Breastmilk IgG engages the murine neonatal immune system to instruct responses to gut antigens

Meera K. Shenoy[1,6], Diane M. Rico[1†], Alina K. Lorant[1†], Hamadoun Touré[1], Shannon Gordon[1‡], Luke J. Milburn[1], Jeanette S. Schwensen[2,4,5], Madelyn E. Cabán[1,3], Meghan A. Koch[1,3*]

[1]Basic Sciences Division, Fred Hutchinson Cancer Center; Seattle, WA, 98109, USA.
[2]Translational Science and Therapeutics Division, Fred Hutchinson Cancer Center; Seattle, WA, 98109, USA.
[3]Department of Immunology, University of Washington; Seattle, WA, 98195, USA.
[4]Molecular and Cellular Biology Graduate Program, University of Washington; Seattle, WA, 98195, USA.
[5]Medical Scientist Training Program, University of Washington; Seattle, WA, 98195, USA.
[6]Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR); 8a Biomedical Grove, Immunos, Singapore, 138648, Republic of Singapore.

*Corresponding author. mkoch@fredhutch.org
[†]These authors contributed equally to this work.
[‡]Present address: University of Chicago, Chicago, IL, 60637, USA

One sentence abstract: Ingestion of breastmilk IgG during a discrete postnatal window tunes neonatal immunity to the gut microbiota.

Abstract

Maternal antibodies fundamentally regulate gut immunity in the developing infant, yet the mechanisms underlying this process remain elusive. We found that maternal IgG, ingested in the first week of life, restrained microbiota-dependent adaptive immune responses weeks later, following weaning. This activity was linked to maternal antibodies that could bind bacteria in the neonatal gut and the ability of microbe-IgG complexes to engage Fc and complement-dependent effector functions in offspring. Ingestion of microbiota-specific maternal IgG also limited aberrant neonatal responses to dietary antigens encountered at weaning. These discoveries suggest that maternal IgG engages the immune system of offspring in early postnatal life to modulate mucosal responses and reinforce intestinal homeostasis in the face of dynamic shifts in food and bacterial antigens during development.

Introduction

A fundamental function of the immune system is to promote mutualism between the host and resident microbiota. In the gut, this process enables the host to harness numerous benefits from microbes, including expanded nutrient assimilation, immune education, and resistance to pathogen colonization, while mitigating risks such as toxin exposure, chronic inflammation, and pathobiont invasion (*1-3*). In early life, establishing immune tolerance toward the microbiota while avoiding inappropriate inflammation is complicated by the dynamic nature of the neonatal period—marked by rapid changes in microbiota and food antigen composition. Studies linking perturbations in infant microbiota exposure or assembly with increased risk of metabolic and/or inflammatory diseases underscore the importance of this process (*4-8*). Ingested immediately post-birth, breastmilk is poised to regulate nascent host-microbiota interactions. Along with nutrients, breastmilk provides offspring with live bacteria, maternal immune cells, and immunomodulatory molecules, including cytokines and immunoglobulins (*9, 10*).

Multiple antibody isotypes, including IgA and IgG, are readily detectable in breastmilk, though the relative abundance of these isotypes differs based on species. For example, murine milk contains proportionately higher levels of IgG compared to that of humans (*11*). Pioneering work by Kramer and Cebra revealed that young mice reared by dams lacking B and T cells exhibit increased T cell-dependent germinal center (GC) reactions in gut-associated lymphoid tissues, including Peyer's patches (PP) and mesenteric lymph nodes (mLN), indicating that maternal antibodies regulate homeostatic gut immunity (*12*). In subsequent studies, we solidified the importance of breastmilk antibodies (rather than maternal T cells) in this process by using mothers with a targeted disruption of the mu heavy chain gene ($\mu MT^{-/-}$). These mice lack mature B cells and are deficient in antibodies. Wild-type C57Bl/6 (B6) offspring reared by $\mu MT^{-/-}$ dams exhibit similar immune dysregulation, characterized by heightened GC T follicular helper (Tfh) and GC B cell frequencies and numbers in the mLN and PP (*13*). This response is transient, with elevated Tfh responses peaking around postnatal days 25-26 (p25-26) and normalizing to wild-type levels at later time points. Additional studies in humans and mice have shown that breastmilk antibodies readily bind infant gut microbes and can help shape the assembly of the microbiome, promote barrier function, restrain inflammatory responses to commensal microbes, and guide the abundance of mucosal T cell subsets (*9, 10, 12-16*).

The prevailing model of immune instruction mediated by maternal antibodies is largely based on studies of IgA-directed regulation of enteric pathogens and certain gut commensal strains in adult mice (*17-23*). However, this model does not account for substantial differences in gut physiology, such as increased barrier permeability in early life, altered microbiota composition, and the presence of other antibody isotypes - such as breastmilk-derived IgG - in the infant gut (*13, 24-27*). As such, the mechanisms underlying antibody function at the maternal-offspring interface remain unclear. Here, we developed a murine model enabling precise control over the timing and composition of breastmilk antibodies, the microbiota, and the infant immune system to delineate the mechanisms by which breastmilk antibodies tune offspring immunity to newly encountered mucosal antigens.

Results

Early-life acquisition of breastmilk antibodies restrains mucosal adaptive immune responses following weaning.

Mucosal immunity is heavily influenced by the microbiota, which can vary substantially between different housing conditions and physical locations (*28, 29*). Thus, to confirm that Tfh-driven immune dysregulation is a common feature of breastmilk antibody deficiency, we assessed responses in mice maintained at the Fred Hutchinson Cancer Center in Seattle, WA. Mirroring our prior findings with neonates housed at UC Berkeley, Berkeley, CA (*13*), we found that the acquisition of breastmilk antibodies during the entire pre-weaning period, from birth to 21 days

(p0-p21), prevented mucosal immune dysregulation. Four days after weaning (p25), the offspring of $\mu MT^{-/-}$ dams and B6 sires had elevated Tfh numbers and frequencies in the mLN (marked as $CD4^+Foxp3^-CD44^+PD\text{-}1^+CXCR5^{hi}Bcl6^+$) (**Fig S1A**), compared to control pups of B-cell sufficient, $\mu MT^{+/-}$ dams (**Figs 1A, B**). These differences were also observed in the PP and corresponded with elevated GC B cell responses, as distinguished by the number and frequency of $CD19^+IgD^{lo}B220^+PNA^+GL7^+$ cells (**Figs S1B – D**). We performed similar experiments with a distinct model of B cell deficiency by using $Jh^{-/-}$ dams, which are completely devoid of mature B cells and antibodies due to a targeted deletion of JH gene segments. Further, to rule out strain-specific effects in maternal antibody function, we used $Jh^{-/-}$ on the Balb/c background, which is genetically distinct from the C56Bl/6 background of $\mu MT^{-/-}$ mice. We observed similar immune dysregulation in p25 offspring of B cell-deficient $Jh^{-/-}$ dams and wild-type (WT) sires (**Figs S1E, F**). Having established that heightened mLN Tfh cell abundance was consistently observed in neonates lacking maternal antibodies, we focused on this response as a 'readout' to decipher the mechanisms involved in breastmilk antibody function.

Establishing intestinal homeostasis relies on a series of coordinated events occurring during distinct developmental phases, such as microbiota seeding and diversification, innate immune hyporesponsiveness, and controlled transport of luminal antigens (*30*). To determine whether breastmilk antibody transfer during a specific postnatal window was required to regulate mucosal immunity, we cross-fostered the offspring of $\mu MT^{+/-}$ and $\mu MT^{-/-}$ dams and B6 sires at different ages (**Fig 1A**). The microbiome can vary markedly between non-cohoused, non-littermate animals, driving intestinal alterations that are independent of genetically encoded immune factors, such as maternal antibodies (*31*). Thus, to maximally homogenize the microbiota between birth mothers, offspring, and foster mothers, littermate, cohoused $\mu MT^{+/-}$ and $\mu MT^{-/-}$ dams were only separated briefly, first for breeding with sires and then again before parturition and through offspring weaning. Progeny of $\mu MT^{+/-}$ dams fostered to antibody-deficient $\mu MT^{-/-}$ dams after the first week of life (p7 or later) harbored reduced frequencies and numbers of Tfh and GC B cells compared to pups fostered at birth (p0) (**Fig 1C, Figs S1G, H**). Additionally, $\mu MT^{-/-}$ dam-born pups fostered to antibody-sufficient $\mu MT^{+/-}$ dams at p0 did not exhibit immune dysregulation, whereas elevated mucosal Tfh and GC B cell responses were apparent in littermate counterparts fostered after p7 (**Fig 1D, Figs S1G, I**). These findings indicated that antibody ingestion during the first week of life regulates homeostatic immune development in the gut.

