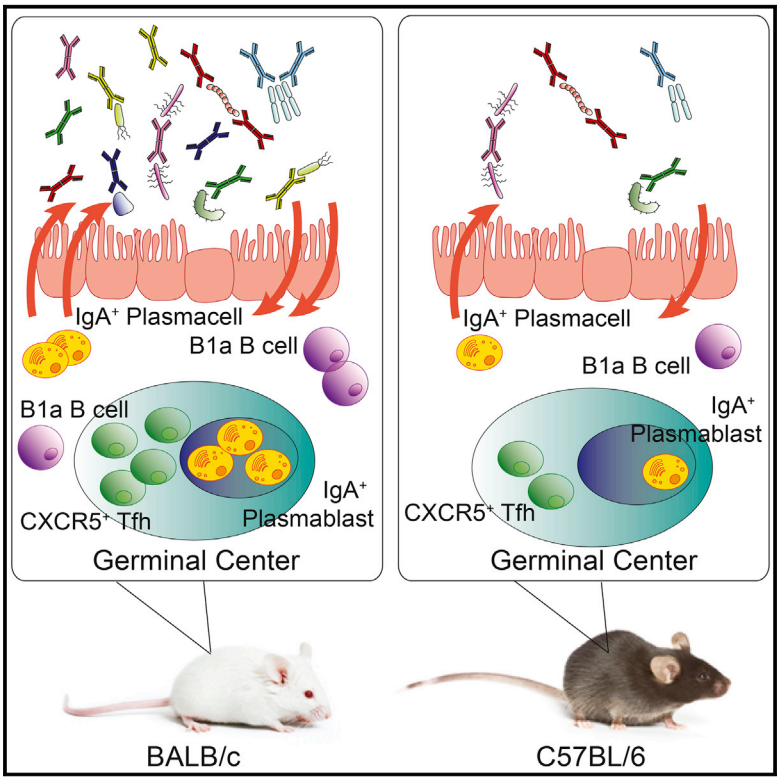
Article

Immunity

BALB/c and C57BL/6 Mice Differ in Polyreactive IgA Abundance, which Impacts the Generation of Antigen-Specific IgA and Microbiota Diversity

Graphical Abstract



Authors

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In Brief

Reduced microbiota diversity has been associated with several pathologic conditions. Rescigno et al. show that the capacity to produce innate IgAs has an impact on microbiota diversity. IgAs can mediate the internalization of non-invasive bacteria and the initiation of a positive feedback loop of IgA production. IgA diversity might be a marker of a healthy condition.

Highlights

- Different mouse strains have diverse predisposition to produce innate IgAs
- Innate IgAs allow a controlled bacterial entrance
- IgA-coated bacteria initiate a positive feedback loop of IgA production
- IgA diversity results in microbiota diversification

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BALB/c and C57BL/6 Mice Differ in Polyreactive IgA Abundance, which Impacts the Generation of Antigen-Specific IgA and Microbiota Diversity

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SUMMARY

The interrelationship between IgAs and microbiota diversity is still unclear. Here we show that BALB/c mice had higher abundance and diversity of IgAs than C57BL/6 mice and that this correlated with increased microbiota diversity. We show that polyreactive IgAs mediated the entrance of non-invasive bacteria to Peyer's patches, independently of CX3CR1$^+$ phagocytes. This allowed the induction of bacteria-specific IgA and the establishment of a positive feedback loop of IgA production. Cohousing of mice or fecal transplantation had little or no influence on IgA production and had only partial impact on microbiota composition. Germ-free BALB/c, but not C57BL/6, mice already had polyreactive IgAs that influenced microbiota diversity and selection after colonization. Together, these data suggest that genetic predisposition to produce polyreactive IgAs has a strong impact on the generation of antigen-specific IgAs and the selection and maintenance of microbiota diversity.

INTRODUCTION

Secretory immunoglobulin (sIg)A are a major component of the epithelial barrier and play a pivotal role in maintaining intestinal homeostasis (Cerutti and Rescigno, 2008; Corthésy, 2013). Dimeric IgAs are secreted after binding to the polymeric Ig receptor and are released together with the secretory component in the intestinal lumen where they bind to the host microbiota (Brandtzaeg, 2003). Several functions have been attributed to IgAs, but the major one is that of immune exclusion (Mestecky et al., 1999). IgAs bind to microbial components and affect the invasive potential of microorganisms by inhibiting their interaction with epithelial cells and their subsequent internalization. On the other hand, IgAs allow bacteria to attach to the mucus that they use as an energy source (Corthésy, 2010).

IgA can also shape the microbiota composition. Mice deleted for activation-induced cytidine deaminase (*Aicda*) lack IgA and this leads to the selected expansion of potentially dangerous microorganisms called pathobionts, such as segmented filamentous bacteria (SFB) (Suzuki et al., 2004). IgAs can reduce the expression of inflammatory epitopes on the microbiota, thus allowing preservation of intestinal homeostasis (Peterson et al., 2007). Recently, a correlation between diversification of IgAs, Foxp3$^+$ T follicular regulatory cells, and microbiota diversity was described (Kawamoto et al., 2014). Hence, the induction of an IgA response to the microbiota is a fundamental step for the maintenance of immune homeostasis.

Another important function of IgAs is to allow a controlled microbial entrance in the host. IgAs can bind to the luminal membrane of microfold (M) cells that are scattered in the follicle-associated epithelium overlying the Peyer's patches (PPs) (Mantis et al., 2002). Trancytosed complexes are then delivered to dendritic cells (Kadaoui and Corthésy, 2007), but the outcome of this interaction is not fully understood.

