The ontogeny of immune tolerance: a model of the early-life gut microbiome and adaptive immunity

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Abstract

To achieve immune and microbial homeostasis during adulthood, the developing immune system must identify which microbes to tolerate and which to defend against. We synthesize the existing literature to develop a mechanistic mathematical model representing the interplay between gut ecology and adaptive immunity in early life. Our results indicate that the inflammatory tone of the microenvironment is the mediator of information flow from pre- to post-weaning periods, addressing an enduring open question in the field. Our model also allows us to evaluate the power of postnatal fecal samples for predicting immunological trajectories; and to explore breastfeeding scenarios when maternal immunological conditions affect breastmilk composition. Our work establishes a quantitative basis to the concept of 'immune education', offering insights into questions of applied relevance.

Introduction

The global burden of immune-mediated diseases is rapidly growing (*1*). Epidemiological data indicate that early life exposures are key determinants of immune-mediated diseases later in life (*2*), such as allergies (*3*), asthma (*4*), type 1 diabetes (*5*), and inflammatory bowel disease (IBD) (*6*). This impact of early life is primarily attributed to interactions between the microbiome and the immune system during a critical developmental window, which enables hosts to establish tolerance to commensal bacteria (*7*), ensuring the maintenance of immune and microbial homeostasis into adulthood, while appropriately defending against pathogens (*8*, *9*). When this crosstalk is perturbed, pathological imprinting may develop, characterized by excessive immune reactivity and increased susceptibility to inflammatory diseases in adulthood (*10*). Both inherent microbial factors and maternal cues determine how microbe-immune interactions unfold during early life: symbiotic commensals provide metabolic products that establish regulatory pathways facilitating a balanced immune response, whereas pathogens stimulate the immune system to develop defense mechanisms. Breastmilk delivers not only microbes and nutrients, but also antibodies that dictate the timing and nature of bacterial antigen presentation to the infant's developing immune system, establishing a transgenerational cycle of immune priming (*11*, *12*). The collective influence of these processes on immune ontogeny and maturation is encapsulated by the term 'immune education' (*13*).

The information available to tackle the establishment of 'immune education' is now considerable (*14*). To date, thousands of papers have been published, ranging from observational data from human populations to experimental perturbations in animal models. However, experimental

methods are inevitably reductionist, focusing on a limited set of mechanisms, while larger scale descriptive analysis rooted in observational data may illuminate patterns, but ultimately yield verbal descriptions lacking mathematical characterization. The time is ripe to integrate these layers of evidence into a systems biology framework that formally accounts for the multiple potential interacting components.

To this end, we introduce a mathematical framework describing the reciprocal imprinting of the human gut microbiome and the antigen-specific endogenous mucosal Secretory Immunoglobulin A (SIgA) response during the first two years of life as the SIgA response integrates both the B-cell and the T-cell arms of immune ontogeny. It manifests the dual functionality of the gut mucosal immune system by selectively neutralizing pathogens while tolerating commensal bacteria beneficial for immune homeostasis (*15*, *16*). Furthermore, SIgA represents the transgenerational aspect of immune priming as maternal SIgA modulates antigen presentation while simultaneously regulating the gut community composition (*11*, *17*).

Our mechanistic modeling strategy blends flexibility with tractability in reflecting the effects of maternal factors, feeding practices, consumer resource dynamics within microbial communities, and multifunctionality of SIgA; and introduces a quasi-stochastic mathematical model of germinal center reactions embedded in a combination of ordinary and delay differential equations. We parameterize our framework using data from infant fecal samples (*18–21*), formally mapping microbial abundance measurements into expectations for the dynamics of the lumen and the germinal centers of the gut, contexts traditionally obscured by the impracticality of direct sampling (*22*).

Overall, our model brings mathematical formalism to the concept of 'immune education', opening the way to investigating persistent questions in early life immunology, including the power of fecal sampling data in predicting immunological outcomes, and the determinants of transmission of immune information from early infancy to adulthood. Finally, it establishes hypotheses regarding breastfeeding practices guided by maternal immunological profiles.

Results

Model outcomes unveil the dynamics in gut lumen, successfully predicting out-of-sample data

Our model encompasses exogenous inputs alongside endogenous dynamics. The former includes the caloric intake from human milk oligosaccharides (HMOs) and plant-derived polysaccharides (PDPs), and maternal secretory Immunoglobulin A (mSIgA) concentrations in the gut lumen transferred through breastmilk; quantified based on different feeding practices of infants (*23–25*) (Eqns. S1.1.18-S1.1.21). HMOs and PDPs differentially modulate growth rates of different bacterial taxa based on their metabolism (*26–30*) (Eqn. S1.1.4). Timing of endogenous immune system activation is dictated by the maturation of Microfold (M) cells, which serve as the primary route for antigen transport to Peyer's Patches (PPs), the key site for the development of the gut adaptive immune response (*31*). M cells appear shortly before weaning, primarily due to decreasing concentrations of maternal steroids in breastmilk (*32*). In the absence of quantitative data, we use mSIgA as a proxy for steroid levels. M cell maturation translates into antigenic sampling — and thus the start of immune response maturation (Fig. 1A).

