk compared to peripheral blood cannot be explained solely by the higher abundance of their target structures, such as virus-infected cells presenting viral peptides in the context of MHC class I. In HIV-positive patients, a greater proportion of antigen-specific T cells is present in milk despite a higher level of target cells for $CD8^+$ cytotoxicity in blood [52,74]. Another possibility is the regulated migration of specific cell types from the mother to milk, aiming to establish immunologically functional MNMc in the offspring's tissues. Through milk, the infant could acquire leukocytes specific to pathogens encountered by the mother [29], leading to the intergenerational transfer of immune memory with significant benefits for the child. The expression profile of T lymphocytes in milk corresponds to the molecules expressed by effector memory T lymphocytes. The majority of $CD8^+$ T lymphocytes in milk express "gut-homing receptors" CD103 [52] and $\alpha_4\beta_7$ [10], as well as the "mucosal homing receptor" CCR9 [10,52]. In the early days after birth, the infant's stomach has a higher pH, allowing cells from milk to survive and reach the digestive tract, interact with mucosal epithelia, settle there, and potentially penetrate further into the body [56]. The stomach also has a lower amount of digestive enzymes, such as pepsinogen produced by chief cells, which mature two to three weeks after birth in rats [75]. Another survival mechanism for cells passing through the digestive tract is the interaction between milk components and the newborn's saliva, leading to the production of biochemical metabolites that may protect maternal leukocytes from the hostile environment of the digestive tract [41].

In the early stages of the offspring's life, maternal cells can undergo transepithelial migration [76,77] due to loosening of tight junctions in the intestinal epithelium, reaching tissues beyond the gastrointestinal tract. The intestinal epithelium of a newborn is underdeveloped and undergoes maturation during the early phase of life. The maturation of the newborn's intestinal epithelium, including the impact of various macromolecules and the microbiota from milk intake, is discussed in a review article by Rose *et al*. This process involves the interaction of specific microbiota and prebiotics with Toll-like receptors (TLRs), leading to the rearrangement and strengthening of tight junctions in the intestinal epithelium [78]. The permeability of the intestinal epithelium decreases with milk intake, especially during the first seven days of human neonatal life [79]. Furthermore, the expression of "gut-homing receptors" by cells in milk suggests that these cells may originate from the mother's digestive tract [52]. Migration of IgA-secreting plasma cells and T lymphocytes from the gut-associated lymphoid tissue (GALT) and blood to the mammary gland has been observed during the late stages of pregnancy and lactation [80-82].

For example, $CD8^+$ T lymphocytes have been found in the Peyer's patches of mouse pups, persisting until weaning with peak detection between the 14^{th} and 18^{th} day after birth (Fig. 4) [10]. Additionally, they are present in extra lymphoid organs, where they can enhance the local immune responses [52]. Maternal $CD4^+$ T lymphocytes are also able to migrate through the intestinal epithelium into the offspring's tissues. They have been detected in the thymus [83], spleen, mediastinal lymph nodes, and lungs [29]. Although the number of maternal antigen-specific $CD4^+$ T lymphocytes in the thymus was very low, it was evidently sufficient to strengthen the offspring's immunity. When an unimmunized offspring was exposed to a specific antigen, its immune response was quicker due to the presence of antigen-specific $CD8^+$ T lymphocytes. Ghosh *et al*. suggest that antigen-specific $CD8^+$ T lymphocytes develop as a result of the presence of maternal antigen-specific $CD4^+$ T lymphocytes in the offspring's thymus, indicating that maternal cells from milk actively participate in the development of the offspring's antigen-specific T lymphocytes [83].

B lymphocytes

B lymphocytes are present in milk in very small numbers [56], making their analysis challenging. Only a small number of articles refer to their identification in the offspring's tissue, however, based on what we know about the population of B lymphocytes in milk, we can

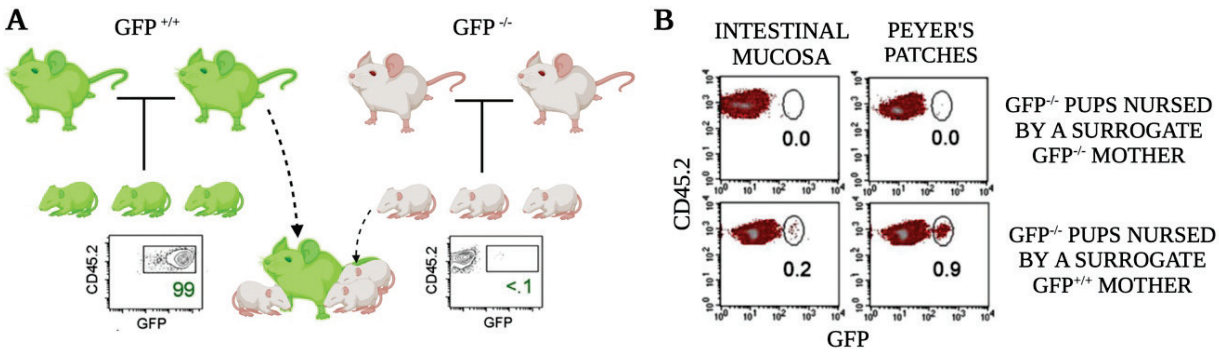

Fig. 4. Detection of maternal cells in the intestinal mucosa and Peyer's patches in 18-day-old mouse pups. (**A**) Two groups of mice, one GFP$^{+/+}$ and one GFP$^{-/-}$, were tested for GFP expression in splenocytes using flow cytometry, confirming that 99 % of cells in GFP$^{+/+}$ mice were GFP^{+}, while GFP$^{-/-}$ mice contained less than 1 % GFP^{+} cells. After birth, GFP$^{-/-}$ pups were nursed by a surrogate GFP$^{+/+}$ mother. (**B**) Maternal GFP^{+} CD45.2^{+} leukocytes were detected in the intestinal mucosa and Peyer's patches of GFP$^{-/-}$ pups using flow cytometry. Adapted from [10]. Created with BioRender.com.

infer their possible functions in the offspring.