M cells also express specific receptors such as the glycoprotein (GP)-2 that mediate the internalization of type I-piliated bacteria such as *Salmonella* Typhimurium (Hase et al., 2009). However, for *Salmonella* to invade a cell, a type three-secretion system (TTSS) encoded by the salmonella pathogenicity island 1 (Spi1) is required. The TTSS is like a syringe that allows the delivery of effector proteins in the cytoplasm of the infected cells, leading to cytoskeleton rearrangements and bacterial internalization (Lostroh and Lee, 2001; Marlovits et al., 2004). Indeed, non-invasive strains of *Salmonella* do not penetrate the PPs, presumably because they are unable to enter M cells (Vazquez-Torres et al., 1999) and do not trigger an IgA response (Martinoli

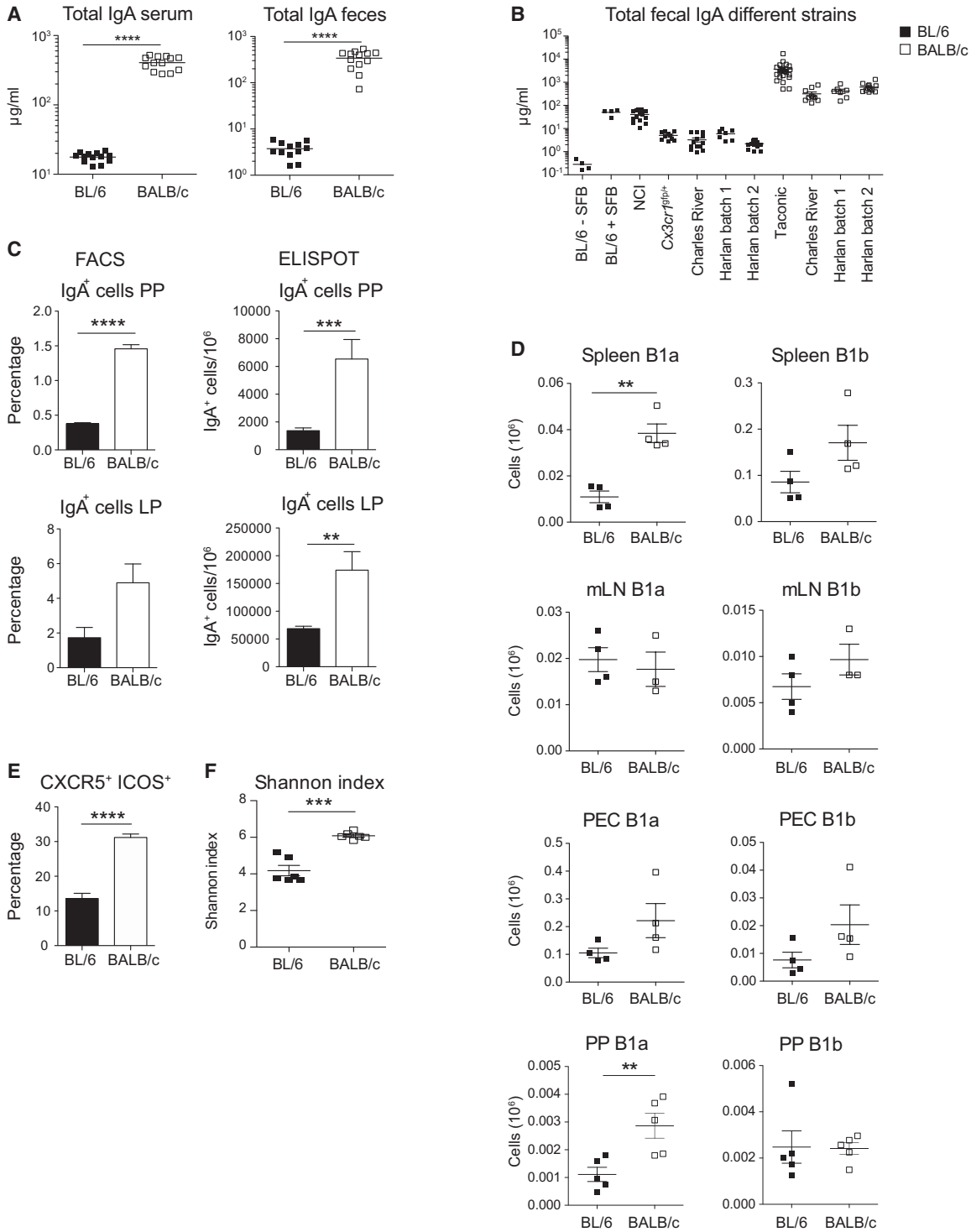

Figure 1. C57BL/6 and BALB/c Mice Fundamentally Differ in IgA Abundance

(A) Total IgA was measured by ELISA in serum and feces of C57BL/6 and BALB/c mice (n = 13).

(B) Total IgA measured in feces of mice from different sources (see Experimental Procedures).

(C) Percentage of IgA$^+$ cells in PPs and LP of C57BL/6 and BALB/c mice measured by flow cytometry (n = 3) or ELISPOT (n = 9 for PPs, n = 3 for LP).

(D) Absolute numbers of B1a (CD43$^+$CD5$^+$) and B1b (CD45$^+$CD5$^-$) among CD19$^+$ IgM$^+$ cells in the spleen, mLNs, peritoneal exudate cells (PECs), and PPs of C57BL/6 and BALB/c mice (n = 4–5). For percentages see also Figure S1.