To fit our model to fecal microbiome data (*18–21*) and the established timeline of immune ontogeny (*14*, *32*), we leverage two observations: i) the infant develops an SIgA profile resembling their nursing mother's (*19*), and ii) the infant's immune response and microbial community structure stabilize after two years (*33*). Specifically, the matured offspring secretes endogenous antibodies with quantitatively similar affinities to bacterial groups as their nursing mother; and relative abundances of bacterial taxa converge to their equilibrium values in host's fecal samples from day 720. We estimate a single set of affinity values shared between the mother and the matured offspring, reflecting maternal antibodies in the gut lumen before activation and after the stabilization of the host's immune response. For this step of inference, we define a composite optimization criterion that integrates the relative and absolute abundances of key taxonomic groups observed in fecal samples of infants and their IgA indexes (indicating enrichment in the SIgA+ and SIgA− fractions). Optimization provides initial estimates which then guide the inference for the interim phase of endogenous immune system maturation. A second composite optimization criterion is applied to match the average SIgA coating ratio (*34*) in the gut lumen and the matured endogenous affinity values to the maternal ones.

To capture immunological and competitive interactions across ontogeny, we selected four bacterial taxonomic groups: *Enterobacteriaceae* encompasses potentially pathogenic bacteria encountered pre-weaning when the endogenous system is not fully functional. *Bifidobacteriaceae* represents early colonizers with anti-inflammatory properties that regulate the microenvironment and provide colonization resistance. *Bacteroidaceae* and *Clostridiales* are post-weaning bacteria with potentially anti-inflammatory properties that contribute to a balanced gut environment when properly regulated by SIgA (Table S1). Taxa are differentiated by their invasiveness (*35*) - their potential to penetrate colonic epithelial cells - and their capacity to modulate the inflammatory tone of the microenvironment (*36*, *37*) via their metabolic products and epithelial conditioning (*38*).

We characterize SIgA's functionality into two mutually exclusive categories: coating (C), which represents SIgA's non-neutralizing functionality, promoting immune 'exclusion' (preventing commensal bacteria from interacting with the immune system) and intestinal residency (*39*, *40*); and neutralizing (N), contributing to the expulsion of bacteria from the gut (*41*). The coating (C) or neutralizing (N) rates per one unit of SIgA concentration are both modeled as a graduated response to affinity levels (*42*) (Eqn. S1.1.1-S1.1.2). This dual function of SIgA creates three subpopulations in the gut lumen — uncoated (SIgA−), coated (SIgA+ (C)), and neutralized (SIgA+ (N)) — of which only the uncoated and coated are metabolically active. The neutralized subpopulation neither interacts with other subpopulations nor stimulates the immune system.

Our model assumes a common set of mechanisms for endogenous SIgA maturation across all commensals, allowing rates of antibody coating and neutralization to emerge from maturing affinity levels. Our quasi-stochastic formulation of germinal center reactions assumes a temporally dynamic distribution of B-cell receptor (BCR) binding variability that dictates B cell fates; mean and standard deviation of binding variability are adjusted at each proliferation, somatic hypermutation, and selection (P-SHM-S) cycle. Naïve B cells migrate to the PPs, are activated depending on the antigen uptake by M cells (Eqns. S1.1.5-S1.1.7), and start participating in GC reactions (*43*). Each P-SHM-S cycle is informed by the microenvironmental stimulation in the

PPs, and the selection pressure imposed by an implicit model of antigen-specific follicular helper and regulatory T cells (Materials and Methods, *Modeling the role of Germinal Centers*, and Eqn. S1.2.8), eventually determining the size and average affinity of the plasma cell pool. Microenvironmental stimulation is modeled as a composite variable (Eqn. S1.1.8) reflecting the cumulative effects of cytokines and microbial products; akin to the inflammatory tone of the local environment (*44*). We infer mSIgA affinities (Fig. 1B) assuming the nursing mother with a balanced microbiome-immune response transfers coating antibodies for the symbiotic commensals (*Bifidobacteriaceae*, *Bacteroidaceae* and *Clostridiales*); and neutralizing antibodies for the pathogenic taxon *Enterobacteriaceae*. Model fit estimates illustrate the trajectory of microenvironmental stimulation (Fig. 1C), average affinity of the endogenous antibodies converging toward maternal values (Fig. 1D), along with the normalized concentrations of antibody levels (Fig. 1E) proportional to the maturation of plasma cells being imprinted as the host ages.

Bacterial colonization is also affected by the oxygen concentration (O2) in the gut lumen (Eqn. S1.1.4). O2 inhibits the growth of obligate anaerobic bacteria and is consumed by facultative anaerobes (Eqn. S1.2.3) (*45*). From an initially normalized level of 1, O2 degrades at a rate inferred during the model fitting process (Fig. 1C).

The final challenge lies in aligning our model output with longitudinal data from the literature. We use relative bacterial abundance and IgA-Seq data derived from infants' fecal samples (*18–21*) as a proxy for the ecological and immunological dynamics within the gut lumen (*46*); however, equating fecal samples with the gut lumen overlooks SIgA's role in microbial turnover (*39*, *40*). We reconstruct the relative abundances per fecal content by calculating the weighted sum of neutralized, coated, and uncoated subpopulations in the gut lumen using distinct observation rates (Eqns. S1.1.14-S1.1.17) which translates the microbial abundances in the gut lumen to abundances detected in fecal samples, allowing us to align the model output with the 16S rRNA sequencing analysis provided in *Tsukuda et al* (*18*). We apply the same reconstruction using SIgA−, SIgA+(C), and SIgA+(N) subpopulations to compute the IgA indexes for each taxon, allowing us to include the IgA-seq profiles of *Enterobacteriaceae* and *Bifidobacteriaceae* provided in *Planer et al.* (*19*). Our estimates led to an average coating ratio of 37.2%, with negative IgA indexes for *Bifidobacteriaceae*, *Bacteroidaceae*, *Clostridiales*, and a positive IgA index for *Enterobacteriaceae* (Fig. 1F).