B lymphocytes in milk exhibit a specific phenotype of class-switched memory B lymphocytes. These are typically memory B lymphocytes, of which more than 70 % have an IgD^{-} CD27^{+} phenotype. They express adhesion molecules such as CD44, integrins $\alpha_4\beta_7$ and $\alpha_4\beta_1$, which are also expressed by B lymphocytes in the gut-associated lymphoid tissue (GALT), suggesting that they may originate from the intestinal lymphatic tissue and migrate to the mammary gland in the same way as T lymphocytes [53,82].

Most B lymphocytes present in milk have undergone terminal differentiation towards plasma cells and are spontaneously activated. These cells mainly secrete IgG [53], in contrast to mammary gland plasma B cells that predominantly express IgA [84]. Cells secreting IgG may enter milk more frequently than cells secreting IgA due to weaker interactions of IgG plasma cells with chemokine receptors such as CCL28. B lymphocytes secreting antibodies against HIV antigens have been found in the milk of some HIV-1 positive women, which potentially reduces the risk of HIV transmission during breastfeeding. Other B lymphocytes present in milk may also contribute to preventing the transmission of viral and bacterial infections from mother to offspring during breastfeeding [53]. Additionally, class-switched memory B lymphocytes could compensate for the low antigen-presenting capacity of macrophages in newborns and aid in the development of their immune system through antigen presentation to developing T cells [85].

Detecting B lymphocytes of maternal origin in the offspring's tissues is challenging due to their low numbers in milk and thus even lower in the offspring's tissues. One of the few studies addressing this topic detected maternal immunoglobulin-secreting plasma cells in mouse offspring. These cells were also detected in B lymphocyte-deficient ($\mu^{-/-}$) mice born to $\mu^{-/-}$ mothers and subsequently nursed with milk from $\mu^{+/+}$ mice, on the 50th day after birth. IgG levels were detected in the serum of 7 out of 10 $\mu^{-/-}$ mice, and IgG-secreting cells originating from milk were identified in the spleen of 2 of these mice. Maternal B lymphocytes expressing typical B lymphocyte markers such as CD19 and B220 were not directly detected in any of the mice, but this does not exclude their presence due to the difficulty of detecting small cell populations [17]. It is worth noting that an alternative explanation for the presence of antibodies in individuals incapable of their production due to genetic modification ($\mu^{-/-}$) is the differentiation of respective B lymphocytes from maternal stem cells residing in the bone marrow. In the study by Darby *et al.*, they did not directly attempt to detect B lymphocytes in the offspring's tissues but focused on the transfer of immunity against *Nippostrongylus brasiliensis* (Nb) from mother to offspring. Since protection against Nb is not dependent on antibodies but directly on B lymphocytes, the presence of immunity against Nb in naive offspring which was breastfed by a mother exposed to Nb serves as indirect evidence of the transfer of maternal B lymphocytes from milk to the offspring's tissues [29].

Stem cells

Not surprising is the presence of stem and progenitor cells in maternal milk, including pluripotent, mesenchymal, neuroepithelial, and hematopoietic cell lineages [86,87]. During the first week after birth, milk serves as a reservoir for hematopoietic and non-

hematopoietic stem cells. $CD34^+$ $CD133^+$ cells, which give rise to myeloid progenitor cells as well as $CD34^+$ $CD133^-$ cells, which give rise to B lymphocytes and erythroid progenitors [88] were found in maternal milk [51]. These stem cells could originate from the bone marrow and reach the mammary gland through the bloodstream [51], subsequently appearing in milk due to mechanical forces during breastfeeding [89]. Hassiotou *et al.* isolated pluripotent stem cells from milk with the potential to differentiate into all three embryonic lineages, including epithelial, mesenchymal, and neural cells, under specific conditions. The population of these stem cells in milk expresses pluripotent genes associated with embryonic stem cells [89], such as *OCT4*, *SOX2*, *NANOG*, and *KLF4*. The expression of these genes was not identified in all samples [86], indicating the presence of cells in different stages of differentiation. Furthermore, it was tested whether milk-derived stem cells can induce teratoma formation in immunodeficient mice, but no tumors developed in any of the 15 injected mice. The oncological safety, combined with the relatively easy acquisition of these cells and their broad differentiation potential, could lead to the development of a therapeutic tool alternative to embryonic stem cell therapies [89].

The physiological function of these milk-derived stem cells in offspring remains a question. Similar to leukocytes, these stem cells are likely to travel through the intestinal epithelium of the offspring into the bloodstream and organs, where they may contribute to tissue homeostasis and potentially tissue regeneration [89]. The migration of milk-derived stem cells (OCT4 or NANOG positive) into the offspring was proven by the identification of maternal stem cells in the stomach, thymus, and liver three weeks after birth [12].