Maternal antibodies have been described to promote intestinal barrier function in offspring at weaning (*15*). However, we found no difference in the accumulation of type 3 innate lymphoid cells (ILC3) or the amount of IL-22 in the lamina propria, factors important for maintaining gut barrier function (**Figs S2A – C**). Direct tests of barrier function via gavage of the non-digestible substrate FITC-dextran or a lab strain of E. coli (K-12) also revealed no difference in the translocation of these substances between young maternal antibody-sufficient and -deficient mice (**Figs S2D, E**).

A network of antigen-presenting cells (APCs) directs the differentiation of effector T cells in gut-draining lymphoid tissues. Thus, we considered that differences in mLN APC composition could precede the Tfh immune dysregulation in mice lacking maternal antibodies (*32, 33*). Analysis at p21, prior to the elevated Tfh and GC B cell responses observed in these offspring, revealed only modest alterations in mLN APCs, including type-1 and -2 conventional dendritic cells (cDC1 and cDC2), F4/80+ macrophages, and CX_3CR1^+ mononuclear phagocytes (MNPs; (*34*) **Fig S2F, G**). These findings demonstrated that the absence of maternal antibodies did not impair intestinal barrier function nor broadly alter the distribution of mLN APC subsets.

Kinetics of murine antibody consumption and gut permeability during development

To understand how breastmilk antibodies regulated intestinal homeostasis, we assessed breastmilk consumption and composition over time (**Fig S3A**). Breastmilk intake increased as a function of age (**Fig S3B**), and the total immunoglobulin concentration in milk (the sum of IgG, IgA, and IgM; IgE was not detected) was largely stable during the first two weeks of lactation. Milk from dams 0-7 days postpartum contained more IgG than those sampled later, consisting of approximately 30% IgG1, 30% IgG2b, 35% IgG2c, and 5% IgG3 (**Figs 1E, F**). Extrapolating data on milk intake and antibody concentrations, we estimate pups in our mouse colony consumed 244 μg/day of IgG and 17 μg/day IgA on average in the first week of life (**Fig S3C**).

The gastrointestinal tract undergoes developmentally regulated physiological changes characterized by alterations in antimicrobial peptides, mucus, nutrient transporters, and barrier permeability (*35, 36*). In healthy human infants, gut permeability to small sugars is increased during the first week of life (*37*), and one study reported translocation of IgA dimers (~300 kDa) in term babies orally administered IgA within 24 hours post-birth (*38*). These observations prompted us to assess if the window of breastmilk antibody acquisition required to dampen offspring immunity correlated with gut barrier permeability to ingested IgG. To directly evaluate IgG translocation in the absence of cognate antigens or endogenous maternal- or offspring-derived IgG, we orally administered an isotype-control IgG2b (normalized mg/kg) to μMT$^{-/-}$ progeny of μMT$^{-/-}$ dams. Two hours after treatment, we detected higher levels of (total) serum IgG2b in p0-7 pups compared to older offspring (**Fig 1G**). We observed a similar heightened translocation of ovalbumin-specific IgG1 or 150kDa Fluorescein-conjugated dextran (similar molecular weight to IgG) in young (p0-7) wild-type B6 pups (**Figs 1H, I**), ruling out the possibility that the increase in barrier permeability resulted from developmental defects in μMT$^{-/-}$ animals. Thus, the mouse gut was more permeable to luminal substrates during the first week of life, the same window during which maternal antibodies must be ingested to establish intestinal homeostasis.

Oral IgG can prevent mucosal immune dysregulation in offspring

To define the maternal antibody isotypes required to prevent immune dysregulation, we fractionated IgG from IgA, IgM, and other milk proteins using Protein A columns (**Fig 2A**). We confirmed the efficient isolation of IgG subclasses and overall purity of fractionated milk IgG (>98%), whereas the flow-through (mΔIgG) was enriched for IgA (**Fig 2B**).

We then performed 'add-back' experiments wherein we fed littermate, maternal antibody-deficient offspring fractionated milk antibodies, thus allowing a direct comparison of co-housed littermates born to and reared by the same mother, and avoiding potential confounding variables introduced by maternal microbiota, antibody concentrations, or housing conditions (*31*). We fed maternal antibody-deficient offspring 0.5 μg of milk IgG (mIgG), 1μg milk IgA (mΔIgG, also harboring other proteins), or 1μg BSA (irrelevant protein antigen) daily from p0 to p7 (**Fig 2A**). At p25, neonates fed mIgG exhibited reduced mLN Tfh cell responses compared to pups administered BSA or mΔIgG (**Figs 2C**, **S4A**). Low pregnancy rates from timed breedings and unanticipated pup attrition due to natural behaviors (e.g., cannibalism) made it difficult to generate large numbers of offspring for each experimental group (*39, 40*). Thus, for this experiment and others where obtaining a high *n* was difficult, we show two or more combined replicates. We obtained similar results to mIgG using IgG purified from sera of wild-type C57Bl/6 mice in our mouse colony (sIgG), wherein administration of sIgG, but not serum depleted of IgG (sΔIgG; of which IgM was the dominant isotype), BSA, or PBS, resulted in fewer mLN Tfh cells in p25 progeny of μMT$^{-/-}$ dams and B6 sires (**Figs 2D, E, S4B**).

Although the magnitude of the immune dysregulation varied between litters, mLN Tfh cell responses were consistently lower in pups fed sIgG compared to littermate controls given sΔIgG (**Figs 2F, S4C**). Administration of sIgG to offspring reared by μMT$^{-/-}$ dams did not impact the

accumulation of other differentiated mLN CD4 T cell populations that serve important functions in mucosal immunity, including T-bet^{+} (T helper type 1, Th1) and RORγt^{+} (Th17) effector subsets nor Foxp3^{+} regulatory T cell subsets (*41, 42*) (**Fig S4D, E**).

Oral IgG was also able to dampen mLN Tfh responses using a distinct model of maternal antibody deficiency achieved by breeding B cell-deficient C57/Bl6 Jh$^{-/-}$ dams with B6 sires (**Fig S4F**). The ability of IgG to prevent mucosal immune dysregulation indicated that the absence of antibodies, rather than an unrelated perturbation in the milk or the behavior of B cell-deficient mothers, triggered aberrant intestinal immunity. Dose-response experiments revealed that as little as 1 μg/day sIgG during p0-7 was sufficient to fully restrain mesenteric Tfh cell responses in p25 offspring of μMT$^{-/-}$ dams and B6 sires (**Figs 2G, S4G**). As mouse serum is more plentiful than milk, and milk lipoproteins impede the efficient purification of IgG, we used sIgG for the remaining studies.

The interplay between maternal antibodies, the postnatal microbiome, and homeostatic intestinal immunity

The selective elevation of Tfh cell responses at mucosal (e.g., mLN and PP) but not systemic sites (e.g., spleen (*13*)), prompted us to examine the role of the gut microbiota in driving immune dysregulation in offspring lacking maternal antibodies. We first analyzed 'sterile' mice maintained under germ-free (GF) conditions and thus devoid of gut bacteria. Offspring of GF B6.Jh$^{-/-}$ dams and wild-type sires harbored equivalent, low frequencies and numbers of mucosal Tfh cells, regardless of maternal antibody acquisition (**Figs 3A, S5A**). As a complementary approach, we treated mice with a cocktail of broad-spectrum antibiotics composed of ampicillin, vancomycin, neomycin, and metronidazole (AVNM) immediately prior to the height of the Tfh response (p17-24 (*43*); **S5B**). While AVNM reduced Tfh cell frequencies and numbers in maternal antibody-deficient offspring, this response was still elevated compared to maternal antibody-sufficient progeny that received antibiotics (**Figs S5C – D**). This increase could result from incomplete microbial elimination or changes in microbial composition following AVNM treatment (*43, 44*). Alternatively, it is possible that other mucosal antigens, such as dietary components, contributed to immune dysregulation in mice lacking maternal antibodies. Weaning pups earlier (p18) or later (p25) than the standard of p21 resulted in similar immune dysregulation, indicating that this response was not controlled by the cessation of breastfeeding (**Figs 3B, S5E**). Together, these observations suggested that the microbiota triggered a homeostatic Tfh cell response following weaning and that maternal antibodies controlled the magnitude of this response.