Relative abundance estimates for periods of inactivity (neonatal, early postnatal) or stability (after 2 years) of the endogenous immune system demonstrate the reliability of our model fitting procedure (Fig. 1G). Furthermore, out-of-sample data corresponding to the period of maturation—not used for parameter inference (Fig. 1G, gray area)—was also accurately predicted by the model. This alignment provides indirect validation of the underlying assumptions regarding the immune response's impact on microbial community dynamics, lending credibility to the model estimates of the gut lumen (Fig. 1H) for which direct data is unavailable.

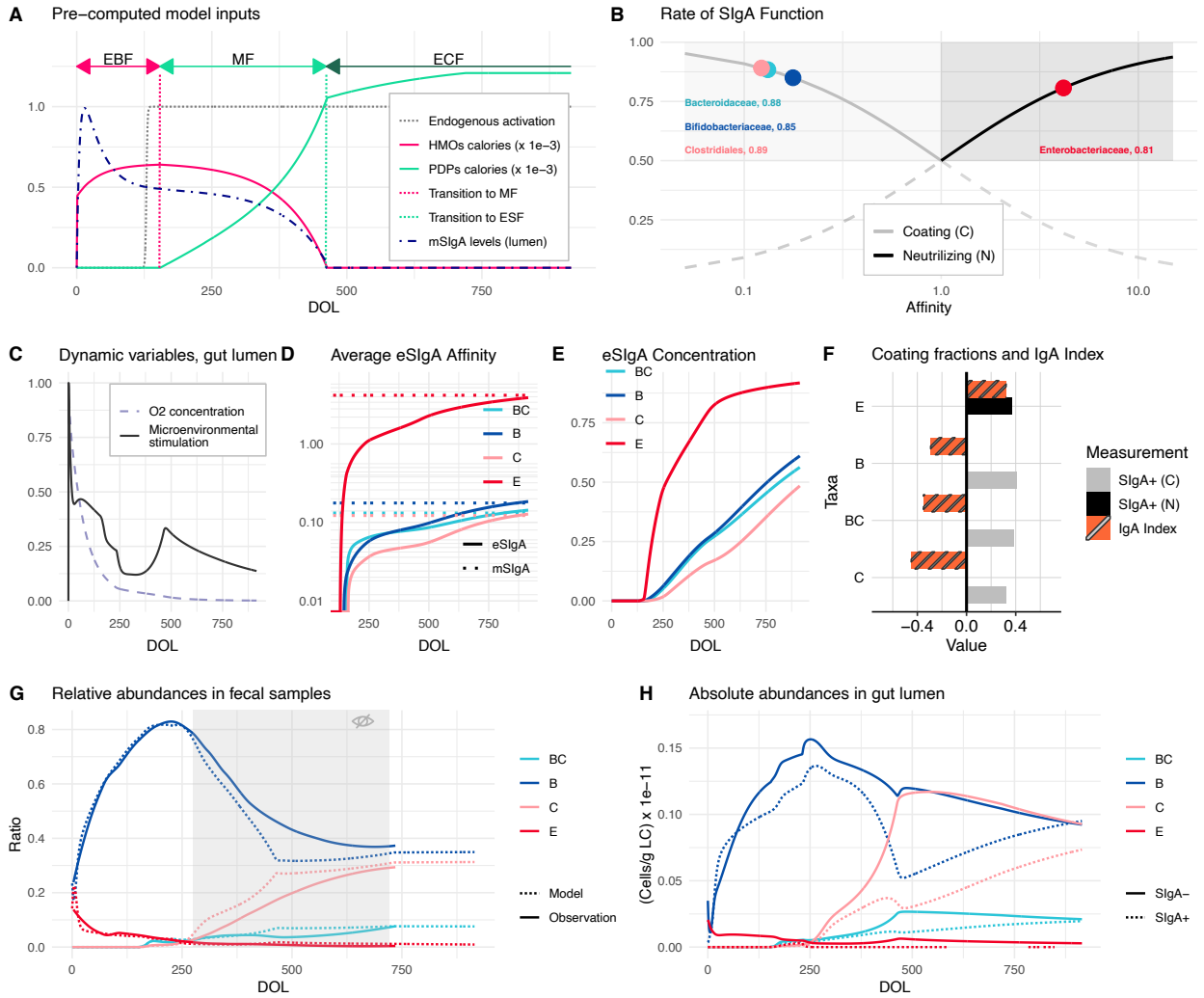

Fig 1. Illustration of Core Mechanisms and Model Fit. (**A**) Model inputs including normalized maternal secretory immunoglobulin A (mSIgA) concentration, human milk oligosaccharide (HMOs) and plant-derived polysaccharides (PDPs) calorie inputs, and timing of the endogenous immune system activation over time. EBM: exclusive breastfeeding; MF: mixed feeding; ECF: exclusive complementary feeding. (**B**) Rate and type of SIgA function relative to the level of antibody affinity, where mSIgA values for key taxa are highlighted. (**C**) Normalized oxygen concentration and microenvironmental stimulation (normalized by its peak value, reflecting the inflammatory tone of the environment) over time. (**D**) Temporal progression of average endogenous SIgA (eSIgA) affinities, (**E**) and the normalized antibody concentrations in the gut lumen for each key taxon. (**F**) Fractions of coated and neutralized bacteria and IgA indexes for each key taxon at DOL 735. (**G**) Relative abundances in fecal samples aligned with observational data, where the gray area highlights the out-of-sample data during parameter inference. (**H**) Absolute abundances in the gut lumen for key taxa distinguished by coating status. Model fit estimates for all parameters with their respective description, unit, and prior distribution are provided in Table S2. DOL: Day of life; E: *Enterobacteriaceae*; B: *Bifidobacteriaceae*; BC: *Bacteroidaceae*; C: *Clostridiales*.

Illustration of the model dynamics are provided in Fig. S1, relative abundance fit estimates aligned with data points are visualized in Fig. S2, and additional assumptions implicit to the model structure are provided in Table S2. A shiny app is available, to allow readers to interact with model parameters and run their own experiments.

IgA-bound Enterobacteriaceae abundance can predict immune phenotypes but only at specific ontogenetic phases

Our ability to develop effective intervention strategies for preventing pathological imprinting hinges on our capacity to accurately predict the trajectory of 'immune education' as the infant gut matures. While there are studies correlating the time course of microbiome composition and IgA-binding patterns with eventual disease susceptibility (*47–49*), the predictive potential of such analyses at different stages of ontogeny remains underexplored. To evaluate the potential of using taxonomic and IgA-seq profiles of fecal samples to predict the trajectory of infant's immune maturation, we systematically explored variation in ecological and immunological outcomes by simulating our model for a total duration of 720 days across a comprehensive