Antigen-specific transfer of maternal immunity

Research on the impact of breastfeeding on offspring's immune development following maternal exposure to specific pathogens has gained significant attention in recent years. One of the examples is the mouse model of experimental infection with the helminth *Nippostrongylus brasiliensis* (Nb). After one week of infection, the female mice were treated and then mated with non-immunized males two weeks later. The immune response characteristics of the offspring were measured (Fig. 5). Firstly, it was found that the number of activated effector $CD4^+$ T cells in the spleen of 14-day-old offspring from immunized mothers was higher than that of offspring from non-immunized mothers. However, when differentiating the effects of pregnancy and breastfeeding, it was determined that milk had an impact on offspring immunity because offspring born to immunized mothers but nursed by non-immunized wild type (WT) mothers did not develop immunity against Nb. Maternal Th2-polarized $CD4^+$ T cells were found in secondary and peripheral immune organs and were identified in the spleen, lungs and mediastinal lymph nodes. These cells influenced offspring protection at the systemic level and the development of their immunity, leading, for example, to the activation of effector B lymphocytes. The establishment of MNMc thus led to the preservation of immune memory against Nb in offspring into adulthood, confirming the hypothesis that the transport of maternal lymphocytes to the offspring's body may provide long-lasting selective immunity to infections experienced by the mother [29]. The conclusions of this research support previous hypotheses regarding the potential influence of maternal $CD4^+$ T cells and maternal milk overall on the development of the offspring's immune system [52,83].

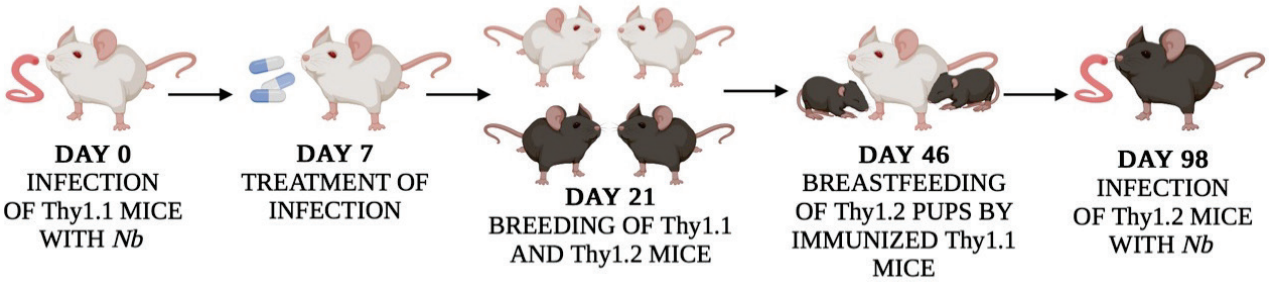

Fig. 5. Experimental mouse model of antigen-specific immunity transfer. BALB/c Thy1.1 mice were infected with *Nippostrongylus brasiliensis* (Nb) and treated with anti-helminth drugs after 7 days. Two weeks after treatment (day 21), they were mated with naive Thy1.1 males (simultaneously, a pair of Thy1.2 mice was mated for pups exchange). Three days after birth (day 46), the offsprings were exchanged, and naive Thy1.2 pups were nursed by a surrogate immunized Thy1.1 mother. At the age of 8 weeks (day 98), these pups were infected with Nb, and their immune response was examined. Adapted from [29]. Created with BioRender.com.

Role of maternal regulatory cells in the MMc maintenance

As already mentioned, the retention of maternal cells in the offspring's body depends on the development of regulatory T lymphocytes specific to non-inherited maternal antigens (NIMA) [9]. However, maternal milk also contains a population of maternal regulatory T lymphocytes that can independently influence the offspring's immune system and promote tolerance towards maternal cells and other antigens, such as the emerging gut microbiota. Regulatory T lymphocytes in milk, for instance, express a significant amount of TGF-β [70] and contribute to inducing tolerance or anergy in developing T lymphocytes [90], thereby potentially suppressing immune responses against certain antigens. When regulatory T lymphocytes are directly exposed to the corresponding antigen, they expand more rapidly than $CD8^+$ T lymphocytes recognizing the same antigen, thus suppressing their development at an early stage [91]. Therefore, maternal regulatory T lymphocytes specific to NIMA antigens contained in milk could significantly impact the early phase of the offspring's immune system development, controlling the expansion of $CD8^+$ T lymphocytes specific to NIMA antigens and enabling the preservation of maternal microchimerism into adulthood.

Conclusions and Future Directions

Maternal-fetal microchimerism established during pregnancy and lactation has a demonstrable impact on the development of the offspring's immune system and its protection against infections in the first weeks to months of life as maternal cells primarily consist of populations of leukocytes and stem cells with functional potential in the offspring.

Maternal effector immune cells migrate to the offspring, compensating for the “inadequate” function of neonatal leukocytes and actively protecting the newborn from pathogens. Maternal T lymphocytes appear to play the most significant role in the development of the offspring's immune system. They constitute the largest population of maternal cells detected in various tissues of the offspring and clearly influence its immune system. If the mother encounters a specific pathogen before or during pregnancy, her antigen-specific T lymphocytes can subsequently migrate to the offspring's immune organs, participating in the development of its immune system at both central and peripheral levels. They contribute to the development of the offspring's T lymphocytes in the thymus or activate effector B lymphocytes in lymph nodes. In addition to direct protection and immune system development, maternal cells are capable of partially compensating for the offspring's immunopathologies. In addition to leukocytes, pluripotent stem cells with the differentiation potential migrate to the offspring's tissues and give rise to differentiated maternal cells with functional potential throughout the individual's life.

