Selective Transfer of Maternal Antibodies in Preterm and Term Children

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Abstract

Preterm newborns are more likely to suffer from infectious diseases at birth compared to children delivered at term. Whether this is due to compromised cellular, humoral, or organ-specific development remains unclear. Recent studies have shown that while preterm children have an aberrant cellular immune response, these infants receive similar maternal anti-viral IgG repertoires compared to term children, albeit at lower concentrations. These data point to selectivity in placental transfer at distinct gestational ages, to ensure that children are endowed with the most robust humoral immunity even if born preterm. To begin to define the mechanism by which preterm selective transfer may occur, the overall quantity and functional quality of an array of vaccine-, endemic pathogen-, and common antigen-specific antibodies were assessed across a cohort of 11 preterm and 12 term-delivered mother:infant pairs from birth through week 12. Although higher antibody levels were present in term infants, the overall functional profiles of antigen-specific antibodies were very similar. Temporal transfer differences were ascertained across distinct antibody subpopulations, with early transfer of functional antibodies capable of binding to FcRn and FcγR2-3 receptors followed by the transfer of distinct IgG subclasses. These results provide new insights on maternal:fetal immunity, highlighting novel immune axes that may be manipulated to enhance neonatal immune transfer of antibodies through gestation.

Introduction

On average, pregnancy lasts 40 weeks, long-enough to allow for the full growth, development, and maturation of the neonate. In the US alone, more than 10% of babies are born at less than 37 weeks of gestation, with 2.75% born at less than 34 weeks of gestation (1). Babies born earlier than 39 weeks have a greater incidence of respiratory complications, low blood sugar, feeding problems, as well as an elevated risk of developing diseases over time (2,3). While rates of preterm births are declining in the developed world, preterm births are on the rise in the developing world (4), with 15 million preterm births world-wide each year (5), with premature birth representing the second leading cause of death among children under 5 years of age across the globe (6).

In the last weeks of pregnancy, significant development of the brain, lungs, liver, and skin occur in parallel to significant weight gain, that collectively ensure the survival of the neonate outside the uterus. However, even when fully developed, neonates are born with a largely naïve immune system, that must quickly adapt to the new environment and prevent infections (7). To help their offspring, mothers actively transfer copious levels of IgG antibodies to infants in utero, to passively immunize the neonates against pathogens previously encountered by the mother. Thus, the final months/weeks of gestation are critical for final developmental events and to fully immunologically arm the neonate to prepare for the non-sterile life outside utero.

Commonly attributed to developmental and immune prematurity, preterm babies are more susceptible to infections (2). This includes infections in the lungs, skin, kidneys, eyes, bladder, and gastrointestinal tract. Specifically, higher susceptibility in preterm infants has been reported for Varicella-Zoster virus (8) and viral respiratory infections (2) even when transferred antibody levels are comparable. However, whether this increased susceptibility of preterm children is related to an impairment in the immune system or due to incomplete transfer of protective antibodies from the mother to neonate remains unclear. If the latter is true, vaccine strategies could be developed for pregnant women to induce enhanced transfer of antibodies to infants against pathogens that disproportionately affect preterm neonates.

Maternal immunity is largely transferred across the placenta in the form of IgGs, actively captured from the maternal circulation and transferred in a neonatal Fc-receptor (FcRn) dependent manner to the infant (9–12). Placenta transfer occurs throughout pregnancy, with the highest levels of antibodies transferred in the third trimester (13). Preterm infants have been shown to possess lower antigen-specific IgG levels than full term infants (14). However, despite the lesser quantities of antibodies in preterm infants, a recent high-dimensional analysis of the transferred antibody repertoire found comparable antibody repertoires and even comparable neutralizing activity in preterm compared to term infants, arguing for selective transfer of particularly functional antibodies early in pregnancy (15). These data challenge the dogma of non-specific antibody transfer and suggests that the placenta may selectively transfer functionally enhanced antibodies earlier in pregnancy.

Early models suggested that placental transfer was largely an unbiased process mainly driven by FcRn affinity for different IgG subclasses (IgG1>IgG4>IgG3>IgG2), indiscriminately transferring any antigen-specific antibody to the neonate (9,16,17), but more recent data point to the preferential transfer of unique antibody specificities (18,19), that may be driven via Fc-glycan preferences of FcRn and/or other collaborating Fc-receptors expressed within the placenta (20). However, whether placental Fc-preferential transfer occurs constantly throughout pregnancy or evolves over time to select more functional antibodies and empower neonates with differential immunity, should they be born early, remains unclear.

Here, we comprehensively profiled the Fc-binding profiles of antibodies in mothers, cord blood and postnatal samples from children born at different gestational ages across 24 pathogen/antigen-specific antibody populations. The data demonstrate that while overall IgG1 transfer increases with gestational age, preterm newborns have received levels of the most functional antibodies at birth comparable to full term, through selective Fc-receptor driven transfer. These data support a model of dynamic placenta development such that functionally enhanced antibodies are selectively transferred via FcRn early in pregnancy in collaboration with additional Fc-receptors. Collectively, this transient selective transfer process provides infants with the most functional antibody responses at birth, poised to fight infection even if born prior to full gestation.

Results

Fc-evolution in early life

Transferred IgG increases with the trimesters (13), resulting in highest levels of antibody transfer in the third trimester of gestation (9,16,17). Consequently, preterm infants are believed to have a compromised immune response due to lower levels of antibody transfer (30,31). However, recent studies, focused on the evaluation of neutralizing titers rather than binding antibody titers, pointed to equivalent levels of neutralizing antibody transfer in preterm infants compared to full term neonates, despite the transfer of higher antibody titers with increased gestational age (15). Given our emerging understanding for potential selective transfer of antibodies across the placenta, we comprehensively profiled the humoral immune response across 24 vaccine or pathogen-derived antigens (**Table 1**) in a cohort of 11 preterm and 12 term mother:child pairs over the course of the first 3 months of life (**Figure 1**)(7).