range of exclusive breastfeeding (EBF) and mixed feeding (MF) durations. Across 91.1% of all feeding scenarios, the host's gut mucosal immune response matured to tolerate commensals by simultaneously developing coating SIgA against all taxa (Fig. 2A), although the underlying rate of coating varied (Fig. 2B). The infant immune response diverges to a hyperreactive profile—developing neutralizing antibodies against symbiotic commensals rather than coating them—across 3.6%, 9.5%, and 8.4%, of all possible feeding scenarios for *Bifidobacteriaceae*, *Bacteroidaceae*, *Clostridiales* respectively (Fig. 2B), and 8.9% of all possible feeding scenarios for at least one of the commensals (Fig. 2A). These results suggest robust convergence towards a tolerogenic profile for the gut mucosal immune response across a wide spectrum of feeding patterns, in line with previously reported stereotypical convergence of the systemic immune response (*2*).

Using this generated data, we investigated how well the matured immunological status after 2 years can be predicted using the total and SIgA-bound *Enterobacteriaceae* abundance from fecal samples collected at various stages of ontogeny. We fitted three generalized linear models to this binary outcome (tolerant or hyperreactive) including as covariates i) total, ii) SIgA-bound and iii) total and SIgA-bound *Enterobacteriaceae* abundances per day. The third model significantly improved prediction over both the first (deviance reduction 68501, $p < 2.2e-16$) and second model (deviance reduction 58251, $p < 2.2e-16$), confirming that combining total and SIgA-bound abundances yields a significant marginal gain in predicting tolerance versus hyperreactivity. Total *Enterobacteriaceae* abundances had the lowest predictive capacity across time (Fig. 2C, 2E), which can be explained by considering the two compensatory mechanisms regulating it: *i)* ecological competition preventing pathogen overgrowth and *ii)* early activation of the endogenous immune system (earliest at DOL 30) when maternal antibodies are not sufficiently neutralizing. In contrast, SIgA-bound *Enterobacteriaceae* abundance alone during the first 15 days of life provides valuable information regarding the immunological trajectory (Fig. 2D, 2E), as this quantity reflects the efficiency of maternal antibodies (*48*).

Higher levels of SIgA-bound *Enterobacteriaceae* abundance flip from reflecting tolerant to reflecting hyperreactive immunological states over the time course of ontogeny (Fig. 2D). Lower SIgA-bound *Enterobacteriaceae* observed for hyperreactive phenotypes during early phases of

ontogeny are indicative of insufficient *Enterobacteriaceae* neutralization by maternal antibodies. As the host matures, this pattern alters, with the hyperreactive phenotype exhibiting an increase in SIgA-bound *Enterobacteriaceae* abundance relative to the tolerant state, echoing the enrichment in SIgA-coated *Enterobacteriaceae* in adults with IBD-like phenotypes (*50*, *51*). Interestingly, samples collected during the transition from exclusively maternal to exclusively endogenous SIgA presence in the gut lumen provided the least information regarding the immunological trajectory of the host, demonstrating non-monotonic temporal patterns in the predictive value of 16S rRNA and IgA-seq analyses.