(legend continued on next page)

et al., 2007; Vazquez-Torres et al., 1999). However, they are still capable of entering the host via the CX3CR1$^+$ mononuclear phagocytes (Hapfelmeier et al., 2008) to reach the mesenteric lymph nodes (mLNs) and induce an IgG response (Martinoli et al., 2007). Hence, if the bacteria are not invasive they are unable to reach the PPs and trigger an IgA response. How then is the IgA response to the microbiota induced for strains that are unable to enter the PPs?

In this study we show a mouse strain dependence in the abundance of polyreactive IgAs, which correlated with microbiota diversification. We show that IgAs either endogenous (specific or polyreactive, depending on the analyzed strain and its immunization status) or exogenously provided allowed non-invasive bacteria to gain access to PPs for the induction of bacteria-specific IgAs, independently of CX3CR1$^+$ mononuclear phagocytes or the microbiota.

RESULTS

C57BL/6 and BALB/c Mice Fundamentally Differ in IgA Abundance

The microbiota plays an important role in the generation of IgA responses (Round and Mazmanian, 2009) and some bacterial species, such as SFB, are potent inducers of IgA (Talham et al., 1999; Umesaki et al., 1999). It has been recently shown that colonization of the mouse gut by *Lactobacillus* species is dependent on the mouse genetic background (Buhnik-Rosenblau et al., 2011). Therefore, we compared the abundance of IgAs in the feces and serum of two mouse strains to address whether there was a relationship between microbiota and IgA abundance. We found that BALB/c mice had much higher concentrations of total IgA in both serum and feces compared to C57BL/6 mice (Figure 1A). To assess whether the observed difference in IgA was due to gut microbiota differences associated with the vendor source, we compared IgA abundance in C57BL/6 and BALB/c mice from different sources (Figure 1B). All BALB/c strains had much higher total IgAs than any C57BL/6 strain, regardless of their source. We also analyzed samples from C57BL/6 mice known to be SFB positive or negative. As expected, presumably SFB-positive mice such as C57BL/6 mice from the National Cancer Institute had much higher IgAs in their feces compared to mice from other sources. Similarly, BALB/c mice from Taconic that are known to be SFB positive (Ivanov et al., 2009) had considerable higher IgAs than other BALB/c strains.

In agreement with the difference in total IgA amounts, we found that BALB/c mice had higher numbers of IgA$^+$ cells in both PPs and the lamina propria (LP) compared to C57BL/6 mice (Figure 1C). BALB/c mice also had a higher percentage and/or number of B1a B cells both in the spleen and PPs as compared to C57BL/6 mice (Figures 1D and S1), which might contribute to the high concentration of innate IgAs in BALB/c mice. However, BALB/c mice also had a much higher percentage of CD4$^+$ T cells with a T follicular helper cell phenotype (CXCR5$^+$ICOS$^+$) in their PPs, which could indicate also a contribution of T-cell-dependent IgA induction (Figure 1E). We evaluated the IgA repertoire diversity in the small intestinal LP of C57BL/6 and BALB/c mice, analyzing complementarity determining region-3 (CDR3) diversity by next generation sequencing approach. The diversity was measured with the Shannon Index combining the amount of different CDR3 sequences and their frequency within the repertoire. Results show that BALB/c mice IgA repertoire is highly diversified compared to C57BL/6 mice (Figure 1F). Altogether, these results indicate that BALB/c mice are characterized by a higher abundance of IgAs, which are more diversified. This is independent on the vendor source and reflects an increased number of IgA$^+$ cells in the PPs and LP and a higher abundance of B1a B cells in the PPs and spleen.

C57BL/6 and BALB/c Mice Respond Differently to *Salmonella* Typhimurium

Innate IgAs have been shown to play an important role in controlling *Salmonella* Typhimurium infection (Wijburg et al., 2006), so we wondered whether, because of the dissimilar basal quantity of IgAs, the two strains had a different capacity to respond to a lethal *Salmonella* infection. We compared the survival in response to *Salmonella* infection and found that naive BALB/c mice were more protected than C57BL/6 mice (Figure 2A). Consistently, naive BALB/c mice had much higher amounts of innate IgA antibodies that could bind to *Salmonella* both in serum and feces than naive C57BL/6 mice and that might inhibit *Salmonella* invasive potential (Figure 2B). We then analyzed the capacity of the two mouse strains to mount an IgA response to *Salmonella* and we used a bacterial strain that is invasive but that has a metabolic defect in the production of aromatic amino acids and does not survive within the host (invasive *S.* Typhimurium aroA). *S.* Typhimurium aroA induced similar concentration of *Salmonella*-specific IgA in the feces of C57BL/6 mice and BALB/c mice, but it induced much less *Salmonella*-specific IgA in the serum of BALB/c mice compared to C57BL/6 mice (Figure 2C). These results suggest that when challenged, C57BL/6 mice can mount a potent IgA response (even higher than BALB/c mice).