As expected, IgGs, IgM, IgAs, and Fc-receptor binding antibodies were highly heterogeneous among mothers of both preterm and term babies (**Figure 1**). However, expected patterns were observed across these isotypes over time. Specifically, IgG and IgG subclasses were detectable at week 0 in the cords, and then declined by week 4 and 12 in peripheral blood as the antibodies waned. Conversely, as expected, substantial levels of IgM and IgA1 responses were only detected in the mothers, were absent in the cord, and IgM and IgA1 began to evolve in a limited number of infants by 3 months following birth. Interestingly, Fc-receptor binding antibodies were detectable in the mothers, were detectable in the cords at birth, but then decayed rapidly to very low levels by the third month of life, with a more rapid and profound decline in the preterm infants. Similar profiles were detected for antigen-specific antibody dependent cellular phagocytosis (ADCP) and antibody dependent neutrophil phagocytosis (ADNP), with variable but detectable functions detected across antigens in the mothers as well as the cord. However, the decline in transferred functional antibodies was more similar across the preterm and full-term baby sets, with some novel ADCP responses appearing to particular antigens by 3 months of life.

Despite the similarities in transfer and decay across the mother:infant groups, a correlation matrix of all antibody features between mother and cord hinted at differential coordination of antibody transfer to pre- and full-term infants (**Figure S1A-B**). Specifically, while antibody profiles were more highly coordinated in full-term mother:cord pairs, antibody transfer relationships were less coordinated in preterm dyads. Of note, the pneumo- and pertussis-specific antibody features were positively correlated in term mother:cord pairs, but were not correlated in the preterm pairs. Similarly, correlations were largely negative (not statistically significant) in preterm pairs. Thus collectively, these data highlight less coordinated antibody transfer profiles in preterm infant pairs decay profiles across pre- and full-term dyads.

Convergence of antibody profiles over time

Accumulating data points to striking differences in cellular immune function and frequencies in neonates compared to their mothers', irrespective of gestational age, that are lost over the first few months of life (7). To begin to examine whether similar patterns may exist within the humoral immune response, we used an unsupervised principal component analysis (PCA) to examine overall antibody profiles across the timepoints and samples (**Figure 2A**). As expected, separation was observed across timepoints, where mothers and cord samples scattered together, but a progressive divergent shift was observed for infant antibody profiles with time from birth (**Figure 2A**). Moreover, while the level of variation in maternal:cord samples was significant, less variation was observed in antibody profiles at weeks 1-4, with renewed variation appearing at 3 months following birth.

Interestingly, to determine whether this unsupervised approach could pick up any variation between full-term and preterm babies, each principal component (PC) was examined systematically. PC1 picked up variation within groups (**Figure 2A**). Conversely, PC5 showed interesting partial discriminatory power between preterm (pink) and term (green) infants (**Figure 2B**). PC2-PC4 captured variation in the data that was not related to groups or preterm and fullterm status (**Supplemental Figure 2A-C**). Interestingly, the greatest variation in antibody profiles across the full- and pre-term infants occurred at birth, and these differences were lost overtime. Thus, collectively, these

data highlight the differences in preterm infant immunity at birth, that wanes in both groups and converges during the first few weeks of life.

To further gain a sense of what contributes to this variation between the two groups of infants, we next examined differences in the transfer and kinetics of decay of antibody subclass, isotype, and Fc-receptor binding profiles over time (**Figure 2C**). As expected, evidence of preferential transfer of IgGs was observed in the full-term babies compared to IgA and IgM transfer. Conversely, less selective transfer was observed among the IgGs in premature infants, albeit IgAs and IgMs were also deliberately not transferred. As expected, IgG transfer was linked to enhanced FcRn binding in full-term infants after birth, coincident with a small, but heterogeneous, increase in FCGR3 binding antibodies and more persistent FCR2 binding antibodies in the full-term infants. Importantly, the robust increase in FcRn binding in full-term infants was disproportionate to the level of transferred IgG1 levels, pointing a qualitative shift in antibody quality selectively trapped and transferred to infants at birth. In contrast, preterm infants exhibited a diminished FcRn binding signature, and more limited FCGR3 binding antibody levels right after birth, likely related to diminished IgG1 transfer, but a more similar FCGR2 binding profile over time. These data highlight expected antibody isotype subclass differences across full- and preterm infants linked to unexpected Fc-receptor binding profiles over time.

Selective impaired transfer of particular antigen-specific antibodies in preterm children

To further define the specific features that were most highly perturbed in PC1 (variation across time) and PC5 (variation by gestational age), loadings on each PC were plotted for each measured variable, where each dot represents an antigen-specificity grouped by each Fc-measurement. As anticipated, greatest temporal changes were observed among IgG/IgG1 antibodies across nearly all antigen-specificities, representing the dominant transferred antibody population from mother:infant (**Figure 3A**). In addition, these IgG differences tracked with significant alterations in Fc-receptor binding profiles, that evolve uniquely over time. Less variation in FcRn, largely involved in early transfer, followed by FcγR (**Figure 3A**) was observed over time, although some variation was notable across specificities.

Gestational age variation was examined using PC5 scores (**Figure 3B**). Strikingly less variation was observed in this dimension, although variation was largely restricted again to IgG/IgG1 and Fc-receptor binding profiles. Interestingly, variation along PC5 was largely uniform for each Fc-receptor highlighting conserved principles of Fc-receptor mediated transfer across gestational ages. However, differences in transfer were noted for a specific subset of antigen-specificities. The top two antigen-specificities, which were consistently higher in preterm (positive loading on PC5) across Fc measurements, included nBosd8 (Bovine milk allergen) and Diphtheria.

To therefore define the particular specificities that were differentially transferred across gestational age groups, a multi-level discriminant analysis (ML-PLSDA) (20,32) was used in mothers and their infants in each group across IgG levels (**Figure 3C-F**). Robust separation was observed across full-term mothers and infants in antibody profiles (**Figure 3C**). The top antibody features that drove this separation were all enriched in cord blood and included higher levels of tetanus, norovirus, influenza, polio, respiratory syncytial virus (RSV), pertussis toxin, adenovirus, pneumo, cytomegalovirus (CMV), and auto-antigen (histone H3)-specific antibodies in the full-term cords (**Figure 3D**). Separation was also observed in mother:cord antibody profiles in preterm infants (**Figure 3E**), however the antibody profiles that drove this separation were associated with higher levels of antibodies in the maternal blood (**Figure 3F**). These data point to antigen-specific variation in transfer rates across the mother:cord dyads. However, what governs these transfer differences selectively at different stages of gestation remain unclear but could point to opportunities to enhance transfer.