Antibodies are key regulators of gut microbiota diversity and composition (*16, 45-47*), leading us to explore whether alterations in microbiome assembly correlated with immune dysregulation in maternal antibody-deficient offspring. 16S rRNA gene sequencing of ileal and colonic contents (**Fig S6A**) did not reveal substantial differences in the diversity of microbes in each sample (alpha diversity) nor between-group distances (beta diversity) between age-matched offspring of μMT$^{+/-}$ or μMT$^{-/-}$ littermate dams and B6 sires (**Figs S6B – E**). However, the abundance of a small subset of taxa differed between p7 offspring of μMT$^{+/-}$ and μMT$^{-/-}$ dams, and this variation increased with age (**Figs 3C, D** and **S6F – H)**. Thus, the absence of all breastmilk antibodies correlated with minor alterations in the assembly of the postnatal microbiome. Analysis of p20 progeny of μMT$^{-/-}$ dams and B6 sires fed sIgG or BSA during the first week of life similarly failed to reveal differences in alpha or beta diversity as a function of feeding (**Figs 3E,** and **S7A – C**). Instead, litter was a driving factor (**Figs 3F, S7D**). Indeed, the provision of IgG resulted in the differential abundance of only a single ileal taxon and two colonic taxa (**Figs 3G, S7E**), which were distinct from those identified in p21 offspring of μMT$^{+/-}$ and μMT$^{-/-}$ dams. Taken together with results from littermate mice in **Fig 2F** and **Fig S4C**, these data demonstrated that the ability of early-life IgG to regulate neonatal immunity was robust to natural microbiome variation between

litters. Additionally, the similarity in microbiota community structure between IgG- and BSA-fed littermates indicates that breastmilk IgG could regulate neonatal immunity without altering the composition of the developing microbiome.

Early life IgG-microbe immune complexes prevent intestinal immune dysregulation

The acquisition of maternal antibodies via breastfeeding, rather than placental transfer, was required to prevent Tfh-driven immune dysregulation (**Fig 1B – D** and (*13*)). Serum analysis of p7 offspring of $\mu MT^{+/-}$ or $\mu MT^{-/-}$ dams and B6 sires cross-fostered at birth revealed that mice receive a substantial amount of maternal IgG via breastfeeding as opposed to during gestation (**Figs 4A, S8A**). We found that maternal antibody-deficient pups fed sIgG harbored even lower serum IgG titers compared to offspring that only received maternal antibodies *in utero* (**Figs 4A, S8A**). As feeding pups sIgG was sufficient to reduce mucosal Tfh responses, these findings rule out the possibility that the immune dysregulation observed in pups nursed by antibody-deficient dams resulted from insufficient amounts of circulating IgG and indicated that the route of acquisition (i.e., oral) underlies the immunoregulatory function of maternal IgG.

These observations prompted us to hypothesize that maternal IgG must bind to neonatal gut microbes to regulate mucosal immunity. We assessed the antibody binding profiles of milk and serum using microbiota-flow cytometry (mFLOW, (*13*) **FigS8B**). To avoid pre-coating of infant bacteria by maternal antibodies, we used gut contents from $\mu MT^{-/-}$ pups (p6-8). IgG antibodies from either milk or serum bound a substantial fraction of the neonatal microbiota (**Figs 4B – D**). Though all IgG subclasses were detectable in breastmilk, the IgG response to pup-derived microbiota in both milk and serum was largely composed of IgG2b and IgG3 subclasses **(Fig 4E**, **S8C)**, consistent with our previous report using serum and microbiota from adult animals (*13*). When matched for concentration, milk IgG tended to bind a larger fraction of infant gut bacteria, while paired serum IgG bound a larger proportion of bacteria derived from 3-month-old $\mu MT^{-/-}$ adult mice (**Fig S8D**). Additionally, we confirmed that fractionated IgG used for feeding studies retained the ability to bind to neonatal gut microbes (**Fig S8E**).

We queried whether efficient binding to intestinal microbes was required for oral IgG to prevent neonatal mucosal immune dysregulation. Using serum IgG from germ-free mice (GF-sIgG), which harbor reduced titers of microbiota-reactive antibodies, including to the infant microbiota (**Fig 4F** (*13, 48*)), we fed progeny of $\mu MT^{-/-}$ dams and B6 sires 1 μg/day GF-sIgG, SPF-sIgG, or BSA between p0-7. Though normalized by concentration, GF-sIgG was unable to restrain mucosal immune dysregulation, as evidenced by the heightened mLN Tfh responses in these animals compared to pups fed SPF-sIgG (**Figs 4G, Fig S8F**).

Since IgG from germ-free mice retained some ability to bind the microbiota, it is possible that higher amounts of GF sIgG would be sufficient to limit subsequent T cell responses. Thus, we attempted complementary experiments by administering commercially available 'non-specific' monoclonal antibodies. We identified IgG1 and IgG2a (allelic variant of IgG2c) clones that did not react to the infant microbiota; however, all IgG2b and IgG3 clones we tested exhibited some amount of binding (**Fig S8G**). The commensal-reactive IgG antibody response is largely comprised of IgG2b and IgG3 responses (**Fig 4E**). As IgG subclass composition is a core determinant of antibody function (*49, 50*), we opted to administer a cocktail of IgG antibodies containing non-specific IgG1 and IgG2a as well as IgG2b and IgG3 clones that demonstrated low microbiota binding. Compared with mice given sIgG, mucosal Tfh responses tended to be higher in pups given the IgG isotype cocktail; yet this difference was not statistically significant (**Fig S8 H, I**). This partial effect could be ascribed to the non-negligible, microbiota binding by IgG2b and

IgG3 antibodies in the cocktail. Nevertheless, the equivalent immune dysregulation in offspring administered BSA or the isotype cocktail suggested that antigen binding in the postnatal gut is important for breastmilk IgG to shape mucosal T cell responses in offspring.

We investigated whether antibody-bacteria immune complexes were sufficient to prevent immune dysregulation in pups that did not receive breastmilk antibodies. To recapitulate the homeostatic T-independent, anti-commensal IgG responses observed in mice (*13*), we immunized mice genetically deficient in all T cell subsets (TCR$\beta\delta^{-/-}$) with *E. coli* strain BL21 (BL21). While not substantially altering the distribution of serum IgG subclasses, immunization elicited a modest increase in IgG1, IgG2c, and IgG2b and a substantial increase in IgG3 titers (**Figs S9A, B**). Flow cytometry analysis of antibody binding to bacterial cells (bFLOW; (*13, 51*) **Fig S9C**) revealed that the BL21-specific response was dominated by IgG2b and IgG3 isotypes, which stably bound to BL21 for up to 1 week (**Figs S9D, E**). Notably, immunization did not elicit BL21-specific IgG1, whereas a slight IgG2c response was generated. Importantly, serum from antibody-deficient $\mu MT^{-/-}$ and naive TCR$\beta\delta^{-/-}$ mice exhibited similar IgG binding profiles, demonstrating that naïve TCR$\beta\delta^{-/-}$ mice in our colony did not harbor 'natural' IgG capable of binding this strain (**Figs S9D, E**).

Like administration of sIgG, feeding pups BL21 coated or 'complexed' with IgG from immune mice (Immune-BL21) during the first week of life reduced mucosal Tfh responses in maternal antibody-deficient offspring at p25 (**Figs 4H, I** and **Fig S9F**). In contrast, Tfh numbers and frequencies remained elevated in mice given BL21 incubated with sera from naïve mice (naïve-BL21). As IgG from naïve TCR$\beta\delta^{-/-}$ mice did not bind to BL21 (**Figs S9D, E**), these data indicated that early life encounter of IgG-bacteria immune complexes was sufficient to restrain T cell immune dysregulation following weaning. Mucosal Tfh responses tended to be lower in pups given polyclonal sIgG compared to those given BL21-IgG immune complexes, perhaps reflecting that breastmilk IgG must bind multiple types of infant gut bacteria to maximally dampen immune dysregulation (**Figs 4I** and **Fig S9F**). Collectively, these data indicate that maternal IgG binds to and forms immune complexes with microbes in the infant gut to tune neonatal immunity.

Maternal IgG engages neonatal IgG sensors to restrain microbiota-dependent adaptive immunity.