Due to the low number of maternal cells settling in the individual and the difficulty in detecting them, data on specific cellular populations and their functions in the offspring are still lacking. Future discoveries of additional phenomena related to maternal microchimerism will not only be relevant to immunology but also to other fields such as tissue regeneration, cancer biology, various biological therapies, and organ transplantation.

To address the fundamental concern regarding the potential physiological role of maternal-fetal microchimerism, it is essential to employ a synergistic approach involving state-of-the-art technologies. This approach combines live cell *in situ* imaging of cells originating from the maternal source in newborns, precise characterization of phenotypes through methodologies such as single-cell transcriptomics, and determination of clonality utilizing techniques such as the confetti mouse model or Next-Generation Sequencing (NGS) for sequencing recombined genes, specifically T-cell receptors (TCRs), and immunoglobulins. For potential extrapolation to human physiology, modifications to the mouse model are essential. This may involve introducing genetic and microbiome variability to emulate natural populations. Alternatively, a more suitable model may need consideration, potentially surpassing the limitations posed by the mouse model. Anticipated advancements in comprehending microchimerism-related phenomena could be facilitated by exploring the spiny mice model. This model, with a placental structure akin to humans, an extended gestation period, and a reproductive strategy reflective of general K reproduction [92], holds promise for providing breakthrough insights.

Conflict of Interest

There is no conflict of interest.

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References

1. Nelson JL. The otherness of self: microchimerism in health and disease. Trends Immunol 2012;33:421-427. https://doi.org/10.1016/j.it.2012.03.002
2. Loubière LS, Lambert NC, Flinn LJ, Erickson TD, Yan Z, Guthrie KA, Vickers KT, Nelson JL. Maternal microchimerism in healthy adults in lymphocytes, monocyte/macrophages and NK cells. Lab Invest 2006;86:1185-1192. https://doi.org/10.1038/labinvest.3700471
3. Vernochet C, Caucheteux SM, Kanellopoulos-Langevin C. Bi-directional Cell Trafficking Between Mother and Fetus in Mouse Placenta. Placenta 2007;28:639-649. https://doi.org/10.1016/j.placenta.2006.10.006
4. Stelzer IA, Urbschat C, Schepanski S, Thiele K, Triviai I, Wieczorek A, Alawi M, ET AL. Vertically transferred maternal immune cells promote neonatal immunity against early life infections. Nat Commun 2021;12:4706. https://doi.org/10.1038/s41467-021-24719-z
5. Wang Y, Iwatani H, Ito T, Horimoto N, Yamato M, Matsui I, Imai E, Hori M. Fetal cells in mother rats contribute to the remodeling of liver and kidney after injury. Biochem Biophys Res Commun 2004;325:961-967. https://doi.org/10.1016/j.bbrc.2004.10.105
6. Boddy AM, Fortunato A, Wilson Sayres M, Aktipis A. Fetal microchimerism and maternal health: A review and evolutionary analysis of cooperation and conflict beyond the womb. BioEssays 2015;37:1106-1118. https://doi.org/10.1002/bies.201500059
7. Rao AS, Thomson AW, Shapiro R, Starzl TE. Chimerism after whole organ transplantation: its relationship to graft rejection and tolerance induction: Curr Opin Nephrol Hypertens 1994;3:589-595. https://doi.org/10.1097/00041552-199411000-00005
8. Stelzer IA, Thiele K, Solano ME. Maternal microchimerism: lessons learned from murine models. J Reprod Immunol 2015;108:12-25. https://doi.org/10.1016/j.jri.2014.12.007
9. Dutta P, Molitor-Dart M, Bobadilla JL, Roenneburg DA, Yan Z, Torrealba JR, Burlingham WJ. Microchimerism is strongly correlated with tolerance to noninherited maternal antigens in mice. Blood 2009;114:3578-3587. https://doi.org/10.1182/blood-2009-03-213561
10. Cabinian A, Sinsimer D, Tang M, Zumba O, Mehta H, Toma A, Sant'Angelo D, ET AL. Transfer of Maternal Immune Cells by Breastfeeding: Maternal Cytotoxic T Lymphocytes Present in Breast Milk Localize in the Peyer's Patches of the Nursed Infant. PLoS One 2016;11:e0156762. https://doi.org/10.1371/journal.pone.0156762
11. Stevens AM, Hermes HM, Kiefer MM, Rutledge JC, Lee Nelson J. Chimeric Maternal Cells with Tissue-Specific Antigen Expression and Morphology Are Common in Infant Tissues. Pediatr Dev Pathol 2009;12:337-346. https://doi.org/10.2350/08-07-0499.1
12. Hassiotou F, Heath B, Ocal O, Filgueira L, Geddes D, Hartmann P, Wilkie T. Breastmilk stem cell transfer from mother to neonatal organs. FASEB J 2014;28(Suppl 1):216.4. https://doi.org/10.1096/fasebj.28.1_supplement.216.4
13. Dutta P, Burlingham WJ. Stem cell microchimerism and tolerance to non-inherited maternal antigens. Chimerism 2010;1:2-10. https://doi.org/10.4161/chim.1.1.12667
14. Cuddapah Sunku C, Gadi V, de Laval de Lacoste B, Guthrie KA, Nelson JL. Maternal and fetal microchimerism in granulocytes. Chimerism 2010;1:11-14. https://doi.org/10.4161/chim.1.1.13098
15. Mauer AM, Athens JW, Ashenbrucker H, Cartwright GE, Wintrobe MM. Leukokinetic Studies. Ii. A Method For Labeling Granulocytes In Vitro With Radioactive Diisopropylfluorophosphate (DFP32). J Clin Invest 1960;39:1481-1486. https://doi.org/10.1172/JCI104167
16. Wrenshall LE, Stevens ET, Smith DR, Miller JD. Maternal microchimerism leads to the presence of interleukin-2 in interleukin-2 knock out mice: Implications for the role of interleukin-2 in thymic function. Cell Immunol 2007;245:80-90. https://doi.org/10.1016/j.cellimm.2007.04.002
17. Arvola M, Gustafsson E, Svensson L, Jansson L, Holmdahl R, Heyman B, Okabe M, Mattsson R. Immunoglobulin-Secreting Cells of Maternal Origin Can Be Detected in B Cell-Deficient Mice. Biol Reprod 2000;63:1817-1824. https://doi.org/10.1095/biolreprod63.6.1817
18. Fujimoto K, Nakajima A, Hori S, Irie N. Whole embryonic detection of maternal microchimeric cells highlights significant differences in their numbers among individuals. PLoS One 2021;16:e0261357. https://doi.org/10.1371/journal.pone.0261357