Differences in preterm and term antibody profiles diminish over time

To begin to define the specific differences, and drivers of differences in antibodies transferred to full- and preterm infants, we next compared the overall antibody profiles across the two groups of mother:infant pairs over time (**Figure 4**). Classification models were generated per time point, initially using a least absolute shrinkage and selection operator (LASSO) to down-select features (to avoid overfitting) followed by Orthogonalized Partial Least Squares Discriminant Analysis (OPLSDA) using the LASSO-selected features. Robust separation was observed in infant profiles in the cord (**Figure 4A**) that persisted but became less different over the first weeks (**Figure 4D**) and months of life (**Figure 4G**). The discriminating antibody profiles at birth were associated with higher levels of antibody features (Fc-receptor binding and levels) in the term infants (**Figure 4B**). Given that the models select a minimal set of antibody features that are most distinct across the groups, we next examined the co-correlates of the model selected features to gain deeper insights into the antibody profiles differences across the infant groups. Strikingly nearly all the model selected features were tethered to additional FcR binding antibody features (**Figure 4C**) pointing to the pivotal selective role and collaboration between FcRn and FcγRs in placental transfer at birth.

Similarly, antibody profiles after 1 week of life also demonstrated robust separation (**Figure 4D**), marked largely by enhanced antibody levels in the full-term infants (**Figure 4E**). However, a few features were enriched among preterm infants (**Figure 4E**). To gain further insights into the additional markers that tracked with the model-selected features, two large networks appeared around the features that marked the full-term antibody profiles, composed of high IgG titers to nearly all tested antigens, as well as largely persistent FcγR binding antibodies to EBV, mumps, polio, RSV, VZV, and rubella. Moreover, a robust cluster of pneumo-specific and mumps-specific antibody features were also noted in the full-term infants 1 week after birth, highlighting robust maintenance of these antibody-specificities to the newborn. Conversely, preterm infants exhibited a highly selective enrichment of all antibody qualities to nBosd8, a milk-allergen (**Figure 4F**).

Despite the normalization of antibody profiles with time across the babies (**Figure 1** and **2**), antibody profiles still differed across full- and preterm infants after 3 months of age (**Figure 4G**), marked by a very small number of features in the full-term neonates (**Figure 4H**). The features marked a robust persistence of pneumo-specific antibodies of multiple qualities in full-term infants as well as the presence of a highly persisting cluster of IgG3-antibodies to Adenovirus T5, CMV, polio, and peanuts (**Figure 4I**). These data point to early differences in antibodies across pre- and full-term infants, that largely normalize, but that result in rare cases in persisting vulnerabilities in preterm infants to bacterial pathogens known to cause enhanced disease in this vulnerable population. Thus overall, while higher levels of antibodies across specificities clearly distinguish term from preterm infants, this difference is lost overtime, with few, but potentially important, differences persisting between the two groups months after birth.

Evolution of transfer profiles during pregnancy

To ultimately define whether antibody transfer occurs via the same mechanism(s) in pre- and full-term infants, we next plotted transfer ratios for all specificities for each analyzed feature according to gestational age (**Figure 5**). A temporal transfer curve (blue solid line) was then calculated for each feature using linear regression. Specifically, a nominal multinomial logistic regression was used to calculate the probability that cord values would exceed maternal levels, where dashed black lines represent the overall temporal shift (left y axis, **Figure 5A,B**). As expected, total IgG transfer ratios increased with gestational age (**Figure 5A**), largely for IgG1 antibodies. More moderate increases were noted in the transferred slopes of other IgG subclasses IgG2-4, consistent with a total increase overtime but no evidence of preferentially enhanced transfer, as is observed for IgG1 antibodies.

To begin to define the mechanism underlying transfer differences over time, we next examined the transfer profiles for Fc-receptor binding antibodies. A steep and linear increase in FcγR binding antibodies was observed over time (**Figure 5B**) as expected with increases in transferred antibodies with gestational age. Similarly, a steady increase in FcγR2B binding antibodies was observed. Conversely, a more striking logistic growth was observed for FcγR3A,B receptor-binding antibodies (**Figure 5B**).

To gain a clearer sense of the timing of antibody selectivity across gestational ages, the gestational age when the likelihood of the transfer ratios increased above 1 (≥ 1) was calculated using a logistic regression (black dashed lines in **Figure 5A,B**, replotted and overlaid in **Figure 5C**). For each feature, the gestational age when this likelihood exceeded 0.5 was calculated (**Figure 5C**, shown on horizontal axis). Surprisingly, all Fc-receptor binding properties cross the transfer threshold earliest in pregnancy, highlighting the critical selective influence of Fc-receptors in shaping transfer. FcγR3B, followed by FcγR2A, FcγRn crossed the transfer threshold earlier than IgG1 levels. These curves were followed closely by the total IgG and other IgG subclasses, highlighting placental kinetic transfer differences across IgG-subpopulations and IgG subclasses. Despite the higher affinity of IgG3 for many Fcγ-receptors, the preferential transfer of IgG1 throughout gestation argue for a collaboration between FcγRn and Fcγ-receptors expressed within the placenta. Collectively the data therefore argue for early collaboration between FcγRn and FcγRs in driving preferential transfer of highly functional antibodies early in gestation, followed by enhanced overall antibody transfer over time.

Sequential preferential transfer of functional antibodies over gestation

The early Fc-receptor transfer profiles suggested that the placenta may select for highly functional antibodies early during gestation. To test this hypothesis, we next examined the overall levels of functional antibodies across the mother:infant pairs (**Figure 6**). The levels of antibody dependent cellular phagocytosis (monocyte phagocytosis, ADCP), antibody dependent neutrophil phagocytosis (ADNP), and antibody dependent NK cell activating (NK cell degranulation and cytokine secretion, ADNk) inducing antibodies were screened across pertussis, influenza and RSV. Surprisingly, despite differences in the overall levels of IgG-specific antibodies to each of these targets across the pre- and full-term cord samples (**Figure 5A**), antibody effector function was largely concordant across most full- and pre-term infants, with the exception of RSV-specific NK IFNγ secretion (**Figure 6A-E**). For example, nearly equivalent antibody-dependent cellular phagocytosis, driven largely by FcγR2 (33–36), was observed across pre- and full-term cords (**Figure 6A**). Similarly, equivalent levels of

neutrophil-phagocytosis activating antibodies were observed across pre- and full-term infants to all tested pathogens (**Figure 6B**). Conversely, NK cell activating antibodies increased more variably over time, with early robust transfer of Influenza-specific antibodies able to drive NK cell activation in preterm infants (**Figure 6C-E**). Thus, despite lower level IgG-levels early in gestation, these data suggest early phagocytic antibody transfer during gestation.