The Fc domain of mouse IgG immune complexes allows them to shape immune responses by triggering IgG-sensing systems, including binding Fc receptors (FcRs) and activating complement (*52*). Analysis of wild-type pups at p6 revealed broad expression of FcRs and complement receptors by gut myeloid cells (**Figs 5A, S10A – D**). To define the molecular basis by which maternal IgG restrained neonatal immune dysregulation, we fostered Fc$R\gamma^{-/-}$ offspring, which lack the common Fc$R\gamma$ chain required for the expression and signaling of activating FcRs (FcγRI, FcγRIII, and FcγRIV (*53*)), to antibody-deficient $\mu MT^{-/-}$ dams at p0 and fed them sIgG or BSA daily for the first week of life (**Fig 5B**). Administration of sIgG decreased mLN Tfh frequency and trended toward reduced numbers in p25 Fc$R\gamma^{-/-}$ offspring (**Figs 5C, S10E**). IgG also activates complement via C1q, and oral administration of sIgG triggered elevated intestinal C5a levels, a downstream product of complement activation, in infant mice in a C1q-dependent manner **(Fig S10F)**. However, sIgG was able to attenuate mLN Tfh responses in offspring lacking this pathway (C1q$a^{-/-}$ pups; **Figs 5D**, **S10G**).

Since neither IgG-sensing pathway in isolation was necessary for maternal IgG to restrain mucosal Tfh responses, we generated C1q$a^{-/-}$Fc$R\gamma^{-/-}$ mice lacking both pathways. IgG administration to C1q$a^{-/-}$Fc$R\gamma^{-/-}$ offspring fostered to lactating $\mu MT^{-/-}$ dams was unable to prevent adaptive immune dysregulation in these animals, which phenocopied control $\mu MT^{-/-}$-born offspring that did not receive IgG (**Figs 5E**, **S10H**). Thus, IgG sensing by the neonatal immune system was required for maternal IgG to calibrate offspring mucosal immunity.

Early life acquisition of oral IgG broadly regulates infant gut immunity following weaning

The temporal difference between maternal IgG activity, required in the first week of life, and subsequent impact on mucosal immunity following weaning suggested a regulatory mechanism whereby early postnatal immune engagement by maternal IgG served to temper later responses to gut antigens. Consistent with this model, we found that the provision of oral IgG in early life protected mice from experimental colitis induced during the weaning transition. Compared with BSA-treated littermates, sIgG supplementation of progeny of $\mu MT^{-/-}$ dams and B6 sires resulted in greater weight gain, longer colon lengths, and reduced inflammatory cytokine levels induced by oral administration of Dextran Sodium Sulfate (DSS) (**Figs S11A – C**).

To further explore this idea of immune instruction, we assessed whether maternal IgG regulated infant adaptive immune responses to newly encountered food antigens using a well-established protocol of dietary ovalbumin (OVA) administration (*54, 55*). During the first week of life, we fed maternal antibody-deficient offspring BSA or purified sIgG isolated from naïve mice that did not harbor OVA-reactive IgG (**Fig S11D**). At weaning, we adoptively transferred naïve OVA-specific CD4 T cells into neonates and fed them dietary OVA (**Fig 5F**). Analysis one week later revealed the expected increase in OVA-specific $Foxp3^+$ Treg cells in the mLN of mice given dietary OVA. This expansion did not differ between pups given IgG or BSA, indicating that the absence of maternal antibodies did not impair peripheral Treg induction to food antigens (**Figs 5G, S11E, F** and (*55*)). Food antigens have been shown to elicit the differentiation of a small subset of Tfh cells, as well as a distinct population of $FR4^+CD73^+CD44^{hi}$ activated cells that lack expression of other key transcription factors such as RORγt and T-bet (termed Th^{lin-} (*56*)). We observed an increased accumulation of OVA-specific Tfh cells, and a concomitant decrease in Th^{lin-} cells in the mLN of OVA-treated pups that did not receive IgG (**Figs 5H – J, S11G, H**). Elevated food-specific Tfh responses also correlated with an increase in intestinal titers of OVA-specific IgE, a key Tfh-dependent mediator of allergic disease, as well as circulating OVA-specific IgG1 titers (**Figs 5K, S11I**). These data demonstrated that oral acquisition of maternal IgG in the first week of life dampens the differentiation of Tfh cell and associated IgE responses to food antigens. As the IgG fed to pups did not bind the dietary antigen used in this model, our results indicated that ingestion of microbiota-reactive, maternal IgG broadly shapes infant adaptive immune responses to newly encountered mucosal antigens during the weaning transition.

Discussion

Breastmilk antibodies are critical for establishing intestinal homeostasis, yet the mechanisms underlying this process remain elusive. Here, we demonstrated that neonatal sensing of breastmilk IgG-microbiota immune complexes in early postnatal life was sufficient to guide subsequent mucosal immune responses following weaning, including dampening Tfh responses to microbiota-derived and dietary antigens and alleviating disease severity during experimental colitis. As our work focused on early life, how breastmilk IgG influences long-term health outcomes remains an open question. Nevertheless, our discovery of a temporal difference between maternal IgG activity in the first week of life and the subsequent impact on intestinal immunity following weaning provides a regulatory mechanism compatible with dynamic and sometimes unpredictable changes in microbiota assembly and food antigen exposure typical of the weaning transition (*57-59*).

The immunoregulatory activity of oral IgG correlated with binding to the infant microbiota. IgG with reduced reactivity to gut microbes failed to completely restrain immune dysregulation, suggesting that microbial recognition is essential. However, future studies using truly non-microbiota-reactive IgG are warranted to confirm this result. Though the milk- and serum-purified IgG used in our studies contained all IgG subclasses, the murine commensal-reactive IgG response is largely composed of IgG2b and IgG3 subclasses (*13, 48*). It is possible that these isotypes are required for breastmilk IgG to dampen immunity, an idea supported by the reduced

mLN Tfh responses observed in offspring fed *E. coli* primarily coated with IgG2b and IgG3. Prior work has indicated that the transfer of maternal IgG1-food antigen complexes via breastfeeding induce food antigen-specific Treg cells and protect offspring from allergic disease (*60*). Whether IgG binding to dietary antigens (i.e., food-IgG complexes) can also broadly limit mucosal T cell responses to other microbiota and dietary-derived antigens remains unknown.

Although maternal IgA also binds offspring microbiota, milk IgA did not restrain adaptive immune responses in maternal antibody-deficient offspring as effectively as IgG. This result may reflect differences in necessary concentration or in microbial targeting breadth, as IgG can target a more expansive proportion of the microbiota compared to IgA (*13*). Additionally, the engagement of IgG-sensing systems in offspring, including activating Fc receptors and complement components, further distinguishes oral IgG from other isotypes abundant in milk. Though activating FcγRs do not bind IgA or IgM, IgM effectively triggers complement. However, previous work indicates that milk IgM alone is unable to prevent mucosal immune dysregulation in murine offspring (*13*). Engagement of IgG sensing systems provides contextual cues to APCs that guide diverse downstream effects such as increasing phagocytosis, expression of costimulatory molecules, and antigen presentation to T cells (*52, 61, 62*). Considering the breadth of FcγR and complement receptor expression by infant gut myeloid populations, future studies investigating how breastmilk IgG shapes the function of these cells to regulate T-dependent mucosal immunity are warranted.

While our feeding approach demonstrated that IgG was sufficient to attenuate immune activation in maternal antibody-deficient pups, our findings do not preclude contributions from other milk isotypes in regulating host-microbiota interactions in the natural setting. Breastmilk IgA can facilitate postnatal microbiota assembly, support intestinal epithelial cell function, and limit microbial translocation and immune activation (*16, 27, 47, 63, 64*). IgM, though less studied, may augment complement-mediated lysis of milk bacteria, thus shaping the composition of microbes that seed the infant gut (*65*). Coupled with our work, these observations suggest that breastmilk antibody isotypes operate through distinct, yet potentially complementary, mechanisms to establish intestinal homeostasis.

IgG is also readily detectable in human breastmilk (*66, 67*), albeit at substantially lower concentrations than in mice. Despite this low abundance, several reports have described functions of human milk IgG in regulating infant immunity at both mucosal and systemic sites (*22, 68-70*). Like mice, healthy humans generate circulating microbiota-reactive IgG antibodies (*66, 67*); yet their transfer via breastfeeding and function in guiding mucosal immunity remain unknown. Moreover, humans encode a receptor for IgA (Fc alpha receptor; FcaR1 (*71*)), which may allow breastmilk IgA to promote intestinal homeostasis via mechanisms parallel to those of mouse IgG. Dissecting the specific features of breastmilk, microbiota-reactive IgG that contribute to immune instruction – and how these features differ from other antibody isotypes and across species – remain important areas of investigation.