Besides their exclusion function, IgAs have been shown to also allow a ''controlled'' entrance of IgA-coated bacteria in the PPs (Kadaoui and Corthésy, 2007). We previously described that a non-invasive strain of *Salmonella* induces a similar amount of IgG as compared to the invasive strain but does not allow the establishment of an IgA response in C57BL/6 mice because it does not reach the PPs (Martinoli et al., 2007). Given the higher amount of innate IgAs capable of binding *Salmonella* in BALB/c mice that could mediate bacterial penetrance in the PPs, we wondered whether the two mouse strains differed in their ability of mounting an IgA response to a non-invasive strain of *Salmonella* (aroAinvA). As expected, both *Salmonella* strains induced similar amounts of IgG in the serum of both mice (Figure S2A). However, we found that after three oral immunizations on alternate days, *S.* Typhimurium aroAinvA was able to induce

(E) Percentage of CXCR5$^+$ICOS$^+$ cells among CD4$^+$ T cells in PPs of C57BL/6 (n = 27) or BALB/c (n = 20) mice measured by flow cytometry analysis. Data were pooled from three independent experiments.

(F) IgA repertoire diversity as measured by Shannon index of diversity in the small intestine of C57BL/6 and BALB/c mice (n = 6).

All data are expressed as means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

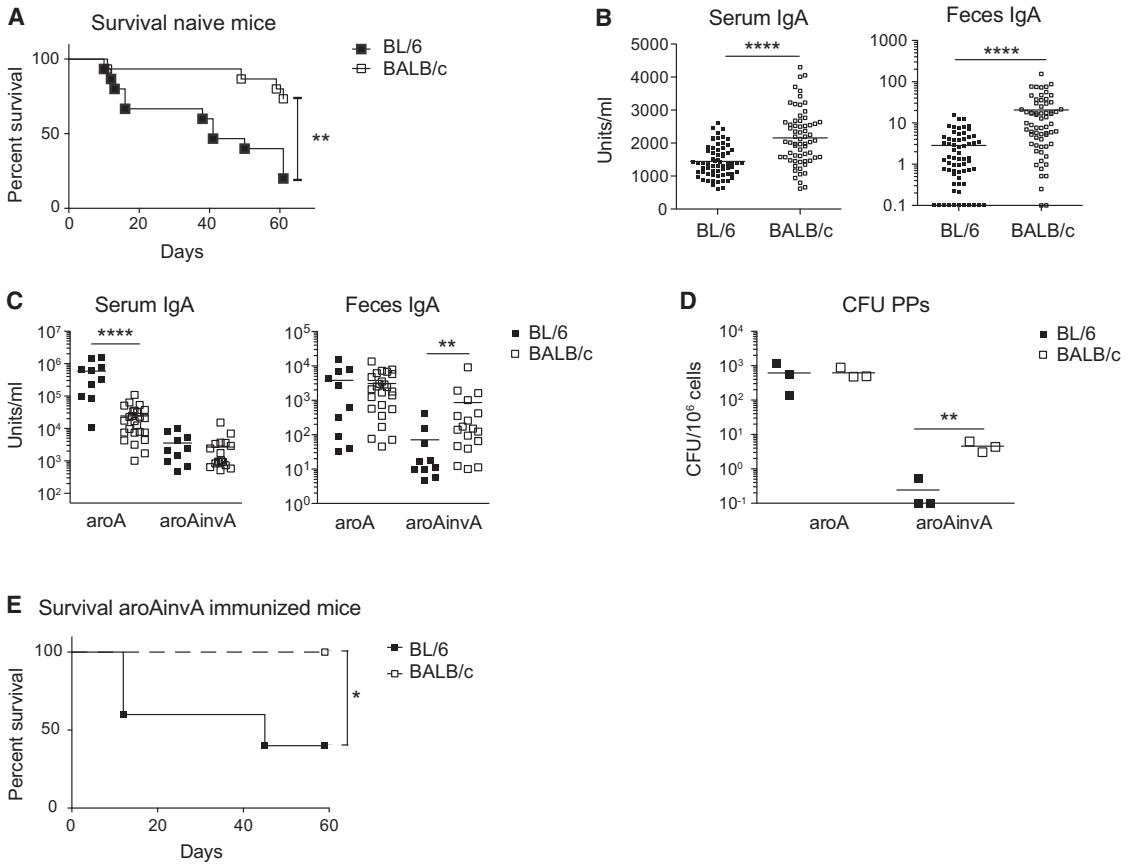

Figure 2. C57BL/6 and BALB/c Mice Respond Differently to *Salmonella* Infection

(A) Naive C57BL/6 and BALB/c mice were challenged orally with 10^6 CFU WT *S*. Typhimurium and survival was monitored for 60 days (n = 15). Data were pooled from two independent experiments.

(B) Quantity of *Salmonella*-reactive IgA antibodies measured by ELISA in serum and feces of naive C57BL/6 or BALB/c mice (n = 65).

(C) C57BL/6 (n = 10) or BALB/c (n = 18–25) mice received orally 10^9 CFU *S*. Typhimurium aroA or aroAinvA on days 0, 2, and 4. After 5 weeks, *Salmonella*-specific IgA was measured by ELISA in serum and feces. Data were pooled from four independent experiments. For serum IgG, see also Figure S2A.

(D) C57BL/6 and BALB/c mice (n = 3) received orally 10^9 CFU *S*. Typhimurium aroA or aroAinvA. After 62 hr, PPs were isolated and the number of intracellular bacteria was determined by plating cell lysates after gentamicin treatment. Representative of two independent experiments. See also Figure S2B.

(E) C57BL/6 and BALB/c mice (n = 5) were immunized orally with *S*. Typhimurium aroAinvA on days 0, 2, and 4. After 5 weeks, mice were challenged with 10^7 CFU WT *S*. Typhimurium and survival was monitored for 60 days. Representative of two independent experiments. See also Figure S3.

