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Spotlight

Our Mothers' Antibodies as Guardians of our Commensals

Stephanie C. Ganal-Vonarburg[1,†,*] and Andrew J. Macpherson[1,†,*]

Mucosal secretory IgA protects the mammalian body from invasion of commensal bacteria colonizing the intestine. A recent murine study in *Cell* shows that commensal-specific antibodies of the IgG isotype are also present in the serum and that maternal commensal-specific IgA and IgG are crucial for maintaining host–microbe mutualism in the offspring.

Mammalian body surfaces, such as the intestine, the airways, and the skin, are colonized with vast numbers of microorganisms – the commensal microbiota. They help to digest food, protect from pathogens, and mature the immune system [1]. However, the high numbers of microorganisms, located predominantly in the intestine, also harbor potential risks for the host. Both the innate and adaptive immune systems are able to recognize microbe-associated molecular patterns, and foreign antigens from the microbiota may exert immune responses that can harm the host, leading to undesirable inflammation. The host has evolved several defense strategies aiming to balance the host–microbe relationship and limit undesirable anti-commensal immune responses. These include innate mechanisms such as the production of mucus and antimicrobial peptides by intestinal epithelial cells, as well as adaptive immune responses including the induction of intestinal regulatory T cells and microbiota-specific antibodies. The antibody isotype immunoglobulin (Ig) A is predominantly produced at mucosal sites and is secreted into the intestinal lumen. Importantly, it maintains intestinal homeostasis by limiting the translocation of bacteria from the intestinal lumen to the inside of the body [2,3].

Barton and colleagues now show in an elegant paper in *Cell* that the protective effect of antibodies in host–microbe mutualism extends beyond mucosally produced IgA and includes microbiota-specific serum IgG isotypes, which can be transmitted to newborn mice postnatally via the mother's milk [4].

In this study, Koch *et al.* demonstrate that fecal microbiota in specific pathogen-free (SPF) mice can be stained with IgG2b and IgG3 from the serum of the same mouse [4]. This clearly indicates that there is not only a mucosal, but also a systemic, immune response to commensal microbiota, in line with other recent publications where low levels of anti-commensal IgG can be detected in the serum of mice [5,6]. The commensal-specific IgG observed by the authors did not constitute natural antibodies but were instead specifically induced because they were absent in germ-free mice. Although independent of T cells, their generation was largely dependent on toll-like receptor (TLR) signaling, as shown by strain combinations experiments with T cell-deficient (*Tcrb*$^{-/-}$ *Tcrd*$^{-/-}$) and TLR signaling-deficient (*Myd88*$^{-/-}$ *Trif*$^{-/-}$) mice. Similarly to IgA^{+} plasma cells, IgG2b- and IgG3-secreting plasma cells were mainly located at mucosal sites such as Peyer's patches (PP) and mesenteric lymph nodes (MLN). Barton and colleagues FACS-sorted the fecal bacteria that were bound by serum IgG and analyzed them by 16S rRNA sequencing. This approach showed that serum IgG was able to bind to a broad range of intestinal bacteria, covering all orders present in the fecal microbiota, and even exceeding the binding capacity of intestinal IgA. Under healthy conditions, gut bacteria do not reach the bloodstream in significant numbers and are kept within the boundaries of the intestinal immune system because the MLN act as a firewall that limits the passage of commensal bacteria to systemic tissues [2]. Nevertheless, inducing systemic immunity against commensal bacteria seems to be a smart way for the host to protect itself during inevitable situations of disrupted intestinal barrier function, such as from infection or inflammation.

Commensal-specific IgG serum antibodies were detectable as early as 2 weeks after birth, decreasing at the age of weaning, and increasing again in adult mice. This observation suggested that IgG2b and IgG3 antibodies detected in the neonates were of maternal origin. In fact, in mice, maternal IgG reaches the offspring via the placenta, and postnatally through maternal milk. The neonatal Fc receptor (FcRn) is involved in transporting IgG across the placenta and into the milk. Via an elegant approach, the authors used wild-type and B cell-deficient ($\mu MT^{-/-}$) heterozygous breeding where either the dam or the offspring was deficient in B cells. Antibody-deficient offspring born to a wild-type mother produced microbiota-specific IgG only in the first few weeks after birth, clearly demonstrating that these commensal-directed antibodies are present in early life and must have been of maternal origin. Likewise, in the absence of maternal B cells, significantly fewer commensal-specific IgG antibodies were present in young pups after birth. Maternal IgG transfer provided obvious functional benefits because pups born to B cell-deficient dams exhibited increased numbers and frequencies of CD4^{+}CD44^{+} effector T cells in PP and MLN, with increased proinflammatory cytokine production following dextran sodium sulfate-induced colitis. Intestinal mucosal CD4^{+} T cell subsets have been shown to be sensitive to alterations in the composition of commensal microbiota [7]. Therefore, the observed differences in CD4^{+} T effector cell numbers in the offspring of $\mu MT^{-/-}$ and wild-type dams could have been due