Although our work centers on T-dependent responses to dietary antigens and the microbiota, breastmilk IgG may also restrain responses to other gut antigens acquired in early life, including vaccines or enteric pathogens (*72*). Mucosal Tfh cells and associated GC-derived antibodies can shape microbiota composition and function, cross-react with enteric pathogens and dietary antigens, and influence pathological responses in the contexts of barrier breach, cancer, and inflammatory disease (*24, 64, 73-75*). Notably, a substantial proportion of the adult intestinal B cell pool in mice originates from precursors activated during the weaning period, suggesting that perturbations to B cell responses during this window could have durable impacts (*76*). The exaggerated Tfh and GC responses in mice not receiving oral IgG could serve as a compensatory mechanism to restore intestinal homeostasis or, conversely, contribute to adverse health outcomes in certain contexts. For instance, heightened generation of food-specific IgE in the absence of oral IgG could increase susceptibility to allergic diseases among non-breastfed individuals (*77, 78*).

Our findings reveal a function of maternal IgG in directing tolerogenic mucosal immunity responses through neonatal recognition of IgG-microbe immune complexes, expanding the scope of maternal IgG beyond its traditional function in providing passive defense to infants against virulent microorganisms (*23*). Further understanding the maternal-offspring interactions that shape immune education will advance strategies to promote beneficial responses to innocuous microbes and environmental antigens and prevent pathological responses throughout life.

Materials and Methods

Mice Specific pathogen-free (SPF) C57Bl/6J (Jax000664), μMT$^{-/-}$ (B6.129S2-*Ighm*^{tm1Cgn}/J, Jax002288), *C1qa*$^{-/-}$ (B6(Cg)-*C1qa*$^{tm1d(EUCOMM)Wtsi}$/TennJ, Jax031675), and TCRβδ$^{-/-}$, (B6.129P2-*Tcrb*^{tm1Mom} *Tcrd*^{tm1Mom}/J, Jax002122) mice were purchased from the Jackson Laboratory. Jh$^{-/-}$ mice on the Balb/c background were purchased from Taconic (1147). *Fcrg*$^{-/-}$ mice generated using C57Bl/6 embryonic stem cells by Dr. Takashi Saito were obtained from a material transfer agreement (RIKEN). Germ-free C57Bl/6 mice were purchased from Taconic (Tac-B6) and germ-free and SPF colonized C57Bl/6 Jh$^{-/-}$ mice were provided by Dr. Andrew Macpherson (Bern, Switzerland).

All mice were bred and maintained at an American Association for the Accreditation of Laboratory Animal Care (AAALAC)-accredited animal facility at the Fred Hutchinson Cancer Center (SPF animals) or UC Berkeley (germ-free animals) and housed in accordance with the procedures outlined in the *Guide for the Care and Use of Laboratory Animals*. All experiments with mice were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee at the Fred Hutchinson Cancer Center (protocol # 51030) or UC Berkeley (protocol # 2015-02-7222-3). All mice were maintained under a 12-h light-dark cycle (7 a.m. to 7 p.m.) and given a standard chow diet (Teklad 2918) and neutral water (pH 6.5-7.5) *ad libitum* unless otherwise indicated.

For cohousing experiments, mice were combined at 3–4 weeks of age until breeding age. For breeding of littermate or cohoused dams, females were separated into individual cages and bred for 2–3 nights. Dams were then “re-cohoused” for approximately 18 days. Pregnant females were separated and housed individually until parturition. Age-matched offspring of both sexes were used in experiments. In some cases, pups were tattooed at birth using a 25G needle (Ketchum).

Tissue processing

Mice were euthanized with CO_2, and the mLN and PP were collected and placed into 0.5mL cold complete media (RPMI 1640 supplemented with 2mM L-glutamine, 1mM sodium pyruvate, 100U/ml penicillin, 100mg/mL streptomycin; all from Gibco) supplemented with 5% or 3% (v/v) Fetal Calf Serum (FCS; Cytiva)) in a 6 well plate. Lymphoid tissues were digested by adding 5mL complete media (without FCS) containing 0.5mg/mL DNAseI (Sigma-Aldrich) and 1mg/mL Collagenase III (Stem Cell Technologies) to each well and incubating plates for 20-30min at 37°C. Small intestinal tissues were cut into 1inch pieces, placed into 5mM EDTA (Sigma-Aldrich) with 0.15mg/mL DTT (Sigma-Aldrich) in complete media, and stirred at 37°C. Small intestine tissue suspensions were strained through kitchen strainers (Amazon.com) and shaken for 30 seconds by hand in 5mM EDTA in complete media three times to remove epithelial cells, then digested by incubating tissues with 0.5mg/mL DNAseI and 0.2 mg/mL Liberase Blendzyme TL (Millipore Sigma) for 30min at 37°C. The enzymatic reaction was stopped with the addition of 10 ml cold complete media containing 3% FCS and tissues were passed through 70 μm (mLN, small intestine) or 100 μm (PP) cell strainers. The rubber end of 1mL syringe plungers were used to gently mash remaining tissue pieces through the filter, after which the filter was rinsed with

complete media to enhance cell recovery. Cells were pelleted at 475x*g*, the supernatant was aspirated, and the pellet was resuspended in complete media containing 3% FCS prior to downstream analysis. The pellet from small intestine prep was resuspended in 37.5% Percoll (Cytivia), centrifuged at 690x*g* for 20 min without brake. The cell pellet was washed once in complete media containing 3% FCS, cells were then pelleted at 475x*g*, the supernatant was aspirated, and the pellet was resuspended in complete media containing 3% FCS prior to downstream analysis.

Flow cytometry

For microbiota flow cytometry, intestinal contents were dissected out, placed in 1mL sterile PBS in a microfuge tube and vortexed vigorously. Contents were centrifuged at 200x*g* and supernatant containing bacteria transferred to a separate microfuge. Bacteria were pelleted at 3210x*g*, rinsed with sterile PBS, pelleted again at 3210x*g* and OD_{600} was measured. Bacteria were resuspended at approximately 5×10^7 bacteria/mL in sterile-filtered Bacterial Staining Buffer (BSB; PBS with 1% bovine serum albumin (BSA; Fisher) and 0.05% sodium azide (Sigma-Aldrich)). Bacterial concentration was estimated as an OD of 1 = $5x10^8$ bacteria/mL. 25 μL of diluted serum or breastmilk antibodies was mixed with 25μL microbial suspension in a v-bottom plate and incubated for 30min at 4°C. 100μl of BSB was added to each well, and cells were pelleted by centrifugation at 3210*g* for 5min. Secondary staining with fluorochrome-conjugated or biotinylated antibodies diluted in BSB followed by streptavidin-PE-Cy7 diluted in BSB was performed (20min each at 4°C). Cells were centrifuged at 3210*g* for 5min and resuspended in sterile-filtered PBS with SYBR Green (Invitrogen) and analyzed by FACS. A similar protocol was used for bacterial flow cytometry, except that an overnight culture of *E. coli* (BL21) was used as a source of microbes, rather than intestinal contents, and resuspended at 10 x 10^6 /mL., then plated at 2.5 x 10^5/ well. See **Table S1** for a list of antibody clones/dyes and concentrations used.

For flow cytometry of leukocytes, approximately 2-5x10^6 cells were incubated in 40 μl of fixable viability dye (Tonbo Biosciences) diluted in PBS. This and all subsequent steps were performed in the dark at room temperature unless otherwise stated. After 15 minutes, 10 μl of Fc block diluted in FACS buffer (PBS supplemented with 2% (v/v) FCS and 2mM EDTA; Sigma-Aldrich) was added to cells. After 5 minutes, 50 μl of the extracellular antibody stain diluted in FACS buffer was added. After 30min, 100μl FACS buffer was added to each well, cells were centrifuged at 475*g* for 5min, and fixed and permeabilized using the Foxp3/Transcription Factor Staining Buffer Set (ThermoFisher Scientific) for 20min at 4°C. Cells were centrifuged at 685*g* for 5min, and 100μl of the intracellular antibody stain diluted in Perm Wash (ThermoFisher Scientific) was added. After 30-45min at 4°C, cells were washed with 150μl PermWash, centrifuged at 685*g* for 5min, and resuspended in 200 μl of PermWash. Analysis was performed no later than 18 hours post-staining using a BD FACSymphony A5 High-Parameter Cell Analyzer. Beads (UltraComp eBeads, Invitrogen) stained with individual antibodies were used for compensation, except for the viability dye control, wherein compensation was performed with leftover cells incubated with the viability dye alone. See **Table S1** for a list of antibody clones/dyes and concentrations used. To obtain cell counts, a separate aliquot of cells was mixed with beads (Accucheck Counting Beads, Invitrogen) and diluted in 4',6-Diamidino-2-Phenylindole (DAPI, Sigma Aldrich) and analyzed immediately on a BD FACSymphony A5 High-Parameter Cell Analyzer or BD Canto. All data were analyzed in FlowJo, version 10.