Accordingly, to define whether particular functions were transferred preferentially, transfer slope analysis was performed (**Figure 6F**). For phagocytic functions (ADCP and ADNP), slopes were relatively stable (**Figure 6F**), consistent with the early transfer of FcγR2 (**Figure 5B-C**), suggesting that transfer increased overtime, aimed at populating the infant early on with functional antibodies. Conversely, the regressed ADNK curves were nearly flat and even decreased for antibody mediated NK cell chemokine secretion (MIP1b) with gestational age, suggesting a potential perpetual selection, from very early gestation, aimed at populating the infant with these highly functional antibodies. Moreover, logistic regression of the probability of transfer >1 (black dashed lines in **Figure 6F**) suggested that antibody-mediated NK activating antibodies are likely to be preferentially transferred as early as week 24 of gestation (**Figure 6G**). These data suggest that functional antibodies are transferred early and consistently throughout pregnancy, to ensure in utero protection and increased probability of survival. Moreover, these data also point to a potentially preferential transfer of NK cell activating antibodies earliest in gestation, potentially linked to the enhanced maturity of neonatal NK cells in early life (37).

Selective antigen-specific antibody decay

Finally, we aimed to define the decay patterns of antigen-specific antibodies overtime following birth across both pre- and full-term infants by comparing antibody profiles across each set of timepoints (**Figure 7**). As expected in the fullterm infants, at birth, the presence of higher levels of particular antibody populations were enriched in the cord, due to preferential transfer across the placenta, marked by enhanced levels of Fc-receptor binding antibodies to norovirus, tetanus, *S. pneumoniae*, poliovirus, hepatitis A, mumps, and allergens, total pertussis antibody titer as well as ADNP-capable antibodies to rubella (**Figure 7A**). As expected, with time, antibody levels decayed, highlighting enriched antibody levels at early timepoints (C>W1 and W1>W12). However, the decay occurred disproportionately across antigen-specificities (**Figure 7C and D**). Interestingly, ADNP inducing antibodies to influenza, FcγR3A-NK cell activating antibodies to tetanus and Varicella zoster virus, FcRn binding antibodies to adenovirus T40 and norovirus, and overall levels of *S. pneumoniae* antibodies were preferentially lost over the first week of life (**Figure 7C**). Further loss of total IgGs to mumps, adenovirus T40, and Fc-receptor binding antibodies to influenza, RSV, Varicella zoster virus, EBV, and peanut allergen were observed later overtime, whereas FcRn binding bovine milk allergen slightly increased by the third month (**Figure 7E**). These data suggest an early preferential loss of the most functional antibodies and a potentially slower decay in overall levels of IgG in full-term infants.

Unlike full-term comparisons, higher levels of antibodies were observed in the maternal circulation compared to cord (**Figure 7B**), marked by higher levels of cow milk-, influenza-, measles-, tetanus- and diphtheria-specific antibodies and ADNP-inducing antibodies to RSV in the maternal blood (**Figure 7B**). An early loss of ADNP antibodies was observed to RSV. Loss of FcγR3A-NK cell activating tetanus antibodies occurred soon after birth along with an early loss of total tetanus-, influenza-, adenovirus-, norovirus-, and human histone H3-specific antibody decay (**Figure 7D**). In premature infants, the next 3 months was associated with a decay in FcγR binding antibodies to influenza, Norovirus, and cow milk (**Figure 7F**). Additionally, total IgGs declined to norovirus over this second phase, and a loss of ADCP was also observed for RSV. Interestingly, IgG3 antibodies to HepA and pollen were lost in this later phase following birth. These data highlight a selective loss of some antibody subpopulations early (ADNP and tetanus/adeno) and a later decline in Fc-receptor binding, functional, IgG3 antibodies following several months of birth, highlighting different potential windows of vulnerability following birth across full- and pre-term infants.

Thus, collectively, our findings confirm the presence of higher transferred antibodies in full-term infants but point to placental mechanism(s) that aim to transfer functional antibodies early during pregnancy to empower the neonate to fight infection even if born prematurely. The data also point to differential kinetic vulnerabilities in pre- and full-term infants that have critical implications for the design of next generation of vaccines or therapeutics able to leverage the unique filtering features of the placenta that clearly evolves over the course of pregnancy to empower neonates with the greatest chance of survival after birth.

Discussion

In 2018, 5.3 million deaths occurred in children under 5 years of age, with 47% of these reported in the first month of life (38). The leading cause of death in these children was attributed to complications of preterm birth, making the early months of life an especially vulnerable period for those born early. Preterm infants are particularly susceptible to infection, especially vulnerable to pathogens affecting the respiratory tract (2). While immature mucosal and cellular immunity are likely key to this vulnerability, preterm infants receive fewer

placentally transferred antibodies, that have also been implicated in compromised immunity. However, recent data suggest that despite these lower titers, preterm newborns possess similar anti-viral antibody repertoires and equivalent levels of neutralizing antibodies, suggesting that despite reduced placental transfer, placental mechanisms must exist to ensure robust transfer of broadly reactive and functionally relevant antibodies (15). Using a systems serology approach, we observed significant differences in the overall magnitude, but not the functional or Fc-receptor binding quality of antibodies across pre- and full-term neonates, arguing for conserved and very early Fc-receptor mediated selection of the most functional antibodies for transfer to neonates.

The early predominant transfer of functional antibodies might occur evolutionarily to equip the infant with the highest quality, rather than quantity of antibodies if born early. This change in sieving may occur via differential expression of Fc-receptors or via the changes in the localization of Fc-receptor expression. Importantly, multiple Fc-receptors have been observed in the placenta (39–42), and recent data have noted co-localization of FcγR3A with FcRn on syncytiotrophoblasts that may explain the preferential transfer of NK-cell activating antibodies (20). However, here the data point to earlier and steady transfer of both NK cell activating and phagocytic antibodies over time, pointing to the selection of sub-populations of IgG1s from maternal circulation to populate the neonatal system. Given the sieving occurs largely on IgG1 antibodies, and not additional IgG subclasses that bind to Fcγ-receptors with higher affinity, these data suggest that FcRn must be involved in the selection. As FcRn typically functions as a homodimer (43–46), requiring two receptors to trap an antibody, it is also plausible that FcRn may form a heterodimer with additional Fcγ-receptors within syncytiotrophoblasts, aimed at capturing the most functional IgG1-antibodies from maternal blood. Conversely, as gestation progresses, the collaboration between FcRn and other Fc-receptors may change. This evolutionary placental process may endow neonates with the greatest probability of survival against infections.