All data are expressed as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Salmonella-specific IgA in the feces of BALB/c but not of C57BL/6 mice (Figure 2C). This suggests that when there are innate IgAs capable of binding *Salmonella*, even if the bacterium is not invasive, it is still capable of inducing an IgA response, presumably because it reaches the PPs. Consistently, we found that non-invasive *Salmonella* was capable of entering the PPs of BALB/c mice after oral feeding at 14 hr after infection, whereas hardly any bacteria were found in the PPs of C57BL/6 mice (Figures 2D and S2B).

To establish whether oral immunization with non-invasive *Salmonella* could induce protective immunity, C57BL/6 and BALB/c mice received *S.* Typhimurium three times on alternate days and were challenged 5 weeks later with wild-type *S.* Typhimurium (Figure 2E). All immunized BALB/c mice survived, but about 60% of immunized C57BL/6 mice did not survive the infection, suggesting that although mice had a similar IgG amounts, only IgAs confer full protection.

IgA-Coated Non-invasive *Salmonella* Typhimurium Enters Peyer's Patches and Induces Adaptive Immune Responses

As mentioned above, we hypothesized that innate *Salmonella*-binding IgAs might facilitate the entrance of non-invasive *Salmonella* into the PPs, which triggers a positive feedback loop of induction of *Salmonella*-specific IgAs. To verify this hypothesis we tested whether IgA-coated bacteria would indeed gain more access to PPs in vivo and would trigger an IgA immune response in C57BL/6 mice. *S.* Typhimurium aroAinvA was incubated in vitro with IgA specific for *Salmonella* (secretory IgA) or left untreated (aroAinvA) and injected into the duodenum of C57BL/6 mice. The next day PPs, mLNs, and spleens were collected and the number of intracellular bacteria was determined by plating of cell lysates after gentamicin treatment. Mice that received IgA-coated non-invasive *Salmonella* had much more bacteria in their PPs but less in the mLNs

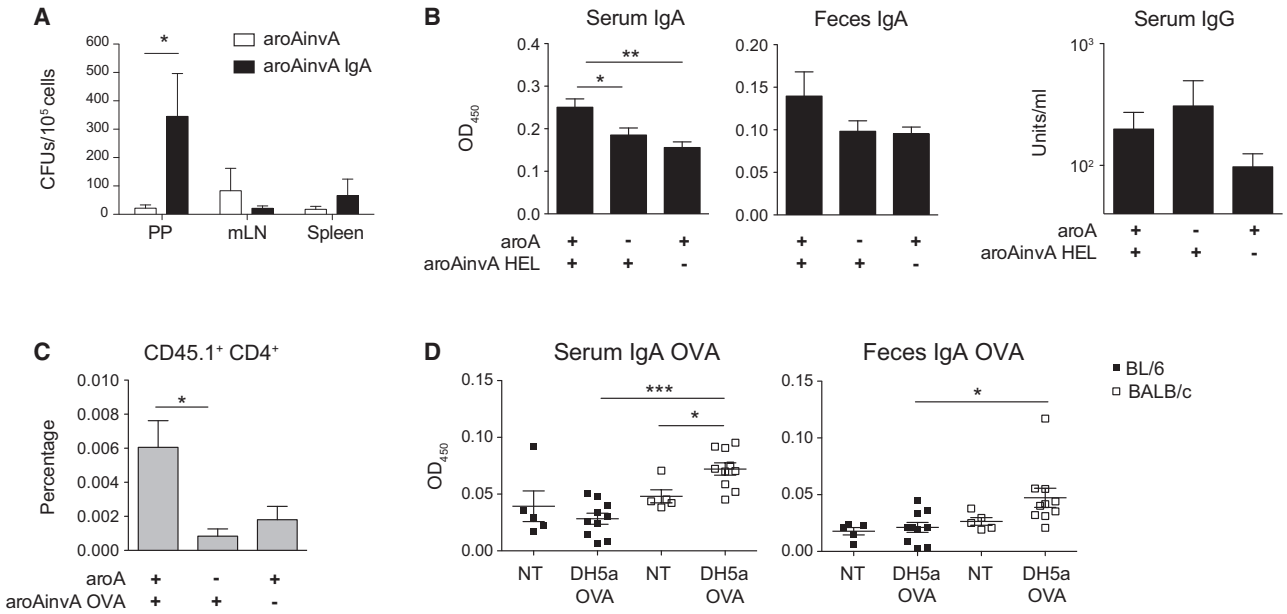

Figure 3. IgA-Coated Non-invasive *Salmonella* Enters PPs and Induces Adaptive Immune Responses

(A) *S.* Typhimurium aroAinvA was left untreated (aroAinvA) or incubated with *Salmonella*-specific Sal4 sIgA (aroAinvA IgA) and injected into the duodendum. The next day PPs, mLNs, and spleen (SP) were collected and intracellular CFUs were determined by plating.

(B) Mice (n = 5) received 10^9 CFU *S.* Typhimurium aroA orally on days 0, 2, and 4. After 5 weeks, mice received orally three times every 48 hr 10^9 CFU non-invasive *S.* Typhimurium-expressing HEL on the cell surface (aroAinvA HEL). 2 weeks later, HEL-specific antibodies were measured by ELISA in serum (IgG and IgA) and feces (IgA). Control groups (n = 4) received only the first or second immunizations.