19. Berry SM, Hassan SS, Russell E, Kukuruga D, Land S, Kaplan J. Association of Maternal Histocompatibility at Class II HLA Loci with Maternal Microchimerism in the Fetus. Pediatr Res 2004;56:73-78. https://doi.org/10.1203/01.PDR.0000129656.10005.A6
20. Kaplan J, Land S. Influence of Maternal-Fetal Histocompatibility and MHC Zygosity on Maternal Microchimerism. J Immunol 2005;174:7123-7128. https://doi.org/10.4049/jimmunol.174.11.7123
21. Kinder JM, Jiang TT, Ertelt JM, Xin L, Strong BS, Shaaban AF, Way SS. Cross-Generational Reproductive Fitness Enforced by Microchimeric Maternal Cells. Cell 2015;162:505-515. https://doi.org/10.1016/j.cell.2015.07.006
22. Mold JE, Michaëlsson J, Burt TD, Muench MO, Beckerman KP, Busch MP, Lee T-H, ET AL. Maternal Alloantigens Promote the Development of Tolerogenic Fetal Regulatory T Cells in Utero. Science 2008;322:1562-1565. https://doi.org/10.1126/science.1164511
23. Hossain S, Mihrshahi S. Exclusive Breastfeeding and Childhood Morbidity: A Narrative Review. Int J Environ Res Public Health 2022;19:14804. https://doi.org/10.3390/ijerph192214804
24. Karlmark KR, Haddad ME, Donato X-C, Martin GV, Bretelle F, Lesavre N, Cocallemen J-F, ET AL. Grandmaternal cells in cord blood. eBioMedicine 2021;74:103721. https://doi.org/10.1016/j.ebiom.2021.103721
25. Shimamura M, Ohta S, Suzuki R, Yamazaki K. Transmission of maternal blood cells to the fetus during pregnancy: detection in mouse neonatal spleen by immunofluorescence flow cytometry and polymerase chain reaction. Blood 1994;83:926-930. https://doi.org/10.1182/blood.V83.4.926.926
26. Hall J, Lingenfelter P, Adams S, Lasser D, Hansen J, Bean M. Detection of maternal cells in human umbilical cord blood using fluorescence in situ hybridization. Blood 1995;86:2829-2832. https://doi.org/10.1182/blood.V86.7.2829.2829
27. Piotrowski P, Croy BA. Maternal Cells are Widely Distributed in Murine Fetuses in Utero1. Biol Reprod 1996;54:1103-1110. https://doi.org/10.1095/biolreprod54.5.1103
28. Su EC, Johnson KL, Tighiouart H, Bianchi DW. Murine Maternal Cell Microchimerism: Analysis Using Real-Time PCR and In Vivo Imaging. Biol Reprod 2008;78:883-887. https://doi.org/10.1095/biolreprod.107.063305
29. Darby MG, Chetty A, Mrjden D, Rolot M, Smith K, Mackowiak C, Sedda D, ET AL. Pre-conception maternal helminth infection transfers via nursing long-lasting cellular immunity against helminths to offspring. Sci Adv 2019;5:eaav3058. https://doi.org/10.1126/sciadv.aav3058
30. Ståhlberg A, El-Heliebi A, Sedlmayr P, Kroneis T. Unravelling the biological secrets of microchimerism by single-cell analysis. Brief Funct Genomics 2018;17:255-264. https://doi.org/10.1093/bfgp/elx027
31. Fujimoto K, Nakajima A, Hori S, Tanaka Y, Shirasaki Y, Uemura S, Irie N. Whole-embryonic identification of maternal microchimeric cell types in mouse using single-cell RNA sequencing. Sci Rep 2022;12:18313. https://doi.org/10.1038/s41598-022-20781-9
32. Camacho-Morales A, Caba M, García-Juárez M, Caba-Flores MD, Viveros-Contreras R, Martínez-Valenzuela C. Breastfeeding Contributes to Physiological Immune Programming in the Newborn. Front Pediatr 2021;9:744104. https://doi.org/10.3389/fped.2021.744104
33. Ross SH, Cantrell DA. Signaling and Function of Interleukin-2 in T Lymphocytes. Annu Rev Immunol 2018;36:411-433. https://doi.org/10.1146/annurev-immunol-042617-053352
34. Schorle H, Holtschke T, Hünig T, Schimpl A, Horak I. Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. Nature 1991;352:621-624. https://doi.org/10.1038/352621a0
35. Letterio JJ, Geiser AG, Kulkarni AB, Roche NS, Sporn MB, Roberts AB. Maternal Rescue of Transforming Growth Factor-β 1 Null Mice. Science 1994;264:1936-1938. https://doi.org/10.1126/science.8009224
36. Kanaan SB, Gammill HS, Harrington WE, De Rosa SC, Stevenson PA, Forsyth AM, Allen J, ET AL. Maternal microchimerism is prevalent in cord blood in memory T cells and other cell subsets, and persists post-transplant. Oncoimmunology 2017;6:e1311436. https://doi.org/10.1080/2162402X.2017.1311436
37. Kanold AMJ, Westgren M, Götherström C. Cellular Subsets of Maternal Microchimerism in Umbilical Cord Blood. Cell Transplant 2019;28:522-528. https://doi.org/10.1177/0963689718779783
38. Bertrand JY, Jalil A, Klaine M, Jung S, Cumano A, Godin I. Three pathways to mature macrophages in the early mouse yolk sac. Blood 2005;106:3004-3011. https://doi.org/10.1182/blood-2005-02-0461