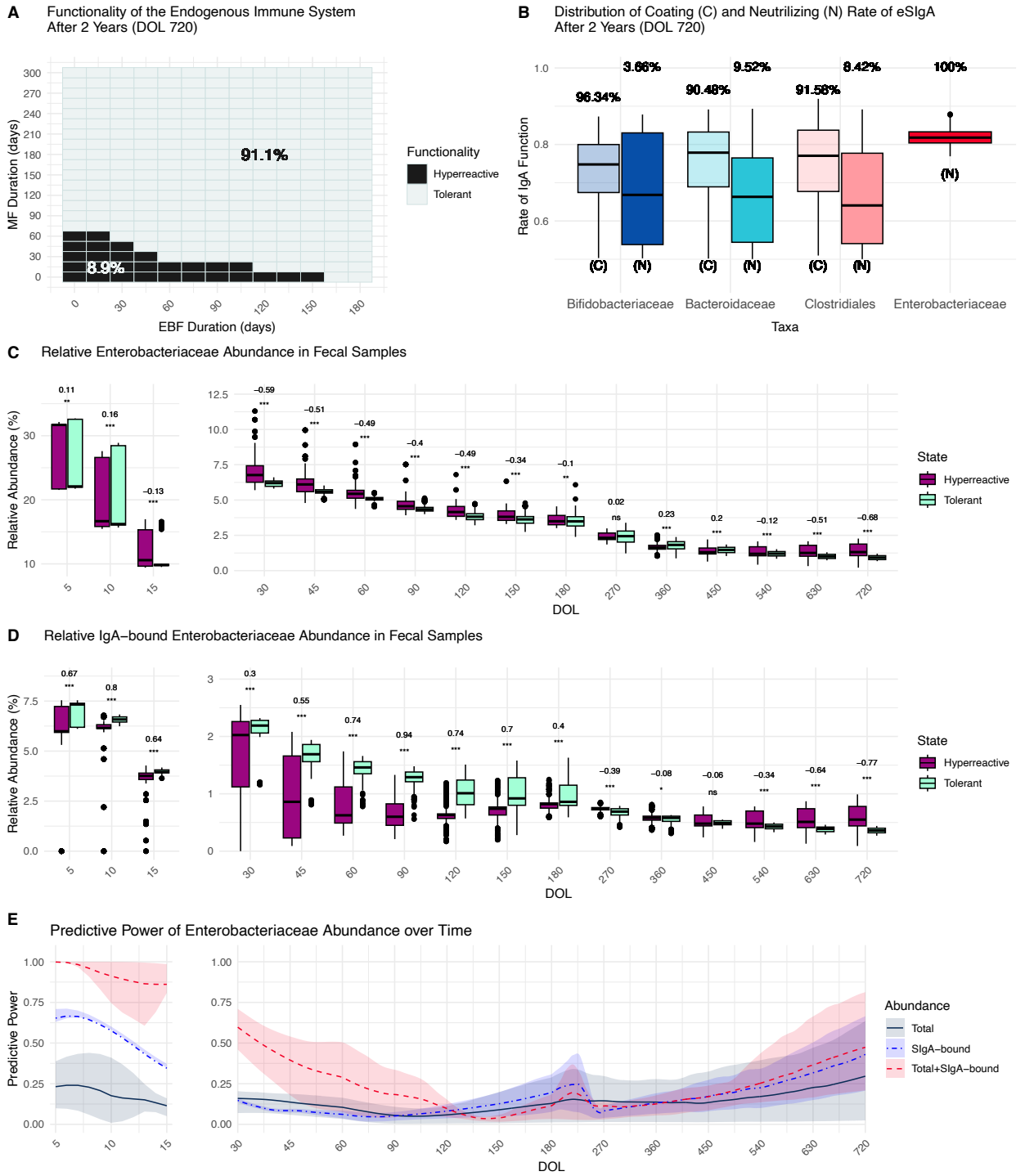

Figure 2. Immunological outcomes and *Enterobacteriaceae* abundances for the first 2 years of life for different combinations of exclusive breastfeeding (EBF) and mixed feeding (MF) durations. (**A**) Immune functionality across a spectrum of EBF and MF durations, where 'tolerant' corresponds to developing non-neutralizing but coating antibodies against all symbiotic commensals; and 'hyperreactive' otherwise. EBF and MF durations vary from 0 to 180 and to 300 days respectively (**B**) Distribution of coating (C) and neutralizing (N) rates of endogenous SIgA at DOL 720. Distribution of (**C**) total and (**D**) SIgA-bound *Enterobacteriaceae* abundances over

the first two years of life segregated by tolerant and hyperreactive states. Dataset is balanced for each time point to reflect the typical sample size of recent cohort studies (*52*). A Wilcoxon rank-sum test is used to calculate the effect size (rank-biserial correlation from -1 to +1; values closer to ±1 indicating stronger associations) and the significance (*** for $p < 0.001$, ** for $p < 0.01$, * for $p < 0.05$, . for $p < 0.1$, and ns for $p \geq 0.1$), displayed at the top of each boxplot. (**E**) Mean predictive power of *Enterobacteriaceae* abundance segregated by data types. Predictive power of 0 indicates no better prediction of a tolerogenic/hyperreactive phenotype than random chance; 1 indicates perfect accuracy. Shaded areas represent the interquartile range covering the 25th to 75th percentiles. DOL: Day of life.

Ecology versus the microenvironment: what carries the information?

An enduring mystery in mucosal immunology is how postnatal influences on the immune system persist into adulthood, even though many core cell types are not present when these initial influences occur (*11*). One way to test whether this persistent influence is also reflected in our model is to investigate the impact of EBF duration on affinity maturation: antigenic sampling is delayed if M cells remain immature as a result of persistently high levels of mSIgA, and this postpones the activation of B and T cells until the end of this period. Thus, we expect the influence of the EBF period – if any – to rapidly fade unless other components of the system have enduring effects, since mSIgA has a strict half-life and the delivery of HMOs ceases instantaneously with the cessation of breastfeeding (Fig. S3).