(C) C57BL/6 CD45.2 mice (n = 6) received orally three times (days 0, 2, 4) 10^9 CFU *S.* Typhimurium aroA. 5 weeks later, mice were injected i.v. with 10^6 OT-II CD45.1 $CD4^+$ T cells. The next day and 3 and 5 days later, mice received orally 10^9 CFU non-invasive *S.* Typhimurium-expressing OVA (aroAinvA OVA). 7 days after the first booster immunization, PPs were isolated and the percentage of $CD45.1^+CD4^+$ T cells was assessed by flow cytometry analysis. Control groups received only the first (n = 3) or second (n = 4) immunizations.

(D) C57BL/6 and BALB/c mice were either left untreated (NT, n = 5) or received orally three times (days 0, 2, 4) 10^{10} CFU *E. coli*-expressing OVA (n = 10). 5 weeks later, OVA-specific IgA response was measured in the serum and feces of the mice.

All data are expressed as means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.

compared to mice that received non-invasive *Salmonella* (Figure 3A).

Next we determined whether the increased uptake of IgA-coated non-invasive *Salmonella* by PPs would lead to the production of more *Salmonella*-specific IgAs. Mice were first immunized orally with *S.* Typhimurium aroA to induce the presence of *Salmonella*-specific sIgA in the gut. 5 weeks later, mice were immunized orally with non-invasive *S.* Typhimurium aroAinvA expressing hen eggwhite lysozyme (HEL) on the cell surface. Thus, antibodies against HEL could be induced only by the non-invasive strain. HEL-specific antibodies were measured 2 weeks later. We found higher HEL-specific IgA in the serum of mice that received both bacteria compared to mice that received only the invasive or only the non-invasive strains (Figure 3B). A similar trend was observed for HEL-specific IgA in the feces. As expected, *S.* Typhimurium aroAinvA HEL induced similar amounts of HEL-specific IgG in the serum, whether or not the mice were previously immunized with *S.* Typhimurium aroA (Figure 3B).

Next we investigated the ability of IgA-coated non-invasive *Salmonella* to activate $CD4^+$ T cells. C57BL/6 $CD45.2^+$ mice were immunized orally with *S.* Typhimurium aroA to induce *Salmonella*-specific sIgA in the gut. After 5 weeks, 10^6 $CD4^+$ T cells derived from $CD45.1^+$ OT-II mice were adoptively transferred to the immunized mice and the next day the mice were orally immunized with *S.* Typhimurium aroAinvA-expressing ovalbumin (OVA). 7 days later, PPs were isolated and analyzed by flow cytometry to determine the percentage of donor-derived $CD45.1^+$ $CD4^+$ T cells in the PPs. Mice that received both immunizations had a significantly higher percentage of $CD4^+$ T cells compared to mice that received only the first or second immunizations, suggesting that the binding of IgA to OVA-expressing non-invasive *Salmonella* enabled the bacteria to activate the expansion of OVA-specific $CD4^+$ T cells in the PPs (Figure 3C).

We then evaluated whether we could recapitulate these results using a physiological non-invasive commensal microbe. We orally fed both C57BL/6 and BALB/c mice with a commensal strain of *Escherichia coli* engineered to express OVA and we followed the IgA response to OVA over time. Only BALB/c mice were capable of inducing an OVA-specific IgA response both in the feces and blood in response to *E. coli* OVA administration (Figure 3D), indicating that the observed results were not a peculiarity of non-invasive *Salmonella*. Together, these results suggest that naive BALB/c mice have high concentrations of innate IgA antibodies, which mediate the entrance of bacteria in the PPs and enable the mice to generate a feedback loop of IgA induction.

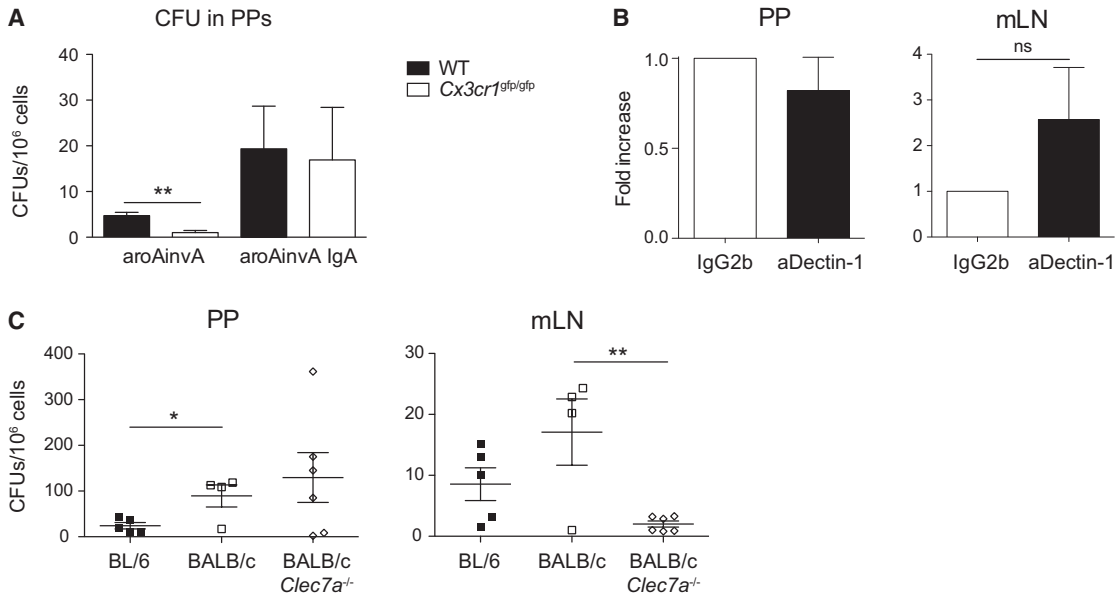

Figure 4. The Entrance of IgA-Coated Bacteria Is Dependent on neither Dectin-1 Expression nor on CX3CR1$^+$ Monocytes

(A) *S.* Typhimurium aroAinvA was left untreated (aroAinvA) or incubated with *Salmonella*-specific Sal4 sIgA (aroAinvA IgA) and injected into the duodenum of C57BL/6 mice (WT) or *Cx3cr1*$^{gfp/gfp}$ mice (n = 6). The next day the number of intracellular bacteria in PPs was determined by plating. Data were pooled from two independent experiments. Data were normalized by dividing each point by the mean of the group with the lowest CFUs (*Cx3cr1*$^{gfp/gfp}$ + aroAinvA). See also Figures S4, S5A, and S5B.