39. Balounová J, Šplíchalová I, Dobešová M, Kolář M, Fišer K, Procházka J, Sedlacek R, ET AL. Toll-like receptor 2 expression on c-kit+ cells tracks the emergence of embryonic definitive hematopoietic progenitors. Nat Commun 2019;10:5176. https://doi.org/10.1038/s41467-019-13150-0
40. Witkowska-Zimny M, Kaminska-El-Hassan E. Cells of human breast milk. Cell Mol Biol Lett 2017;22:11. https://doi.org/10.1186/s11658-017-0042-4
41. Laouar A. Maternal Leukocytes and Infant Immune Programming during Breastfeeding. Trends Immunol 2020;41:225-239. https://doi.org/10.1016/j.it.2020.01.005
42. Boes M, Cerny J, Massol R, den Brouw MO, Kirchhausen T, Chen J, Ploegh HL. T-cell engagement of dendritic cells rapidly rearranges MHC class II transport. Nature 2002;418:983-988. https://doi.org/10.1038/nature01004
43. Pačes J, Knížková K, Tušková L, Grobárová V, Zadražil Z, Boes M, Černý J. MHC II - EGFP knock-in mouse model is a suitable tool for systems and quantitative immunology. Immunol Lett 2022;251-252:75-85. https://doi.org/10.1016/j.imlet.2022.10.007
44. Muzumdar MD, Tasic B, Miyamichi K, Li L, Luo L. A global double-fluorescent Cre reporter mouse. Genesis 2007;45:593-605. https://doi.org/10.1002/dvg.20335
45. Crago SS, Prince SJ, Pretlow TG, McGhee JR, Mestecky J. Human colostral cells. I. Separation and characterization. Clin Exp Immunol 1979;38:585-597.
46. Babu ST, Niu X, Raetz M, Savani RC, Hooper LV, Mirpuri J. Maternal high-fat diet results in microbiota-dependent expansion of ILC3s in mice offspring. JCI Insight 2018;3:e99223. https://doi.org/10.1172/jci.insight.99223
47. Hassiotou F, Hepworth AR, Metzger P, Lai CT, Trengove N, Hartmann PE, Filgueira L. Maternal and infant infections stimulate a rapid leukocyte response in breastmilk. Clin Transl Immunol 2013;2:e3. https://doi.org/10.1038/cti.2013.1
48. Molès JP, Tuaillon E, Kankasa C, Bedin A-S, Nagot N, Marchant A, McDermid JM, Van de Perre P. Breastmilk cell trafficking induces microchimerism-mediated immune system maturation in the infant. Pediatr Allergy Immunol 2018;29:133-143. https://doi.org/10.1111/pai.12841
49. Basha S, Surendran N, Pichichero M. Immune responses in neonates. Expert Rev Clin Immunol 2014;10:1171-1184. https://doi.org/10.1586/1744666X.2014.942288
50. Hassiotou F, Geddes DT, Hartmann PE. Cells in Human Milk: State of the Science. J Hum Lact 2013;29:171-182. https://doi.org/10.1177/0890334413477242
51. Valverde-Villegas JM, Durand M, Bedin A-S, Rutagwera D, Kankasa C, Tuaillon E, Nagot N, ET AL. Large Stem/Progenitor-Like Cell Subsets can Also be Identified in the CD45 - and CD45 +/High Populations in Early Human Milk. J Hum Lact 2020;36:303-309. https://doi.org/10.1177/0890334419885315
52. Sabbaj S, Ghosh MK, Edwards BH, Leeth R, Don Decker W, Goepfert PA, Aldrovandi GM. Breast Milk-Derived Antigen-Specific CD8+ T Cells: An Extralymphoid Effector Memory Cell Population in Humans. J Immunol 2005;174:2951-2956. https://doi.org/10.4049/jimmunol.174.5.2951
53. Tuaillon E, Valéa D, Becquart P, Al Tabaa Y, Meda N, Bollore K, Van de Perre P, Vendrell J-P. Human milk-derived B cells: a highly activated switched memory cell population primed to secrete antibodies. J Immunol 2009;182:7155-7162. https://doi.org/10.4049/jimmunol.0803107
54. Pačes J, Grobárová V, Zadražil Z, Knížková K, Malinská N, Tušková L, Boes M, Černý J. MHC II-EGFP Knock-in Mouse Model. Curr Protoc 2023;3:e925. https://doi.org/10.1002/cpz1.925
55. Ikebuchi R, Fujimoto M, Moriya T, Kusumoto Y, Kobayashi K, Tomura M. T cells are the main population in mouse breast milk and express similar profiles of tight junction proteins as those in mammary alveolar epithelial cells. J Reprod Immunol 2020;140:103137. https://doi.org/10.1016/j.jri.2020.103137
56. Wirt DP, Adkins LT, Palkowetz KH, Schmalstieg FC, Goldman AS. Activated and memory T lymphocytes in human milk. Cytometry 1992;13:282-290. https://doi.org/10.1002/cyto.990130310
57. Pillay J, Den Braber I, Vrisekoop N, Kwast LM, de Boer RJ, Borghans JAM, Tesselaar K, Koenderman L. In vivo labeling with 2H2O reveals a human neutrophil lifespan of 5.4 days. Blood 2010;116:625-627. https://doi.org/10.1182/blood-2010-01-259028
58. Yu JC, Khodadadi H, Malik A, Davidson B, da Silva Lopes Salles É, Bhatia J, Hale VL, Baban B. Innate Immunity of Neonates and Infants. Front Immunol 2018;9:1759. https://doi.org/10.3389/fimmu.2018.01759