The placenta is a complex organ consisting of chorionic villi, which are surrounded by chorion. Antibodies in the maternal blood flowing through the intervillous space are first transported through the outer syncytiotrophoblast layer of the chorion to the inner layer of cytotrophoblast progenitor cells and the fetal endothelium into the fetal capillaries. However, the fetal endothelium also expresses FCGR2B, which is thought to play an additional role in sieving antibodies prior to transfer into cord blood (39,47). However, additional Fc-receptors are expressed dynamically in the placenta in the setting of infection (48), likely all contributing to changes in the quality of transferred antibodies. Specifically, dynamic cellular heterogeneity of the placenta was previously shown (48), that collectively could shift the quality of antibodies that are allowed to transit across the maternal/fetal barrier. Along these lines, several infections have been linked to compromised placental transfer (18,49), including HIV (50–52), malaria (51,53–56), and Zika (57) likely occurring both due to altered humoral immunity in the infected mother, but also due to changes in the placenta that may shift the quality of the captured antibodies. Thus defining both the natural mechanisms that lead to placental sieving of antibodies in healthy maternal:fetal dyads as well as how these shift with infection may provide critical clues for the development of next generation vaccines or therapeutics aimed to counteracting infections.

Of all the antibody specificities, only cow-milk allergen specific antibodies were enriched in preterm neonates 1 week after birth compared to full term infants. Exposure to allergens have been controversially linked to allergies in preterm infants (58–61). However, emerging data point to antibodies as a robust surrogate of food allergy. Thus, future studies, aimed at profiling antibody transfer will be able to identify the relationship of food-antibodies and allergy across gestational ages.

Interestingly, previous studies highlighted the transfer of equivalent antibody repertoires and neutralization in pre- and full-term infants (15), largely controlled at the antigen-binding domain level of the antibody (the Fab). However, how Fab selection is regulated via the placenta is unclear. Emerging data point to the co-evolution of the Fab and Fc-domain, in such a way that the Fab and Fc domain often collaborate to mediate protective immunity (62–64). For example, in the context of Influenza infection, several of the most potent protective antibodies require Fc-mediated activity to mediate protection from infection in animal models (65–67). Similarly, many protective HIV-neutralizing antibodies (29,68–70) and bacterial toxin-specific antibodies (71,72) also require Fc-mediated activity in order to mediate protection in animal models. Thus, it is plausible that the placenta aims to leverage the most highly protective antibodies that both neutralize and drive Fc-effector function to maximize protective immunity in the neonate.

Together, these findings suggest that the rules governing placental transfer may change throughout gestation, and that preterm infants may be empowered to fight infection via placental selection of the most functional antibodies required to fight infectious diseases. Whether these levels are sufficient to prevent disease remains unclear, but this selection of functionally enhanced antibodies may provide an evolutionary survival advantage aimed at giving the neonate the best chance possible after birth. Coupled to breast feeding, that provides additional mucosal protection, with the richest antibody transfer occurring at the time of birth in the colostrum (73,74), infants may possess a highly functional barrier against pathogens during the first days of life. Further dissection of the precise rules of antibody-

transfer over the course of gestation, both in health and disease may offer critical insights for the design of next generation vaccines and/or therapeutics able to leverage the unique evolving biology of the placenta to maximally protect infants as they enter the world.

Methods

Cohort

21 mother/child pairs with 11 preterm and 12 full term sample sets were included in this study (7). Samples included cord blood (0 weeks), and peripheral blood from 1 week, 4 weeks, and 12 weeks post birth. Maternal peripheral blood samples were also taken at birth. Gestational ages for preterm newborns ranged from 24 to 29 weeks and full-term from 37 to 40 weeks, which was correlated with birth weight between the two groups (Table 1A). Other characteristics such as maternal age, delivery mode, male-female proportion (Table 1A) and vaccination status (Table 1B) were comparable between the preterm and full-term groups.

Antigen-specific antibody isotype, subclass, and FcR binding

Antigen-specific isotypes, IgG subclasses, and binding to FcγRIIA, IIb, IIIA, IIIb and FcRn were measured as previously described (21,22). Protein antigens (**Table S1**) were covalently coupled to MagPlex® microspheres via a two-step carbodiimide reaction. The beads were activated with 80μL of activation buffer (0.1M NaH₂PO₄, pH 6.2), 10μL of 50mg/ml Sulfo-NHS (N-hydroxysulfosuccinimide, Pierce, A39269), 10μL of 50mg/mL ethyl dimethylaminopropyl carbodiimide hydrochloride (EDC) and incubated for 30 minutes at room temperature. The beads were washed three times in coupling buffer (0.05M morpholinoethanesulfonic acid (MES), pH 5.0), then incubated with protein antigen in coupling buffer for two hours at room temperature. The beads were subsequently washed and blocked with PBS-TBN (PBS, 0.1% BSA, pH 7.4), then washed with PBS-Tween Buffer (PBS, 0.1% BSA, 0.02% Tween 20, 0.05% Azide, pH 7.4). Beads were resuspended in PBS at concentration of 5x10⁶ beads/mL and stored for up to two weeks at 4° C.

To measure isotypes and subclasses, samples were diluted 1:10 in PBS to achieve a final assay dilution of 1:100 when 5μL sample was added to 45μL antigen-coated beads at a concentration of 5x10⁶ beads/mL. Immune complexes were incubated for 16 hours overnight shaking at 900 rpm at 4° C. Unbound antibody was removed via washing six times in Luminex assay buffer with an automated plate washer. PE-conjugated secondary antibodies (IgG, IgG1, 2, 3, 4, IgA1, 2, IgM) were diluted 1:500 in Luminex Assay Buffer, and 40μL were added and incubated for 1 hour shaking at 900 rpm at room temperature. Unbound secondary was washed, and Qsol Buffer was added for reading on the Intellicyt iQue. Relative levels of isotypes and subclasses were calculated as the median fluorescence intensity of PE. Background was determined by a no-antibody control.