(B) BALB/c mice (n = 5) were injected in the duodenum with rat IgG2b or anti-Dectin-1 neutralizing antibody and 6 hr later infected with 10^9 CFU *S.* Typhimurium aroAinvA. 14 hr later, PPs and mLNs were harvested and plated to assess CFU number in organs after gentamicin protection assay. See also Figures S5C and S5D.

(C) C57BL/6 (n = 5), BALB/c (n = 4), and *Clec7a*$^{-/-}$ mice on BALB/c background (n = 6) were infected with 10^{10} CFU *S.* Typhimurium aroAinvA. 14 hr after infection, CFU number were assessed in organs.

All data are expressed as means ± SEM. *p < 0.05, **p < 0.01.

Increasing the Interval between Immunizations Enhances the IgA Response toward Non-invasive *Salmonella* Typhimurium

As mentioned above, the binding of innate IgA by non-invasive *S.* Typhimurium would foster a positive feedback loop of IgA responses in BALB/c mice. Therefore, we wondered whether by increasing the interval of bacterial administration from alternate days to weeks we could trigger an IgA response also in C57BL/6 mice by creating a positive feedback loop. The assumption would be that after oral feeding, a low number of non-invasive bacteria would reach the PPs, allowing the production of a small amount of *Salmonella*-specific IgAs several days later. When the mice receive a second dose of non-invasive *S.* Typhimurium 2 weeks later, these IgAs could bind to the bacteria and allow further entrance of IgA-coated bacteria in the PPs and more IgA production, generating a positive feedback loop.

Consistent with our hypothesis, when the intervals between immunizations with *S.* Typhimurium aroAinvA were extended to 14 days, we observed that 2 weeks after the third immunization, *Salmonella*-specific IgA was detectable in the serum and the feces (Figure S3). As expected, the immunization scheme of three immunizations on alternate days induced very little *Salmonella*-specific IgA and both immunization schemes resulted in equal amounts of *Salmonella*-specific IgG in the serum (Figure S3).

The Entrance of IgA-Coated Bacteria Is Not Dependent on CX3CR1$^+$ Mononuclear Cells nor on Dectin-1 Expression

LP mononuclear phagocytes have been shown to extend protrusions across the epithelium into the intestinal lumen (Rescigno et al., 2001) (this process is dependent on the expression of the chemokine receptor CX3CR1 [Niess et al., 2005]) or to send dendrites through M-cell-specific transcellular pores (Lelouard et al., 2012). Phagocyte extensions can capture and internalize non-invasive *Salmonella* (Chieppa et al., 2006) and are absolutely required for their internalization (Hapfelmeier et al., 2008). To investigate whether CX3CR1$^+$ cells were responsible for the uptake of IgA-coated non-invasive *Salmonella* into PPs, *S.* Typhimurium aroAinvA was left untreated or coated with *Salmonella*-specific IgA in vitro. Next, bacteria were injected into the duodenum of wild-type C57BL/6 mice or CX3CR1-deficient mice on a C57BL/6 background. The next day, PPs were isolated and the number of intracellular bacteria was determined by plating cell lysates after gentamicin treatment (Figure 4A). We recovered a lower amount of non-invasive uncoated *Salmonella* in PPs of CX3CR1-deficient compared to wild-type mice. However, when mice received IgA-coated non-invasive *Salmonella*, no differences were observed between wild-type and CX3CR1-deficient mice. Thus, CX3CR1-dependent dendrite extensions seem to be required for the uptake of non-invasive non-coated, but not coated, *Salmonella* into PPs. Consistently, CX3CR1$^+$ phagocytes