59. Weinberger B, Laskin DL, Mariano TM, Sunil VR, DeCoste CJ, Heck DE, Gardner CR, Laskin JD. Mechanisms underlying reduced responsiveness of neonatal neutrophils to distinct chemoattractants. J Leukoc Biol 2001;70:969-976. https://doi.org/10.1189/jlb.70.6.969
60. Yost CC, Cody MJ, Harris ES, Thornton NL, McInturff AM, Martinez ML, Chandler NB, ET AL. Impaired neutrophil extracellular trap (NET) formation: a novel innate immune deficiency of human neonates. Blood 2009;113:6419-6427. https://doi.org/10.1182/blood-2008-07-171629
61. Hughes A, Brock JH, Parrott DM, Cockburn F. The interaction of infant formula with macrophages: effect on phagocytic activity, relationship to expression of class II MHC antigen and survival of orally administered macrophages in the neonatal gut. Immunology 1988;64:213-218.
62. Ichikawa M, Sugita M, Takahashi M, Satomi M, Takeshita T, Araki T, Takahashi H. Breast milk macrophages spontaneously produce granulocyte-macrophage colony-stimulating factor and differentiate into dendritic cells in the presence of exogenous interleukin-4 alone. Immunology 2003;108:189-195. https://doi.org/10.1046/j.1365-2567.2003.01572.x
63. Geissmann F, Jung S, Littman DR. Blood Monocytes Consist of Two Principal Subsets with Distinct Migratory Properties. Immunity 2003;19:71-82. https://doi.org/10.1016/S1074-7613(03)00174-2
64. Zheng Y, Corrêa-Silva S, de Souza EC, Rodrigues RM, da Fonseca FAM, Gilio AE, Carneiro-Sampaio M, Palmeira P. Macrophage profile and homing into breast milk in response to ongoing respiratory infections in the nursing infant. Cytokine 2020;129:155045. https://doi.org/10.1016/j.cyto.2020.155045
65. Medzhitov R, Schneider DS, Soares MP. Disease Tolerance as a Defense Strategy. Science 2012;335:936-941. https://doi.org/10.1126/science.1214935
66. Leyva-Cobián F, Clemente J. Phenotypic characterization and functional activity of human milk macrophages. Immunol Lett 1984;8:249-256. https://doi.org/10.1016/0165-2478(84)90004-X
67. Geijtenbeek TBH, Kwon DS, Torensma R, van Vliet SJ, van Duijnhoven GC, Middel J, Cornelissen IL, ET AL. DC-SIGN, a Dendritic Cell-Specific HIV-1-Binding Protein that Enhances trans-Infection of T Cells. Cell 2000;100:587-597. https://doi.org/10.1016/S0092-8674(00)80694-7
68. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. J Exp Med 1994;179:1109-1118. https://doi.org/10.1084/jem.179.4.1109
69. Bedin A, Molès J, Rutagwera D, Nagot N, Kankasa C, Tylleskär T, Valverde-Villegas JM, ET AL. MAIT cells, TCR γδ+ cells and ILCs cells in human breast milk and blood from HIV infected and uninfected women. Pediatr Allergy Immunol 2019;30:479-487. https://doi.org/10.1111/pai.13037
70. Cérbulo-Vázquez A, Hernández-Peláez G, Arriaga-Pizano LA, Bautista-Pérez P, Romero-Venado J, Flores-González JC, Figueroa-Damian R, ET AL. Characterization of CD127 − CD25 ++ Treg from human colostrum. Am J Reprod Immunol 2018;79:e12806. https://doi.org/10.1111/aji.12806
71. Rudloff HE, Schmalstieg FC, Mushtaha AA, Palkowetz KH, Liu SK, Goldman AS. Tumor Necrosis Factor-α in Human Milk. Pediatr Res 1992;31:29-33. https://doi.org/10.1203/00006450-199201000-00005
72. Hawkes JS, Bryan DL, James MJ, Gibson RA. Cytokines (IL-1β, IL-6, TNF-α, TGF-β1, and TGF-β2) and Prostaglandin E2 in Human Milk during the First Three Months Postpartum. Pediatr Res 1999;46:194-199. https://doi.org/10.1203/00006450-199908000-00012
73. Hackett RJ, Davis LS, Lipsky PE. Comparative effects of tumor necrosis factor-alpha and IL-1 beta on mitogen-induced T cell activation. J Immunol 1988;140:2639-2644. https://doi.org/10.4049/jimmunol.140.8.2639
74. Sabbaj S, Edwards BH, Ghosh MK, Semrau K, Cheelo S, Thea DM, Kuhn L, ET AL. Human Immunodeficiency Virus-Specific CD8(+) T Cells in Human Breast Milk. J Virol 2002;76:7365-7373. https://doi.org/10.1128/JVI.76.15.7365-7373.2002
75. Tatematsu M, Takahashi M, Tsuda H, Hirose M, Furihata C, Sugimura T. Precocious differentiation of immature chief cells in fundic mucosa of infant rats induced by hydrocortisone. Cell Differ 1975;4:285-294. https://doi.org/10.1016/0045-6039(75)90013-5
76. Ma LJ, Walter B, DeGuzman A, Muller HK, Walker AM. Trans-Epithelial Immune Cell Transfer during Suckling Modulates Delayed-Type Hypersensitivity in Recipients as a Function of Gender. PLoS One 2008;3:e3562. https://doi.org/10.1371/journal.pone.0003562