Dietary OVA administration

$CD4^+$ T cells were isolated by magnetic bead separation (Miltenyi; L3T4). Mice were given 1-10x10^5 CD45.1 OTII CD4+ T cells via retro-orbital injection between p16-p18. The next day, animals were given drinking water containing 1.5mg/mL OVA (grade III) until p25/26. For some experiments, the day after OTII injection, 50 mg of OVA (grade III; A5378; Sigma) was

administered in 200 µl PBS by oral gavage. Animals were harvested on p25/p26 or p40-45 for analysis of T cell and antibody responses, respectively.

DSS-induced colitis

Offspring were cohoused at p20 and provided drinking water with 2% DSS (w/v) (molecular weight 36-50 kDa; Thermo-Fisher Scientific) for 7 days. Body weight and physical appearance were monitored approximately every other day until time of sacrifice. Mice that appeared moribund or experienced ≥25% weight loss were euthanized.

Measurement of Intestinal Permeability

Mice were given 100mg/kg FITC-dextran (4kDa or 150kDa, Sigma), 10mg/kg monoclonal IgG2b (Bioxcel), or 5mg/kg monoclonal ReadyTag anti-ova IgG1 (Bioxcell) diluted in PBS via pipet feeding (p0-13) or oral gavage (p14-18). As a positive control, some animals were given substances i.v. 2 hours later, blood was isolated by decapitation (p0-10) or cardiac puncture (p10-28) and allowed to clot for 15min at room temperature. Samples were centrifuged at 13,000*g* for 15min and serum collected. For FITC-dextran, serum was diluted 1:5 in PBS and measured with excitation and emission wavelengths of 435 and 538nm, respectively, using an Epoch microplate reader (Biotek). Serum IgG2b and anti-OVA IgG1 titers were assessed via ELISA. Fluorescence or antibody levels were interpolated from a standard curve generated via nonlinear 4 parametric logistic regression (Prism, GraphPad).

For bacterial gavage experiments, mice were administered 1-10x10^{10} *E.coli* K12 MG155 containing a plasmid encoding for ampicillin resistance (pTrc-HisC; Invitrogen #V36020) in 150µL PBS via oral gavage at p21. Mice were gavaged with metal animal feeding needles, 22 gauge, 1" (Cadence Scientific). *E. coli K12* cultures were grown overnight in Luria-Bertani (LB) broth (Research Products International) at 37°C, cultures were centrifuged at 4000xg and washed with PBS twice, and OD was attained by spectrophotometer. OD600 = 1.0 was estimated as 1x10^{8} bacteria/mL and cfu was confirmed by plate count. 16-18 hours post-gavage, animals were euthanized, mLN collected into 2mL microtubes containing 0.5mL PBS, and homogenized (Polytron). 250uL of homogenate was plated on LB agar per mouse, plates were incubated at 37°C, and colonies were counted 18-36 hours later. Plate counts were multiplied by 2 to estimate cfu/mLN.

Mouse antibody feeding experiments

Pups were fed daily between p0-p7 (8 days) of life with up to 10µg purified antibodies diluted in PBS, with the exception of the IgG titration feed (Fig 2G, Fig S4F). Pups were administered up to 2µl of diluted antibodies per day via pipet. For isotype cocktail experiments (Fig S8F-H**)**, each pup was given a cocktail comprised of 1µg of IgG1 (BioXcell, RT0267), 5µg of IgG2a (BioXcell, BP0085), 3µg of IgG2b (Sydlabs, PA007130), and 1µg of IgG3 (Biolegend, 401329), reflecting the distribution of subclasses observed in Protein A-purified sIgG (i.e, 10%IgG1, 50%IgG2b, 30%IgG2a/c, 10%IgG3 as per Fig 2D).

Enzyme-linked immunosorbent assay (ELISA)

Approximately 3 cm of the terminal ileum was homogenized at 100 mg/mL in PBS containing protease inhibitor (Roche). Serum, milk, or homogenized ileal tissue was centrifuged 13,000*g* for 10min and supernatant was used to assay immunoglobulin levels or complement proteins by ELISA. Briefly, Nunc Hi Affinity ELISA plates (Thermo Scientific) were coated with isotype-specific antibodies or 50ug/ml OVA (grade VI, Sigma A2512) overnight at 4°C or C5a coating antibody overnight at room temperature. C5a ELISA was performed by following the manufacturer instructions (Biotechne DY2150) using Duoset reagents kit (DY008B). For isotype and OVA-specific ELISA, plates were washed 5X with Wash Buffer (PBS containing 1%BSA (w/v; Fisher)

and 0.05% Tween-20 (v/v; Fisher)) and blocked with PBS with 1% BSA (w/v) and 2% goat serum diluted in PBS (Gibco; v/v) for 1hour at 22-25°C. Plates were washed 5X with Wash Buffer, serial dilutions of samples diluted in PBS with 1% BSA were added, and plates were incubated for up to 2hr at 22-25°C. Plates were washed 5X with Wash Buffer and peroxidase-conjugated secondary antibodies specific to mouse isotypes and diluted in PBS containing 1% BSA were added. After up to 2hr at 22-25°C, plates were washed 5X with Wash Buffer, and developed using 1-Step Turbo TMB (Thermo Fisher) followed by 2N H_2SO_4 Stop solution. Absorbance at 450nm was measured on an Epoch microplate reader (Biotek) within 10min of adding Stop solution. See **Table S1** for a list of antibody clones and concentrations used. Antibody titers were interpolated from a standard curve generated via nonlinear 4 parametric logistic regression (Prism, GraphPad).

IL-22 Detection

Approximately 3cm of the terminal ileum was homogenized at 100 mg/mL in PBS containing protease inhibitor (Roche). Homogenate was centrifuged at 13,000*g* for 10min and supernatant was used to assay IL-22 levels via Bio-Plex Mouse IL-22 kit (Biorad) according to the manufacturer's instructions.

Antibody purification from serum and milk

For milk collection, dams were separated from pups for 2 hours at 3 -18 days post-parturition and anesthetized via nose-cone with isoflurane. Dams were injected intraperitoneally with 250uL of 2 IU oxytocin diluted in 1mL PBS (Thermo-Fisher Scientific), and milk expression was encouraged with hand massage. Milk was collected using microhematocrit capillary tubes, transferred to a microfuge tube and stored at -80°C for subsequent analysis.

For serum collection from adult mice, blood was collected via saphenous vein bleed or cardiac puncture of mice at the time of euthanasia. For neonates, blood was collected after decapitation. To collect serum, blood was allowed to clot at room temperature for up to 60min, centrifuged at 10°C, 13,000*g* for 15min, and the serum supernatant was transferred to a separate microfuge tube and kept at -80°C for subsequent analysis.

Pooled serum or milk antibodies were purified over a Protein A column according to the manufacturer's instructions with minor modifications as described below (Pierce; Thermo Scientific). For milk antibodies, milk was thawed on ice, diluted 1:2 in PBS, centrifuged at 21,000*g* for 15min, and the top (fat) layer was removed by aspiration. The milk supernatant was transferred to a new tube, diluted a subsequent time 1:2 in PBS and centrifuged again at 21,000*g* for 15min. The supernatant (minus fat layer) was used for Protein A fractionation. To ensure recovery of all IgG isotypes, including IgG3, IgG was eluted via 2 sequential washes in Elution Buffer pH=2.8, followed by 1 wash with Elution Buffer modified to pH=4.5. IgG was neutralized immediately following each elution and all fractions were combined. IgG-enriched and -depleted protein fractions were concentrated using a 100,000 MWCO protein concentrator (Pierce; Thermo Scientific). Antibody purity and concentration were assessed by isotype and/or subclass specific ELISA (e.g., IgA, IgG1, IgG2b, etc) and nanodrop protein absorption.

Milk consumption

Pups were fasted for 4 hours by separating them from lactating dams. Pups were then weighed, returned to dams to nurse for one hour, and weighed a second time. Latching was confirmed by visual confirmation and milk consumption was estimated by the difference in weight gain before and after 1 hour of suckling. To maintain pup health and encourage suckling upon return to dam, pups were kept on a heating pad during the fasting period and maintained in a darkened hood. We note that as weight loss due to urination and defecation were not accounted for, reported values may slightly underestimate total milk consumption.

Antibiotic administration

Microbiota depletion was performed as described (*43*). Briefly, animals were gavaged once per day with a cocktail of antibiotics consisting of ampicillin (20mg/kg; Fisher), vancomycin (10mg/kg; Sigma-Aldrich), neomycin (20mg/kg; ThermoFisher), and metronidazole (20mg/kg; Alfa Aesar) between p17-24. Microbiota depletion was confirmed via stool culture on Luria-Bertani and blood agar plates, as well as visual confirmation of enlarged cecums.