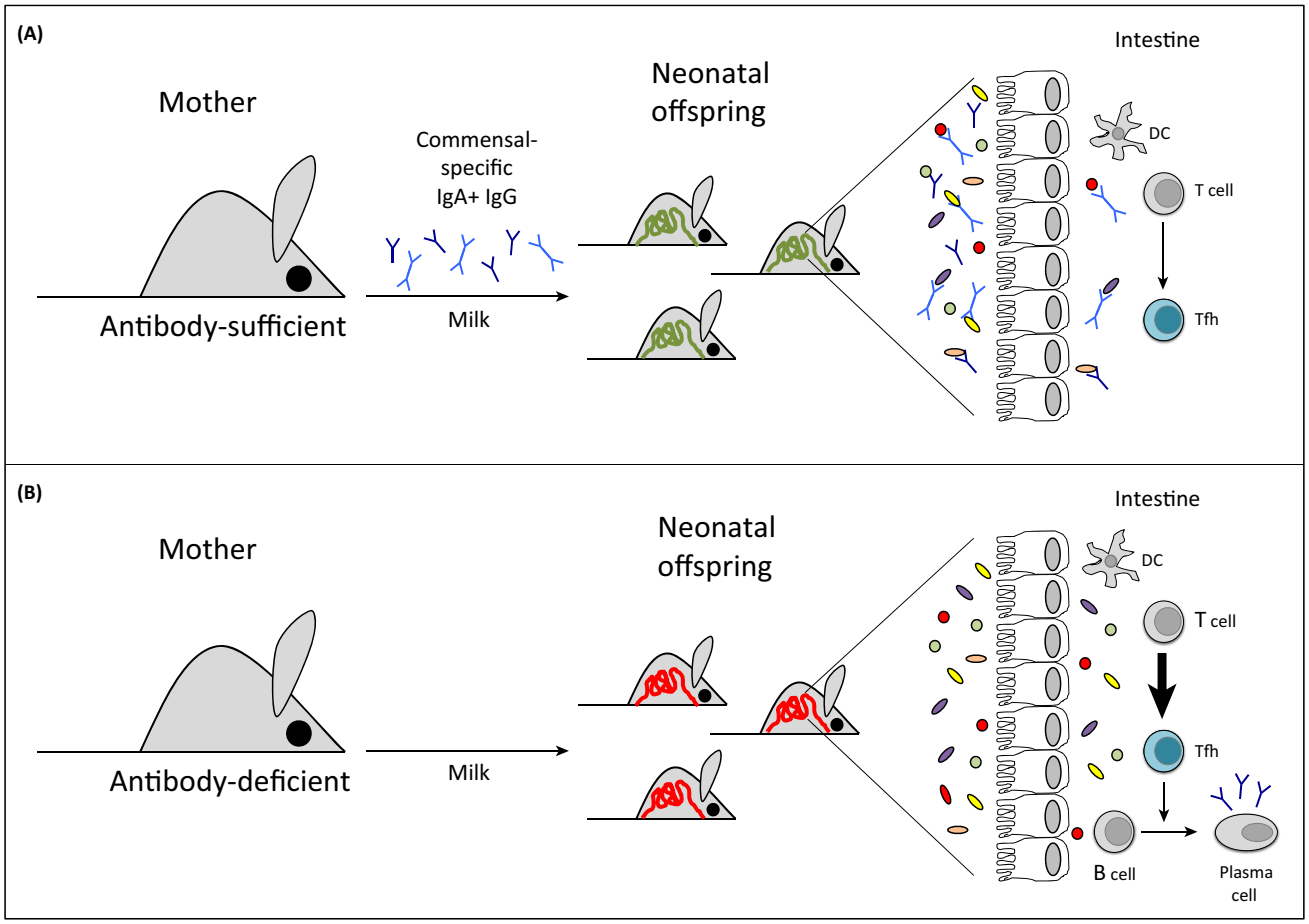

Figure 1. Maternal Commensal-Specific Antibodies Protect the Neonatal Intestine from Bacterial Translocation and CD4$^+$ T Cell Activation in Mice. (A) Commensal-specific antibodies of the immunoglobulin (Ig) A and IgG isotypes are transferred to murine offspring postnatally via maternal milk. These maternal commensal-specific antibodies bind to bacteria of the microbiota in the neonatal intestine of the offspring and limit bacterial translocation across the intestinal epithelium. (B) In the offspring of antibody-deficient mothers, more commensal bacteria invade the neonatal intestinal mucosa and activate CD4$^+$ T cells to become T follicular helper cells (Tfh). This results in a compensatory induction of plasma cells which produce protective endogenous anti-commensal antibodies. Abbreviation: DC, dendritic cells (which are antigen-presenting cells).

to secondary to differences in their microbiota. However, Barton and colleagues have convincingly shown the persistence of the phenotype in co-housing experiments, where both wild-type and $\mu MT^{-/-}$ mice harbored comparable microbiota.