77. Tuboly S, Bernáth S. Intestinal Absorption of Colostral Lymphoid Cells in Newborn Animals. Adv Exp Med Biol 2002;503:107-114. https://doi.org/10.1007/978-1-4615-0559-4_12
78. Rose EC, Odle J, Blikslager AT, Ziegler AL. Probiotics, Prebiotics and Epithelial Tight Junctions: A Promising Approach to Modulate Intestinal Barrier Function. Int J Mol Sci 2021;22:6729. https://doi.org/10.3390/ijms22136729
79. Catassi C, Bonucci A, Coppa GV, Carlucci A, Giorgi PL. Intestinal Permeability. Changes during the First Month: Effect of Natural versus Artificial Feeding. J Pediatr Gastroenterol Nutr 1995;21:383-386. https://doi.org/10.1002/j.1536-4801.1995.tb11955.x
80. Roux ME, McWilliams M, Phillips-Quagliata JM, Weisz-Carrington P, Lamm ME. Origin of IgA-secreting plasma cells in the mammary gland. J Exp Med 1977;146:1311-1322. https://doi.org/10.1084/jem.146.5.1311
81. Rose ML, Parrott DM, Bruce RG. The accumulation of immunoblasts in extravascular tissues including mammary gland, peritoneal cavity, gut and skin. Immunology 1978;35:415-423.
82. Ramanan D, Sefik E, Galván-Peña S, Wu M, Yang L, Yang Z, Kostic A, ET AL. An Immunologic Mode of Multigenerational Transmission Governs a Gut Treg Setpoint. Cell 2020;181:1276-1290.e13. https://doi.org/10.1016/j.cell.2020.04.030
83. Ghosh MK, Nguyen V, Muller HK, Walker AM. Maternal Milk T Cells Drive Development of Transgenerational Th1 Immunity in Offspring Thymus. J Immunol 2016;197:2290-2296. https://doi.org/10.4049/jimmunol.1502483
84. Tanneau GM, Oyant LHS, Chevaleyre CC, Salmon HP. Differential Recruitment of T- and IgA B-lymphocytes in the Developing Mammary Gland in Relation to Homing Receptors and Vascular Addressins. J Histochem Cytochem 1999;47:1581-1592. https://doi.org/10.1177/002215549904701210
85. Lokossou GAG, Kouakanou L, Schumacher A, Zenclussen AC. Human Breast Milk: From Food to Active Immune Response With Disease Protection in Infants and Mothers. Front Immunol 2022;13:849012. https://doi.org/10.3389/fimmu.2022.849012
86. Li S, Zhang L, Zhou Q, Jiang S, Yang Y, Cao Y. Characterization of Stem Cells and Immune Cells in Preterm and Term Mother's Milk. J Hum Lact 2019;35:528-534. https://doi.org/10.1177/0890334419838986
87. Fan Y, Chong YS, Choolani MA, Cregan MD, Chan JKY. Unravelling the Mystery of Stem/Progenitor Cells in Human Breast Milk. PLoS One 2010;5:e14421. https://doi.org/10.1371/journal.pone.0014421
88. Handgretinger R, Kuçi S. CD133-Positive Hematopoietic Stem Cells: From Biology to Medicine. Adv Exp Med Biol 2013;777:99-111. https://doi.org/10.1007/978-1-4614-5894-4_7
89. Hassiotou F, Beltran A, Chetwynd E, Stuebe AM, Twigger A-J, Metzger P, Trengove N, ET AL. Breastmilk Is a Novel Source of Stem Cells with Multilineage Differentiation Potential. Stem Cells 2012;30:2164-2174. https://doi.org/10.1002/stem.1188
90. Chappert P, Schwartz RH. Induction of T cell anergy: integration of environmental cues and infectious tolerance. Curr Opin Immunol 2010;22:552-559. https://doi.org/10.1016/j.coi.2010.08.005
91. Chappert P, Leboeuf M, Rameau P, Lalfer M, Desbois S, Liblau RS, Danos O, ET AL. Antigen-specific Treg impair CD8 + T-cell priming by blocking early T-cell expansion. Eur J Immunol 2010;40:339-350. https://doi.org/10.1002/eji.200839107
92. Carter AM. Animal models of human pregnancy and placentation: alternatives to the mouse. Reproduction 2020;160:R129-R143. https://doi.org/10.1530/REP-20-0354