Immune complex generation and administration

Serum was collected from TCR$\beta\delta^{-/-}$ mice four weeks after i.v. injection of $1x10^6$ *E.coli* (BL21, Thermo Fisher Cat no. C600003) or PBS, and incubated with BL21 overnight at 4°C at a ratio of 1ul serum per $1.25x10^6$ bacteria in 50μl PBS. Bacteria were washed in BSB to remove unbound antibodies, resuspended at $5x10^6$ cfu/μl, and kept at 4°C for 8 days. Immune complex formation and stability was confirmed using bacterial flow cytometry on the first and last day of treatment.

Pups were fed daily between p0-p7 of life with $5x10^7$ bacteria pre-incubated with sera from immune or naïve animals.

16S rRNA Gene Sequencing and Microbial Community Analysis

Amplification and MiSeq Illumina Sequencing of the V4 region of the 16S gene was performed by the Alkek Center for Metagenomics and Microbiome Research (CMMR) at Baylor College of Medicine. Reads were demultiplexed, trimmed and quality checked with bbduk (*79*). Paired-end reads were then merged using bbmap (*79*) and run through the MaLiAmPi workflow to generate Amplicon Sequence Variants (ASVs) using DADA2 (*80, 81*). The Bayesian classifier Pplacer (*82*) was used to estimate taxonomic classification of ASVs using phylogenetic placement with a likelihood cutoff of 90%. This analysis method does not use strict sequence similarity, which is often not sufficient to distinguish between closely related reference sequences with differing taxonomic assignments. Sequences that could not be assigned unambiguously to a single genus were instead assigned at a higher taxonomic level. The degree of taxonomic resolution provided by 16S amplicon sequencing is generally reported in the field at the genus level, with any lower-level results (i.e. species) generally viewed with a level of skepticism. This conservative approach led us to report genus level results in our analysis.

Alpha diversity estimates were generated by MaLiAmPi and Bray-Curtis dissimilarity was calculated using the R package *vegan* (v2.6-4) (DOI:10.32614/CRAN.package.vegan (*83*)), to determine beta diversity estimates. A pseudocount was applied prior to calculating differences in the relative abundance of taxonomic groups and ASVs with fewer than 10 reads across all samples were removed from the dataset. Differential abundance was determined using DESeq2 (*84*), and data was normalized using the “poscounts” size estimator.

Statistics

Statistical significance was determined as indicated in the figure legends with Prism 10 (GraphPad Software Inc). Significance of beta diversity comparisons was determined using PERMANOVA, which was conducted using the adonis2 function in the R package *vegan*.

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Author contributions:

Conceptualization: MKS, MAK

Formal Analysis: MKS, DMR, MAK

Methodology: MKS, MAK

Investigation: MKS, DMR, AKL, HT, SG, LJM, JSS, MEC, MAK

Visualization: MKS, DMR, MAK

Funding acquisition: MKS, MAK

Project administration: MKS, MAK

Writing – original draft: MKS, MAK

Writing – review & editing: MKS, AKL, HT, SG, JSS, MEC, MAK

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Supplementary Materials List

Figures S1-S11

Table S1

Figure Legends

Figure 1. Acquisition of breastmilk antibodies in early life prevents immune dysregulation following weaning. (**A**) (Top Schema) Timed breedings were performed using co-housed, littermate μMT$^{+/-}$ and μMT$^{-/-}$ dams, and B6 sires. Offspring were maintained with their birth mother or cross-fostered at postnatal day 0, 7, or 10, and analyzed at postnatal day 25 (p25). (Bottom) Representative flow cytometric analysis of germinal center T follicular helper cells (GC Tfh cells, CD4^{+}Foxp3^{-}CD44^{hi}PD-1^{+}CXCR5^{hi}Bcl6^{+}) isolated from the mesenteric lymph nodes (mLN) of p25 offspring born to and reared by the indicated dams. Numbers in flow plots indicate percentage of Tfh cells of total CD4^{+} T cells. Cells were gated according to the schematic in Fig S1A (**B**) Proportions (top) and numbers (bottom) of mLN GC Tfh cells in p25 offspring born to and reared by the indicated dams. (**C**) Proportions (left) and numbers (right) of mLN GC Tfh cells in p25 offspring born to μMT$^{+/-}$ dams and fostered to μMT$^{-/-}$ dams at the indicated postnatal timepoints. (**D**) Similar to (C) comparing μMT$^{-/-}$ born offspring fostered to μMT$^{+/-}$ dams. (**E**, **F**) IgA, IgM, and IgG titers (**E**) and IgG subclass distribution (**F**) in breastmilk from wild type (WT) B6 dams sampled at the indicated time periods post-parturition. All dams were 12-16 weeks of age. (**G**) Serum IgG2b levels in μMT$^{-/-}$ pups of the indicated age 2 hours post-gavage with 10mg/kg monoclonal mouse IgG2b antibodies. Lower and upper dotted lines indicate detected IgG2b levels in mice gavaged with PBS or injected intravenously (i.v.) with IgG2b, respectively. (**H**) Serum FITC-dextran (FITC-dex) titers in WT pups of the indicated age 2 hours post-gavage with 10mg/kg FITC-dex (150kDa). Lower and upper dotted lines indicate detected FITC-dex levels in mice gavaged with PBS or injected i.v. with FITC-dex, respectively. (**I**) Serum ovalbumin (OVA)-specific IgG1 levels in WT pups of the indicated age 2 hours post-gavage with 10mg/kg monoclonal mouse anti-OVA IgG1 antibodies. Lower and upper dotted lines indicate detected OVA-specific IgG1 levels in mice gavaged with PBS or injected i.v. with anti-OVA IgG1, respectively.
Error bars indicate the mean ± standard deviation (SD); symbols represent individual mice. Data are representative of three to four independent experiments with ≥ 5 mice per group. Statistical significance was determined using one-way ANOVA and Tukey post-hoc tests, except (**B** and **G** to **I**), wherein unpaired two-tailed Student's *t* test's were used.

Figure 2. Oral IgG is sufficient to restrain Tfh immune dysregulation in offspring lacking maternal antibodies. (**A**) Milk IgG was fractionated from other proteins using Protein A columns; fractions were concentrated, then fed to offspring of μMT$^{-/-}$ dams and B6 sires during the first week of life. Control pups were fed BSA. (**B**) Immunoglobulin distribution in milk (input) and following Protein A fractionation into purified milk (m)IgG and eluate (mΔIgG) components. Milk was isolated from B6 dams at 12-16 weeks of age. (**C**) Proportions of mLN GC Tfh cells in p25 offspring fed 0.5μg mIgG, 1μg mIgA (+ other proteins), or 1μg BSA daily from p0-p7. (**D**) Immunoglobulin distribution in serum (input) and following Protein A fractionation into purified serum (s)IgG, and eluate (sΔIgG). Serum was isolated from wild-type mice at 10-16 weeks of age. (**E**) Proportions of mLN GC Tfh cells in p25 offspring of μMT$^{-/-}$ dams and B6 sires fed 10μg serum-purified IgG (sIgG), IgG-depleted serum (sΔIgG), PBS, or BSA daily during p0-p7 as indicated. (**F**), Similar to (E), comparing proportions of mLN GC Tfh cells in littermate, co-housed pups given 10μg sIgG or sΔIgG between p0-p7. Littermate animals are indicated by symbols, and lines connect the mean of co-housed littermates. (**G**) Similar to (E), comparing p25 mLN GC Tfh frequencies in offspring of μMT$^{+/-}$ dams or of μMT$^{-/-}$ dams fed the indicated concentrations of sIgG or BSA between p0-p7.
For (**B**), milk was pooled from approximately two dozen dams, each with ≥4 pups aged between 4-10 days. Pooled milk was separated by Protein A chromatography, and the antibody composition was determined via ELISA. The indicated fractions from pooled milk were used for all independent experiments. Data are representative of three to four independent experiments with ≥ 3 mice per group, except for **C** and **E**, wherein data are combined from two to four replicate

experiments and representative of four or more independent experiments. Lines on bar graphs indicate the mean of each replicate, and symbols represent individual mice. For (**F**), error bars indicate the mean of each litter, and diagonal lines connect the means of co-housed littermates. For (**G**), error bars indicate the mean ± SD; symbols represent individual mice. Statistical significance was determined using two-way ANOVA with Sidak post-hoc tests for (**C**) and (**E**), one-way ANOVA and Tukey post-hoc tests for (**G**), and an unpaired two-tailed Student's *t* test for (**F**).