We first investigate the differential impacts of EBF and MF durations in determining endogenous SIgA (eSIgA) affinity across a comprehensive range of EBF and MF durations. Given the presence of pre-weaning commensals (*Bifidobacteriaceae*, *Enterobacteriaceae*) in the gut lumen during both EBF and MF, eSIgA affinity maturation towards these taxa will be influenced by the duration of both feeding practices. Conversely, a significant role for EBF duration in the affinity maturation against post-weaning commensals (*Bacteroidaceae*, *Clostridiales*) would align with the persistent postnatal influences described above. As hypothesized, immune responses against pre-weaning and post-weaning commensals are impacted by EBF and MF durations differently shown by a predictor importance analysis (Fig 3A), where we compare the relative importance of EBF and MF durations in explaining the variance in eSIgA affinities. Recapitulating the presented dilemma, the influence of EBF duration remains prominent in explaining the variance in eSIgA affinities against post-weaning commensals, despite MF duration being 1.5 times more influential. This influence is more prominent when EBF is followed by Exclusive Complementary Feeding (ECF) without any intervening MF period, which makes it challenging for the host to develop tolerance against post-weaning commensals (Fig. S3B), emphasizing how maternal antibodies play a critical role in ensuring the controlled exposure of novel antigens to the immune system by coating them.

Although mSIgA itself may not persist, its initial modifications may continue to shape both the ecology and the inflammatory tone of the microenvironment; similarly, HMOs may have shaped the ecology by selecting for bacteria based on their metabolic capacity, leading to a sustained temporal influence. Therefore, we compare the impact of pre-weaning commensal abundance and the microenvironmental stimulation on eSIgA affinities for post-weaning commensals to identify which components of our system carry information from the EBF period onward. We employed the Random Forests method to handle correlation between microbial abundances and

microenvironmental stimulation. Intriguingly, our findings reveal a significantly greater influence of the microenvironmental stimulation over taxonomic composition (Fig. 3B). These findings suggest that the conditions and signals within the gut microenvironment—such as epithelial permeability and the tolerogenic bias of follicular T cells which are directly modulated by the microenvironmental stimulation in our model—play a pivotal role in shaping the infant's immune response relative to the presence or abundance of specific bacterial taxa. Moreover, these results indicate that breastfeeding's influence extends beyond immediate transmission of HMOs, antibodies, and microbes, conditioning the gut microenvironment in ways that produce effects outlasting the transient presence of such factors.

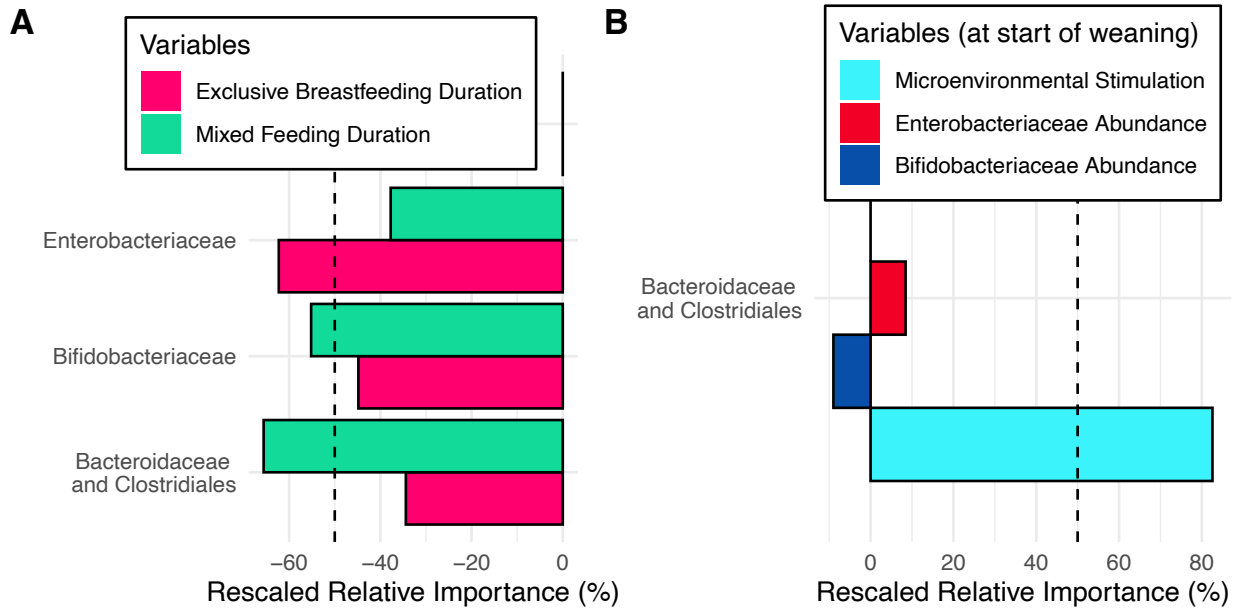

Fig. 3. Impact of different feeding durations and environmental variables in determining the matured eSIgA values for key taxa. (**A**) Relative importance analysis in determining the eSIgA values for key taxa, comparing the impact of EBF and MF durations. (**B**) Relative importance analysis in determining the eSIgA values for post-weaning commensals, comparing the impact of the abundances of pre-weaning commensals and microenvironmental stimulation. Due to the similarity of results for *Bacteroidaceae* and *Clostridiales*, average values are provided in (**A**) and (**B**). Directionality of the importance values are determined using the directionality of the correlation between the outcome variable and the predictors.