To prepare Fc receptors, AVI-Tagged FcγRs and FcR were biotinylated with BirA enzyme (Avidity, BirA500) for 30 minutes rotating at room temperature, and excess biotin was removed with Zeba Spin Desalting Columns, 7 MKCO (Thermo Fisher, 89892). Immediately prior to use, biotinylated FcRs were coupled to Streptavidin-PE for 10 minutes. Immune complex binding to FcRs was then measured and calculated as described for isotypes and subclasses.

Antibody-dependent cellular phagocytosis (ADCP)

Based on the published protocol (23), Rubella, pertussis, influenza, pneumococcus, CMV, and RSV antigens were biotinylated with LC-LC biotin and coupled to yellow-green fluorescent Neutravidin 1μm beads (Thermo Fisher, F8776) for 2 hours at 37° C. Coupled beads were then washed twice with 5% BSA/PBS and stored at 4° C for up to 1 week. Cord and maternal samples were diluted 1:100 and 10μL sample was incubated with 10μL antigen-coupled beads for 2 hours at 37° C to form immune complexes. After washing with PBS to remove unbound non-specific antibody, THP-1 monocytes were added (200 μL/well) at a concentration of 1.25x10⁵ cells/mL and incubated with immune complexes overnight for 16 hours at 37° C. Cells were fixed with 4% PFA and acquired with an Intellicyt iQue. Phagocytosis was measured by a phagocytosis score ((gMFI of bead-positive cells x percentage of bead-positive cells)/1000). Background levels of phagocytosis were measured with a no-antibody control for each antigen.

Antibody-dependent neutrophil phagocytosis (ADNP)

Based on the published protocol (24), Rubella, pertussis, influenza, pneumococcus, CMV, and RSV antigens were coupled to beads, and antigen-coated beads were incubated with sample as described for ADCP. To prepare PBMCs from whole blood, erythrocytes were lysed (Buffer, 150mM NH₄Cl, 10mM KHCO₃, mM Na₂EDTA in 1 mL H₂O), and leukocytes were washed twice with ice cold PBS. PBMCs were added (200 μL/well) at a concentration of 2.5x10⁵ cells/mL. Neutrophils were stained with a Pacific Blue conjugated anti-CD66b

secondary antibody (BioLegend, 305112). Finally, cells were fixed and acquired, and a phagocytosis score was calculated as described for ADCP.

Antibody-dependent Natural Killer (NK) cell degranulation assay

ELISA plates were coated with 100ul/well of pertussis, influenza A, and RSV antigen at 2 µg/mL each and incubated 2 hours at 37° C. Plates were washed three times with PBS and blocked with 5% BSA/PBS overnight at 4° C. NK cells were isolated from Buffy coats from 3 donors using RosetteSep NK cell enrichment kit (StemCell Technologies) and rested overnight in the presence of IL-15. The following day, samples were diluted 1:25 and 50µL/well diluted sample was added to ELISA plates after washing, incubating at 37° C for 2 hours. A CD107 PE-Cy5/BFA/GolgiStop (BD Biosciences, 555802) cocktail was prepared and added directly to NK cells before adding 200 µL/well NK cells at a concentration of 2.5×10^5 cells/mL. Cells and plate-bound immune complexes were incubated for 5 hours at 37° C. After incubation and washing, NK cells were stained for surface markers, CD56 PE-Cy7, CD16 APC-Cy7, and CD3 Pacific Blue (BD Biosciences, 557747, 557758, 558124) in V-bottom plates. Cells were subsequently washed and incubated with PermaA for 15 minutes followed by intracellular staining with fluorescently conjugated MIP1β PE and IFNγ FITC (BD Biosciences, 550078, 340449). NK cell activation and degranulation markers were measured by flow cytometry with the Intellicyt iQue.

Principal Component Analysis (PCA)

A PCA model was constructed using 323 antibody features. Variables were centered and scaled to a standard deviation of 1. In the two-dimensional space of PC1 vs. PC5, temporal trends of samples were observed to be in the direction of PC1, whereas PC5 was observed to partially separate preterm and full term profiles.

Identification of preterm/full term-specific signatures with LASSO and OPLSDA

The minimum signatures of antigen-specific antibody biophysical and functional measurements that could differentiate between preterm and term infants at birth, week 1 and week 12 were identified using the Least Absolute Shrinkage and Selection Operator (LASSO) method (25). This was implemented using Matlab software (version 2018a, Mathworks, Natick, MA) on the data, where each variable was centered and scaled to have a standard deviation of 1. Orthogonalized Partial Least Squares Discriminant Analysis (OPLSDA) (26,27) was then used to visualize and assess the predictive ability of LASSO-selected biomarkers for classifying preterm and full term groups. PLSDA is a multivariate regression technique where linear combinations of features are used to predict the variance in the dependent categorical variables. The model was then orthogonalized such that Latent Variable 1 (LV1) captured the variance in features that are in the direction of the pairwise separation of the groups, while other latent variables described the variation orthogonal to this predictive component. The LASSO+PLSDA feature reduction and discriminate analysis in a cross validated framework was previously described (28,29). 5-fold cross validation was performed on the data (100 random 5-fold cross validation). To assess model significance, permutation test was performed on the cross validated models by randomly shuffling the labels. Networks of features correlated to the LASSO-selected features were then constructed (co-correlate networks). Combinations of these correlates can be considered as possible substitutes for the LASSO-selected features as biomarkers of group separations.

Multi-Level Partial Least Squares Discriminant Analysis (MLPLSDA)

To mathematically identify the key features contributing to the profile differences between maternal and cord blood, MLPLSDA (Westerhuis et al., 2010) model was constructed. MLPLSDA uses the same principles as OPLSDA analysis for multivariate data but also takes advantage of the paired structure of the data (paired maternal and cord blood). Intuitively, this analysis subtracts the effect of inter-pair variability, *i.e.* the heterogeneity between maternal:cord samples and focuses on the effects within maternal:cord samples. The model was constructed using LASSO-selected variables. Variables were centered and scaled to a standard deviation of 1. 5-fold cross validation was performed on the data (Venetian blinds). To assess model significance, a permutation test was performed by randomly shuffling labels.