Figure 3. Impact and assembly of the microbiota in maternal antibody-deficient offspring. (**A**). Proportions of mLN GC Tfh cells in germ-free p25 offspring born to and reared by the indicated B6.Jh$^{-/-}$ or B6 dams bred with the indicated B6.Jh$^{-/-}$ or B6 sires. (**B**) Proportions of mLN GC Tfh cells in p25 offspring born to the indicated dams and weaned on the indicated postnatal days. (**C**) Number of significant differentially abundant taxa (sequence variants) between maternal antibody-sufficient and -deficient offspring isolated from the indicated intestinal sites at the indicated postnatal timepoints. (**D**) Volcano plots of ileal microbiota taxa (sequence variants) at the indicated postnatal timepoints of mice described in (C). The taxonomic families of significantly altered taxa are indicated by symbols/colors. (**E**) Non-metric multidimensional scaling plot displaying the Bray-Curtis distances between ileal bacteria from p20 littermate offspring of μMT$^{-/-}$ dams and B6 sires fed 10 μg/day sIgG or BSA in the first week of life. PERMANOVA analysis: Litter p=0.001, R^2=0.482, F=6.98 and IgG feeding group p=0.929, R^2=0.013, F=0.218. (**F**) Heatmap of genus-level ileal taxa (rows) isolated from individual p20 offspring (columns) described in (E) and ordered by complete linkage clustering. Feed and litter status of each mouse is indicated at the top of the heatmap by colors and symbols, respectively. (**G**) Volcano plot of p20 ileal microbiota species (sequence variants) in mice described in (E). The taxonomic family of the single significantly altered taxa is indicated.
For (**A** to **B**), error bars indicate the mean ± SD; symbols represent individual mice. Data are representative of three independent experiments with ≥ 3 mice per group. Statistical significance was determined using an unpaired two-tailed Student's *t* test (**A**) or one-way ANOVA and Tukey post-hoc tests (**B**). For (**C** to **D**), data are combined from two independent experimental cohorts with samples harvested greater than one year apart, totaling n≥10/group. For (**E** to **G**) data are generated from 3 separate litters with n=9 per group. Symbols in (**E, G**) and columns in (**F**) represent individual mice.

Figure 4. Efficient binding to mucosal antigens is required for early life IgG to restrain mucosal Tfh responses. (**A**) Serum IgG titers of p7 offspring of the indicated dams that were cross-fostered or fed proteins as indicated. (**B** to **D**) Proportion of SYBR+ bacteria bound by IgG as assessed by microbiota flow cytometry (mFLOW). Microbiota was pooled from the small intestine and colon of p6 μMT$^{-/-}$ neonates and incubated with (B, D) sera or (C, D) milk. (B, C) Graphs depict the mean proportion of IgG bound microbiota from 12 independent serum (B) or breastmilk (C) samples. (D) Graphs depict IgG-bound microbiota following incubation with matched sera and milk from two replicate dams. IgG+ SYBR+ bacteria were gated according to the schematic in Fig S8B. (**E**) Proportion of SYBR+ bacteria bound by IgG subclasses following incubation of milk or sera isolated from adult (10+ weeks) mice with small intestinal or colonic microbiota isolated from p6 μMT$^{-/-}$ neonates. (**F**) Similar to (B) but comparing IgG-bound SYBR+ bacteria following incubation with sera of adult (10+ weeks) SPF or germ-free (GF) animals. (**G**) Proportions of mLN GC Tfh cells in p25 offspring of μMT$^{-/-}$ dams and B6 sires fed 1μg sIgG purified from SPF or GF mice, or BSA, as indicated. (**H**) Serum was collected from TCRβδ$^{-/-}$ mice 4 weeks after i.v. injection of BL21 or PBS, and IgG was purified via Protein A fractionation. Timed breedings were performed using co-housed littermate μMT$^{-/-}$ dams and B6 sires, and resulting offspring were fed sIgG, BSA, or BL21 pre-incubated with serum from BL21-immunized (Imm.) or

PBS-immunized (Naïve) mice daily in the first week of life. (**I**) Proportions of mLN GC Tfh cells in p25 offspring of $\mu MT^{-/-}$ dams and B6 sires fed the indicated proteins +/- bacteria.
For (**B** to **F**) Graphs depict the relative median ± SD. For (**A, G**, and **I**) error bars indicate the mean ± SD; symbols represent individual mice. Data are representative of two to four independent experiments with ≥2 mice per group. For **G** and **I**, data are combined from two replicate experiments, representative of three independent experiments, and indicated by symbols. Bars indicate the mean of each replicate experiment, and statistical significance was determined using two-way ANOVA and Holm-Sidak post-hoc tests.

Figure 5. Oral IgG engages Fc-dependent effector functions in the neonate to regulate intestinal homeostasis. (**A**) Median fluorescence intensity (MFI) expression of FcγRII/FcγRIII (CD16/32) by small intestinal cell subsets isolated from WT p7 neonates. Cell subsets comprised epithelial cells ($EpCAM^+CD45^-$) and myeloid $CD45^+B220^-CD90.2^-NK1.1^-SiglecF^-$ populations, including cDC1 ($MHCII^{hi}CD11C^{hi}XCR1^+$), cDC2 ($MHCII^{hi}CD11C^{hi}SIRP\alpha^+$), neutrophils ($Gr\text{-}1^+$), and CX_3CR1^+ MNPs ($MHCII^+CD11c^+CX_3CR1^+Ly6C^{var}$). nd = cell population not detected in the indicated tissue. Cells were gated according to the schematic provided in Fig. S10A (**B**) Timed breedings were performed with $\mu MT^{-/-}$ dams and B6 sires and IgG sensor-deficient dams and sires including $FcR\gamma^{-/-}$, $C1qa^{-/-}$, and $C1qa^{-/-}FcR\gamma^{-/-}$ pairs. At birth, a portion of offspring of $\mu MT^{-/-}$ dams and B6 sires were replaced with age-matched IgG sensor-deficient offspring. All offspring were fed 10μg purified sIgG or BSA daily in the first week of life. (**C**) Proportions of mLN GC Tfh cells in p25 $FcR\gamma^{-/-}$ or $\mu MT^{+/-}$ offspring fed the indicated proteins in the first week of life. (**D**) Similar to (B) comparing $C1qa^{-/-}$ and $\mu MT^{+/-}$ offspring. (**E**) Similar to (B) comparing $C1qa^{-/-}FcR\gamma^{-/-}$ and $\mu MT^{+/-}$ offspring. (**F**) Offspring of $\mu MT^{-/-}$ dams and B6 sires were fed 10μg purified sIgG or BSA daily in the first week of life. At p16-18, offspring were given 1-10x10^5 $CD45.1^+CD4^+$ OVA-specific OTII transgenic T cells and administered OVA in drinking water until analysis at p25/p26. Controls were given regular drinking water. (**G** to **J**) Cells were gated according to the schematics provided in Fig S4D and Fig S11E. (**G**) Proportions of mLN OVA-specific $CD45.1^+V\alpha2^+V\beta5.1^+CD4^+Foxp3^+$ pTreg cells in p25 offspring given the indicated treatments as described in (F). (**H**) Similar to (G) but comparing proportions of mLN OVA-specific $CD45.1^+V\alpha2^+V\beta5.1^+Foxp3^-CD44^{hi}FR4^+CD73^+$ Th^{lin-} cells. (**I**) Similar to (G) but comparing proportions of mLN OVA-specific $CD45.1^+V\alpha2^+V\beta5.1^+$ GC Tfh cells. (**J**) Pie chart depicting fraction of GC Tfh, Treg, Thlin- and 'other' subsets of total activated $CD44^{hi}CD45.1^+V\alpha2^+V\beta5.1^+$ OVA-specific T cells of offspring described in (F). Numbers in legends indicate the mean percentage of events (± SD) of all samples within the experiment. (**K**) Ileal OVA-specific IgE titers of p40 offspring given the indicated treatments as described in (F).
For (**A**, **C** to **E, G** to **I**, and **K**) Error bars indicate the mean ± SD; symbols represent individual mice. Data are representative of two to four independent experiments with ≥2 mice per group. For **A**, data are representative of two independent experiments, with ≥2 samples per group, and 3-4 mice pooled per sample. For **D**, data are combined from three replicate experiments indicated by symbols. For (**C**, **E**), statistical significance was determined using one-way ANOVA and Tukey post-hoc tests. For (**D**), statistical significance was determined using two-way ANOVA and Holm-Sidak post-hoc tests. For (**G** to **I**, and **K**), statistical significance was determined using an unpaired, two-tailed Student's *t* test.