Finally, the authors reported cross-fostering experiments in mice demonstrating that the antibodies present in milk – as opposed to those transferred trans-placentally – were particularly important in inhibiting commensal-specific T cell responses in the offspring. Murine milk contains both IgA and IgG [8,9], and the presence of both maternal IgA and IgG was required to limit intestinal CD4$^+$ T cell responses. Uptake of IgG in the neonatal intestine via the FcRn was not required. This argues that maternal IgA and IgG cooperatively bind to members of the commensal microbiota in the intestine of the neonate, thereby preventing bacterial translocation and subsequent induction of microbiota-directed T cell responses. These findings were indeed consistent with higher numbers of bacteria present in the MLN of pups raised by antibody-deficient mothers (Figure 1).

The 'inflammatory' phenotype observed in pups nursed in the absence of maternal antibodies did not persist in adult mice. Thus, there are apparently compensatory mechanisms, including increased T-dependent germinal center B cell reactions, to produce microbiota-controlling antibodies.

The work by Barton and colleagues highlights the importance of early life events in shaping host–microbe mutualism throughout life, and demonstrates that maternal antibodies offer more than merely passive immunity to pathogens in the neonate. These findings are in agreement with

recent work from our laboratory showing that signals derived from the maternal microbiota are crucial in shaping early postnatal innate immune development and adaptation to colonization by commensals in the offspring [6,10]. In our studies, maternal antibodies acted as carriers, transferring microbe-derived metabolites into milk. Now, Koch *et al.* robustly demonstrate the ability of maternal antibodies to bind to commensal bacteria in the neonatal intestine, thereby offering protection to the offspring while these are being colonized by millions of microbes shortly after birth.

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[1]Maurice Müller Laboratories (DKF), Universitätsklinik für Viszerale Chirurgie und Medizin, Inselspital, Murtenstrasse 35, University of Bern, 3010 Bern, Switzerland
[†]Equal contribution

*Correspondence: stephanie.ganal@dkf.unibe.ch (S.C. Ganal-Vonarburg) and andrew.macpherson@dkf.unibe.ch (A.J. Macpherson).

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Spotlight

Shipping Drug Resistance: Extracellular Vesicles in Ovarian Cancer

Elke Pogge von Strandmann[1,2,*] and Rolf Müller[3]

Ovarian cancer is a deadly disease, largely because of relapse and chemotherapy resistance. Common genetic mechanisms causing drug resistance have not been identified. A recent study unravels a novel and unexpected pathway involving the transfer of microvesicle-encapsulated miRNA from omental adipocytes and fibroblasts, to cancer cells.

Ascites and peripheral blood from ovarian cancer (OC) patients contain substantial amounts of microvesicles (MVs) originating from both tumor and tumor-associated host cells. These MVs possess tumor-promoting and immunosuppressive properties [1,2]. Of note, MVs carry a variety of miRNAs, some of which appear to be associated with an unfavorable clinical outcome [1,2]. However, the molecular mechanisms underlying these findings are unclear and their clinical relevance is difficult to judge. This issue has been addressed for one of these molecules, miR21, in an elegant study by Au Yeung *et al.* recently published in *Nature Communications* [3].

In essence, the authors have identified a novel pathway causing resistance to taxane-based chemotherapy in murine OC; the mechanism comprises the shuttling of MV-encapsulated miR21 from omental cancer-associated adipocytes (CAAs) and fibroblasts (CAFs) to cancer cells [3]. Once taken up by malignant cells, miR21 is released into the cytoplasm where it directly targets and downregulates *APAF-1* mRNA. *APAF-1* encodes apoptotic peptidase activating factor 1 (APAF-1), an indispensable component of the apoptosome mediating cytochrome c-triggered autocatalytic activation of procaspase-9 and the initiation of apoptosis. This pathway plays an essential role in taxane-induced cell death, with a rate-limiting function for APAF-1 [4]. It is thus conceivable that targeting *APAF-1* mRNA by miR21 increases the apoptotic threshold in drug-treated OC cells.

Several key experiments support this model. Fluorescently labeled or FAM-tagged miR21 was used to visualize the transfer of miR21 to OC cells by confocal microscopy. Uptake of miR21 by OC cells was clearly detectable in CAA and CAF co-cultures and also on incubation with isolated vesicles released by these cell lines. Importantly, the transfer was confirmed not only *in vitro* but also intratumorally in a mouse xenograft model of human OC cells co-transplanted with labeled-miR21-transfected mouse embryonic fibroblasts (MEFs). Moreover, enhanced expression of miR21 correlated with an increase in OC cell motility and invasion as well as with decreased sensitivity to, and rate of apoptosis in response to, paclitaxel (Taxol) both *in vitro* and *in vivo* (in mice). Interestingly, APAF-1 was identified by transcriptomics and by functional assays as the prominent miR21 target. This exciting data adds another piece to the emerging picture of how microenvironment-dependent signals supplied by non-malignant host cells (here adipocytes and fibroblasts) can promote tumor growth and metastasis. Within this scenario, MVs are crucial players supporting metastasis, as recently shown for melanoma and pancreatic carcinoma [5–7]. These findings clearly suggest that in-depth characterization of the molecular components transferred by extracellular vesicles (EVs) is urgently required to assess their clinical relevance and their potential as novel drug targets.