Construction of the correlation network of the LASSO-selected features

Spearman correlation of only the LASSO-selected Fc features to all original 323 antigen-specific antibody features were calculated. Each node is a feature and the thickness of the edges between nodes were weighted using significant correlation coefficients. The p-value depicting the significance of these correlations were corrected for multiple comparisons (Benjamini-Hochberg q-value < 0.05, testing the hypothesis of zero correlation). Only correlations with corrected p-values < 0.05 and Spearman correlation coefficients > 0.7 were included. Also, only the features directly and significantly correlated with the LASSO-selected features were included in the figure.

Construction of the correlation heatmap

Spearman correlations of maternal and cord antibody measurements were calculated for preterm and full-term maternal-cord dyad separately. Correlation coefficients for IgG subclasses and binding to Fc receptors across 14 antigens were then depicted in a heatmap (Figure 1B). Statistical significance was then calculated without correcting for multiple comparisons.

Declarations

Data availability

Data are available as supplements (see Supplemental Data).

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Informed consent was obtained from all participants, and for neonates from a parent and/or legal guardian. All experimental protocols were approved by Massachusetts General Hospital Institutional Review Board. All methods were carried out in accordance with relevant guidelines and regulations.

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Tables

Table 1A. Background group characteristics

Group	Preterm, n=11	Term, n=12
Birth weight, grams	1104 ± 226 (601-1398)	3655 ± 509 (3055-4432)
Gestational age, week ^{+days}	27 ⁺⁶ (24 ⁺² – 29 ⁺³)	39 ⁺³ (37 ⁺⁰ – 41 ⁺⁶)
male/female (% male)	4/7 (36)	5/7 (42)
Vaginal delivery (%)	6 (55)	8 (67)
Maternal age, years*	32 ± 6 (24-43)	34 ± 6 (26-44)

*Mean ± SD (minimum-maximum)

Table 1B. Sample characteristics

	preterm	term
mother sample, n=43		
week 1	11	12
week 12	0	2
umbilical coord	7	11
baby sample, n=53		
day 0-1	2	0
week 1	9	9
Week 4	10	
week 12	11	12
Children vaccinated before any sample (%)	9 (82)	9 (75)
Rotatec/Rotarix ^a	1	7
Prevenar 13 ^b	8	9
Infanrix hexa ^c	9	9
Synagis ^d	1	0
Varicellon ^e	1	0

^aRota virus

^bPneumococci

^cDiphtheria, tetanus, pertussis, hep B, polio, Hib

^dPalivizumab, immunoglobulins for respiratory synthycial virus

^eImmunoglobulin for Varicella-zoster virus

Figures

Figure 1

The evolution of the humoral response early in life. The heatmap depicts antigen-specific antibody measurements including antibody-dependent cellular phagocytosis (ADCP, for 5 antigens), antibody-mediated neutrophil phagocytosis (ADNP, for 6 antigens), total IgG, IgM, IgA1-2, IgG1-4, FcRn, FcγR3A, 3B, 2A, and 2B levels across 24 antigens (Methods, Table 1). Each column represents an antigen-specific measurement, always plotted in the same order for each Fc-measurement (listed at the bottom of the figure). Each data type was normalized to have zero mean and unit variance. Red, black, and yellow depict high, mean, and low levels, respectively.

Figure 2

Convergence of antibody profiles over time. (A-B) Principal Component Analysis (PCA) was performed to reduce the dimensions of data from 323 to 5 dimensions to visualize the major axes of variation across the whole dataset. Each dot represents a sample. (A) 80% of the variance was captured by PC1, capturing the major variance in the data, picking up the evolution of the humoral profiles over time. The dot color represents the sample origin (maternal, cord, and time after birth). (B) In contrast to PC1, PC5 captures differences in antibody profiles across the preterm and fullterm infants, where the dot color depicts the gestational group of the sample. (C) Each trendline represents the median of each antigen-specific measurement relative to the median of the maternal level of that antigen-specific measurement. These were calculated separately for preterm (pink) and fullterm (green) infants. One trendline is included per antigen per graph. Median tetanus-, diphtheria-, and pertussis-specific (tdap) IgG3 peak at W12 for both pre-term and full-term children, consistent with the Tdap administration between 6-8 weeks in Sweden.

Figure 3

Antigen-specific transfer varies in preterm infants. (A-B) The graphs depict the loading values on PC1 (A) and PC5 (B) of the PCA scores plot from Figure 2. (A) Loadings on PC1 capture the changes over time. Each dot represents an antigen, and dot colors depict different Fc readouts. (B) Loadings on PC5 reflect differences with gestational age. The order of antigens shown from left to right for all Fc measurements with the exception of ADCP and ADNP are: measles, mumps, rubella, pertussis toxin, influenza, VZV, polio, HepA, pneumo, Adeno T5, Adeno T40, Norovirus, Parvo, VP2, tetanus, Diphtheria, HSV1, HSV2, CMV, EBV, RSV, Human Histone H3, nArah2, nBosd8, nBetv1. Antigen specificities from left to right for ADCP: RSV, pneumo, pertussis toxin, rubella, influenza. Antigen-specificities for ADNP from left to right: rubella, pertussis toxin, influenza, pneumo, CMV, RSV. (C) ML-PLSDA depicts the antigen-specific differences across fullterm mother:cord pairs. The model was orthogonalized such that the separation of maternal and cord antibody profiles was captured on Latent Variable 1 (LV1), capturing 83% of the Y-variation. Conversely, LV2 captures the variability in the antibody features that do not contribute to the separation (Y var = 0%). (D) The bar graph depicts the VIP scores for loadings on LV1. Only the top contributors to the separation with VIP score>1 are shown, all of which are higher in cord blood. A 5-fold cross validation (CV) of the model resulted in CV accuracy of 85%. This model outperformed 93% of label-shuffled random models (Wilcoxon p=0.07). (E) The ML-PLSDA scores plot depicts differences in antibody profiles in mother:cord preterm pairs. In this orthogonalized model, the separation of preterm maternal and cord antibody profiles was captured on LV1, capturing 73% of the Y variation. LV2 captures 0% of Y var variation. (F) VIP scores for loadings on LV1 (VIP scores>1) highlight higher antigen-specific antibody levels in maternal serum. A 5-fold cross validation of the model resulted in a CV accuracy of 69%. The model outperformed 85% of models based on randomly label-shuffled inputs (Wilcoxon p=0.15).