Maternal protection against pathogens depends on mother's immunological profile

Despite the well-established health benefits of breastfeeding, it presents a complex decision-making process for mothers experiencing immune dysregulation (*53*). By meticulously eliminating other possibilities such as genetic and epigenetic factors, microbiota variation, and differences in milk-derived metabolites, Ramanan *et al.* demonstrated that the primary maternal factor allowing for transgenerational immune priming in mice is vertical transmission of maternal IgA (*11*). This observation opens up the possibility of transmission of inflammatory phenotypes via maternal IgA, as well as tolerogenic ones. Indeed, evidence suggests that Inflammatory Bowel Disease (IBD) is significantly more prevalent in the offspring of IBD mothers, who tend to produce breastmilk with higher inflammatory potential and lower antibody levels (*54–56*); and inadequate levels of

maternal SIgA to food allergens have been linked to infantile allergic diseases in breastfed infants, showing a stronger correlation with symptoms than parental atopic history (*57*). However, breastmilk's influence goes beyond immune priming and antibody transfer, also enhancing colonization resistance against pathogens through nutritional and microbial support. Therefore, it is not immediately apparent under what circumstances the benefits of breastfeeding outweigh its risk of inflammatory priming.

To address this question, we compare five different feeding scenarios with varying maternal immunological profiles : *i)* control group recapitulating the scenario in Fig. 1, *ii)* excessive targeting of commensal bacteria by maternal antibodies (high affinity mSIgA against Bifidobacteria, *Bacteroidaceae*, and *Clostridiales*), mimicking an IBD-like phenotype (*58*), *iii)* breastmilk devoid of mIgA but supplying HMOs and Bifidobacteria, indicative of a maternal SIgA deficiency, *iv)* exclusive complementary feeding (ECF), with Bifidobacteria introduced at a lower inoculum size and delayed introduction of post-weaning taxa (*Bacteroidaceae* and *Clostridiales*), mimicking a scenario with no breastfeeding, and *v)* ECF with *Bacteroidaceae* and *Clostridiales* transfer from the start, mimicking additional probiotic supplementation. In all scenarios, *Enterobacteriaceae* are introduced at the same inoculum size to assess the balance between pathogenic and symbiotic commensals (Bifidobacteria, *Bacteroidaceae*, and *Clostridiales*) during the first 30 days of life, a critical period when the infant is particularly vulnerable to pathogen overgrowth.

In all scenarios apart from the control, inflammatory phenotypes were transgenerationally transmitted: excessive commensal targeting by mSIgA led to hyperactive endogenous SIgA maturation in the infant, pointing to the importance of SIgA coating in immune imprinting (Fig S4). However, scenarios differed in degree of pathogen control, demonstrating two opposite trends (Fig. 4). ECF with probiotic supplementation (Fig. 4A, 4C; dashed light blue line) and SIgA-deficient breastfeeding (Fig. 4A, 4C; dashed orange line) initially showed an increase in the pathogenic-to-symbiotic commensal ratio followed by a decrease that brought them to a similar level by day 10, echoing their similarity in leading to NEC susceptibility (*48*). SIgA-deficient breastfeeding had an initial advantage suggesting that HMOs and Bifidobacteria can partially compensate for the lack of mSIgA by directly modulating the gut microbiome composition and function. In contrast, ECF without probiotic supplementation (Fig. 4A, 4C; dashed yellow line) demonstrated a ten fold increase in the pathogenic-to-symbiotic commensal ratio, emphasizing the importance of a diverse microbiome in providing defense against pathogens. Interestingly, the worst outcomes were observed with the IBD-like phenotype (Fig. 4 dotted red line), even though it provides neutralizing antibodies against the pathogenic commensal *Enterobacteriaceae*. The transfer of hyperreactive mSIgAs against symbiotic commensals not only led to inflammatory imprinting but allowed for excessive *Enterobacteriaceae* overgrowth by neutralizing Bifidobacteria, leading to significantly higher relative abundances of SIgA-bound *Enterobacteriaceae* in the fecal samples compared to the control group (Fig. 4B).