Figure 4

Antigen-specific antibody differences across gestational ages normalize over time but may point to immune vulnerabilities. (A) OPLSDA shows the separation between preterm (PT: pink) and fullterm (FT: green) cord blood. Each dot represents an individual sample. The orthogonalized approach ensured that Latent Variable 1 (LV1) captured the separation between PT and FT antibody profiles while simultaneously capturing 93% of the Y-variation, while LV2 captured the antibody profile variances that do not contribute to this separation. 5-fold cross validation was performed, resulting in 100% Cross Validation (CV) accuracy. Permutation test was performed by randomly shuffling the labels. Our model performed significantly better than random (Wilcoxon $p = 0.0007$). (B) The ordered VIP scores on LV1 for all the LASSO selected features are directed (and colored) based on whether the features are enriched in fullterm (green, right) or preterm (pink, left) infants. (C) The network represents antibody features that are highly correlated with the LASSO-selected features across cord samples. (D-E) OPLSDA model shows the differences in antibody profiles between preterm and fullterm infants at week 1, resulting in 95% CV accuracy and permutation test Wilcoxon $p=0.005$. LV1 captured the separation of PT and FT, 92% of the Y-variation. Bar graph shows VIP scores for loadings on LV1 similar to (B). (F) The network represents features that are highly correlated with the LASSO-selected features across week 1 samples. (G-H) OPLSDA model separates preterm and fullterm 12-week infants, resulting in 92% CV accuracy and permutation test Wilcoxon $p=0.006$. LV1 captured 74% of Y-variation. The VIP scores plot highlights fewer differences between the groups. (I) The network represents antibody features that are highly correlated with the LASSO-selected features across 12-week infants. In networks of (C), (F), (I), edges depict correlation coefficients after removing correlation coefficients <0.7 and p -values >0.05 (corrected for multiple comparisons using Benjamini-Hochberg method). The red color of the edges represents positive correlation.

Figure 5

Preferential and early transfer of functional antibodies. (A-B) Transfer ratios (cord/maternal) per person-antigen are shown in pink and green for preterm and full-term newborns, respectively. Each dot represents the transfer ratio of an antigen-specific measurement for a maternal:cord pair plotted based on gestational age. The ratios where maternal levels were below PBS levels as well as measurements with transfer ratios >7 were excluded from the analysis for more stable results. Linear regression lines are overlaid in blue. The regression coefficients for total IgG, IgG1, IgG2, IgG3, IgG4, FcRn, FcR3A, FcR3B, FcR2A and FcR2B were calculated to equal 0.04, 0.07, 0.06, 0.02, 0.06, 0.09, 0.05, 0.05, 0.04, and 0.03, respectively. Additionally, logistic regression was performed and the probability of transfer >1 was calculated and plotted in dashed black curves (right y axis). (C) The individual probabilities for each Fc-readout was plotted separately and overlaid for comparison. The likelihood of transfer ratio ≥ 1 surpasses the likelihood of transfer ratio <1 was marked on the graph with hatched lines for each Fc-readout.

Figure 6

Functional antibodies transfer early in pregnancy (A-E) The dot plots depict the univariate level of (A) ADCP, (B) ADNP, (C) antibody mediated NK cell degranulation (NK CD107a), (D) antibody driven NK cell IFN γ secretion, (E) antibody dependent NK cell chemokine MIP1 β secretion across Pertussis-, Influenza-, and RSV-specific antigens. (F) The dot plots show the transfer ratios by gestational age, with each dot representing a different antigen and sample set organized by gestational groups (preterm-PT: pink and fullterm-FT: green). Fewer antigens were included for the functional profiling due to sample limitations. Regression coefficients were estimated as 0.08, 0.10, 0.01, -1.41, and 0.08 for ADCP, ADNP, NK MIP1 β , IFN γ , and CD107a expression, respectively. Moreover, the likelihood of transfer ratios surpassing the likelihood of transfer ratio <1 were calculated at gestational week 36, 41, 63, 36 and 24, respectively. (G) The individual probabilities for ADCP, ADNP, and NK CD107a were plotted and overlaid for comparison. The probability curves for NK IFN γ and MIP1 β secretion were excluded since the fitted nominal multinomial logistic regression model was not reliably parameterized. The likelihood of transfer ratio ≥ 1 surpasses the likelihood of transfer ratio <1 was marked on the graph with hatched lines.

Figure 7

Similar antigen-specific decay patterns occur following birth in preterm and fullterm infants. Separate ML-PLSDA models were constructed for preterm and fullterm infants using LASSO-selected features to examine the evolution of antibody profile changes over time in the mother:cord (A,B), across cord:week 1 (C,D), and between week 1 and week 12 (E,F). (A) The ML-PLSDA scores plot model

separating maternal and cord antibody profiles in full-term neonates is shown on top. The model captures 100% of the Y-variation. The bar plot depicts the VIP scores for loadings on LV1, which are signed and colored to reflect which group they are enriched in. The dashed line marks the threshold of VIP score=1 above which are the top contributors to the separation. A 5-fold cross validation of (CV) the model resulted in CV accuracy of 100%. This model significantly outperformed label-shuffled random models (Wilcoxon $p=2 \times 10^{-5}$). (B) The ML-PLSDA scores plot shows the separation in maternal and cord antibody profiles in preterm children. The bottom bar plot illustrates the VIP scores for loadings on LV1. The bars point to the direction of higher abundance, which are dominantly found in the maternal serum. A 5-fold cross validation of the model resulted in a CV accuracy of 100%. The model outperformed models based on randomly label-shuffled inputs (Wilcoxon $p=0.0005$). (C,D) Similar models are shown for cord and week 1 profiles for full-term and preterm infants are depicted in Panels with a CV accuracy of 100% for both models, with permutation test Wilcoxon p-values of 0.0002 and 0.001 for preterm and fullterm infants, respectively. (E-F) The ML-PLSDA models show the week 1:week 12 profiles for fullterm and preterm children with CV accuracy of 100% and Wilcoxon p-values for the permutation testing of 0.0001 for both models. IgM, IgA1, and IgA2 are excluded from all analyses to focus on IgG-specific trends.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [FigS1RplotMCSpearmanCorrbothfinalNoMA.pdf](#)
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