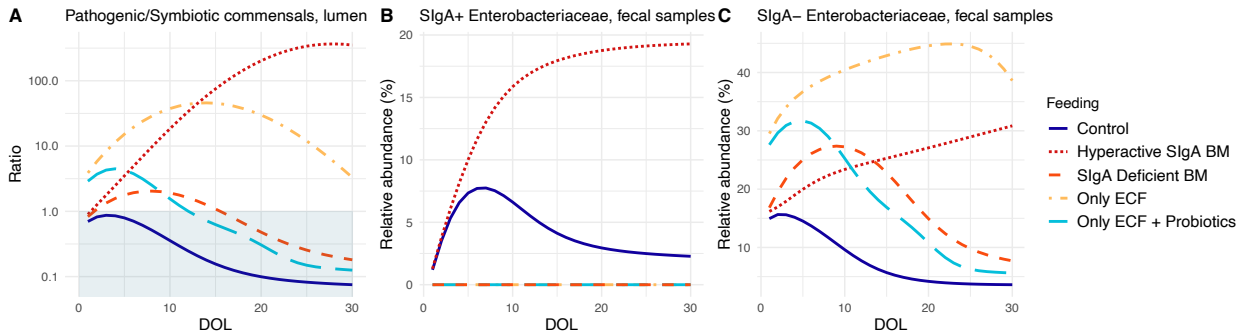

Fig 4. Comparison of various breastfeeding scenarios and their impact on *Enterobacteriaceae* growth. Temporal progression of (**A**) Pathogenic-to-symbiotic commensal (*Enterobacteriaceae* to *Bifidobacteriaceae*, *Bacteroidaceae*, and *Clostridiales*) ratio in the gut lumen, (**B**) relative SIgA-bound (SIgA+) *Enterobacteriaceae* abundance in fecal samples, and (**C**) relative SIgA-free (SIgA-) *Enterobacteriaceae* abundance in fecal samples of the infant for the first 30 days of life (DOL) for four different breastfeeding scenarios : Control, breastmilk (BM) with hyperactive mSIgA, mSIgA deficient BM, and only exclusive complementary feeding (ECF).

Discussion

'Immune education' has proved a powerful concept in understanding the long shadow of early life on health outcomes. Yet, to date, the concept has remained largely qualitative, informally encompassing a wide range of individual components. This diversity and complexity of interactions make integrating these effects by experimental methods alone intractable, quite apart from the question of the degree to which animal models translate to humans. We take the first steps towards formalizing the concept of immune education, leveraging the power of mathematical frameworks to robustly integrate known feedback loops connecting the ecology and immunology of the gut, and inferring core parameters using data on early life immunity and microbial community dynamics.

Our analysis adds a predictive dimension to the interpretation of gut microbial composition data. Traditional studies, employing 16S rRNA and IgA-seq techniques on fecal samples, have primarily focused on identifying associations between early-life microbiome compositions and later immune-mediated health outcomes. We add mechanistic understanding as to why and how the IgA coating patterns vary over the course of ontogeny by disentangling the influences of maternal and endogenous antibodies. We show that IgA-seq analysis of fecal samples collected at early and late stages of ontogeny are informative in predicting immunological outcomes (albeit with opposing patterns of SIgA-bound *Enterobacteriaceae* abundances) but are not informative during the transitional period. These insights call for a discerning approach when using fecal samples from specific postnatal periods to predict disease outcomes.

Mixed feeding duration emerges as a critical determinant of the infant's immune trajectory: the sustained presence of maternal antibodies after weaning enables the introduction of novel antigens to the immune system in a non-inflammatory manner, promoting the development of tolerance. Exclusive breastfeeding still plays a role in shaping the immune response against microbes introduced after its cessation, by its remnant effects on microenvironmental stimulation, or inflammatory tone within the gut lumen and PPs. This emergent phenomenon resolves the

perennial question of how information from the early postnatal phase propagates to further stages of immune development: our model indicates that the information is not specifically carried by either symbiotic or pathogenic taxa, but, rather, results from the cumulative capacity of their SIgA-coated and uncoated subpopulations to modulate the inflammatory tone of the microenvironment.

Beyond these fundamental insights, our analysis sheds light on applied questions including how to approach breastfeeding when maternal immunological conditions such as allergies, IBD, or SIgA deficiency affect breastmilk composition. Both lack of SIgA in breastmilk, or excessive targeting of symbiotic commensals by SIgA can lead to inflammatory imprinting in the infant, indicating that maternal SIgA is the main mechanism driving the transgenerational transmission of immunological phenotypes. Although transfer of *Bifidobacteriaceae* and HMOs cannot entirely compensate for maternal SIgA's role in tolerogenic imprinting, it can partially compensate for the absence of SIgA in pathogen control. However, these benefits do not negate the risk of inflammatory imprinting when maternal SIgA is hyperreactive, disrupting colonization resistance by excessive targeting of symbiotic commensals.

This finding, combined with the influence of microenvironmental stimulation on endogenous SIgA affinity maturation has therapeutic implications for ensuring tolerogenic imprinting when maternal immunity is hyperreactive or insufficient. Our results suggest that interventions should focus on strategies to modulate gut inflammation that go beyond probiotic supplementation. Further strategies might include targeting Toll-like receptors such as TLR4, which has been proposed to tackle preterm birth and fetal inflammatory injury (*59*). Preliminary results from our model support the effectiveness of this intervention (Fig. S5), suggesting that tolerance can be induced by reducing the inflammatory potential of Gram-negative commensals, similar to the effects observed with TLR4 antagonists. However, a complete analysis of such a scenario would necessitate expanding our model to explicitly incorporate cellular immunity.

Our blend of mechanistic and semi-mechanistic modeling formally merging multiple data sources to recapitulate out of sample immunological and ecological trajectories represents a first step towards an integrated model of 'immune education'. Model complexity was tailored to the range of data currently available: expanding data availability will open the way to further validation and model extension, particularly at the intersection between cellular and humoral immunity. Our quantitative analysis yields both fundamental insights into pressing questions in the field and has therapeutic implications relevant in the context of a growing global burden of immune-mediated diseases.

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Supplementary Materials
Materials and Methods
Supplementary Text
Figs. S1 to S5
Tables S1 to S3
References (60–124)