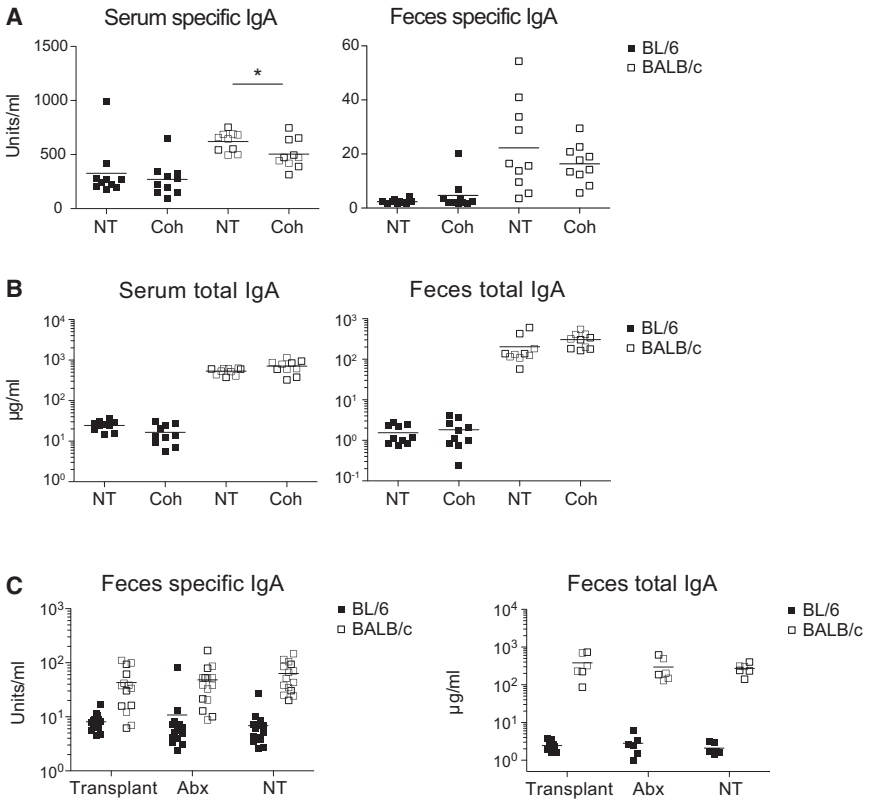

Figure 5. IgA Amounts in C56BL/6 and BALB/c Mice Are Stable after Co-housing or Fecal Transplantation

(A and B) Naive C57BL/6 and BALB/c mice (n = 10) were kept in separate cages (NT) or co-housed for 1 month and *Salmonella*-specific IgA (A) and total IgA (B) was measured by ELISA in serum and feces.

(C and D) C57BL/6 and BALB/c mice were left untreated (NT) or were treated with a mix of antibiotics (Abx) for 14 days.

(C) After treatment with Abx, mice received fecal transplantation with feces from the other (transplant) or the same mouse strain (Abx). After 4 weeks, *Salmonella*-specific IgA (left) and total IgA (right) were determined by ELISA in the feces and compared to untreated mice (NT). For *Salmonella*-specific IgA, feces from each individual mouse were analyzed (n = 14–16); for total IgA, 2–3 fecal samples from the same group were pooled (n = 6–8). See also Figure S6.

All data are expressed as means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

were not required to establish an IgA response to *Salmonella*; rather, their absence favored it (Figure S4). By contrast, IgA-coated bacteria could enter PPs via another route, presumably epithelial cells or M cells. Indeed, we observed that IgA coating enhanced the uptake of non-invasive *Salmonella* directly by epithelial cells in vitro (Figures S5A and S5B).

IgAs can bind to the luminal membrane of M cells that are scattered in the follicle-associated epithelium overlying the PPs (Mantis et al., 2002), presumably via Dectin-1 receptors that allow reverse transcytosis of IgA-antigen complexes (Rochereau et al., 2013). We therefore asked whether Dectin-1 was required to allow IgA-coated bacterial entrance. We used two approaches: we either neutralized Dectin-1 with a specific antibody or used Dectin-1-deficient mice. Using both strategies, we found that we could not affect the penetrance of IgA-coated bacteria in the PPs although Dectin-1 seemed to be required to reach the mLN in *Clec7a* (the gene for Dectin-1) mice (Figures 4B and 4C). These results suggest that Dectin-1 is dispensable for bacterial entrance in the PPs. Consistently we found that epithelial cells expressed very little amounts of Dectin-1, but nevertheless they internalized IgA-coated bacteria (Figures S5C and S5D).

These results suggest that IgA-coated bacteria enter the PPs with an unknown mechanism that is not dependent on CX3CR1$^+$ phagocytes nor on Dectin-1 binding.

IgA Quantities in C57BL/6 and BALB/c Mice Remain Stable after Co-housing or Fecal Microbiota Transplantation

Having observed that C57BL/6 and BALB/c mice have different abundance of IgAs, even though they are equally capable to mount an IgA response to an invasive strain of *Salmonella*, we wanted to assess whether the observed difference in IgA response was microbiota driven. C57BL/6 and BALB/c mice were co-housed for 1 month and polyreactive and total IgA were measured in serum and feces. There was a slight reduction in polyreactive IgA in the serum and feces of co-housed BALB/c mice compared to BALB/c mice that were not co-housed (Figure 5A). However, co-housing had no effect on total IgA amounts (Figure 5B).

In a second set of experiments, we depleted the endogenous microbiota with antibiotics and then swapped the microbiota across strains. Antibiotic treatment reduced bacteria and total fecal IgAs in both mice (Figures S6A and S6B). Next, mice were administered orally feces from the other mouse strain or the same mouse strain as a control. After 1 month, *Salmonella*-specific IgA and total IgA was measured in the feces (Figure 5C). The treatment had little effect on polyreactive or total IgA. Altogether these results indicate that BALB/c and C57BL/6 mice differ in the amount of basal polyreactive IgAs and there is no difference after microbiota exchange or uniformity. However, at this point we had no proof that cohousing or fecal microbiota transplantation (FMT) did result in microbiota exchange.

C57BL/6 and BALB/c Mice Harbor a Mouse-Strain-Specific Microbiota

We analyzed whether we had achieved microbiota exchange between mice through co-housing or FMT. Feces were collected 8 weeks after co-housing or 4 weeks after FMT. The DNA of bacteria from feces was extracted and the hypervariable V5-V6 region of 16S ribosomal RNA (rRNA) was amplified and sequenced.

As expected, the large majority of identified bacterial species belonged to the phyla Firmicutes and Bacteroidetes for both mice (Figure 6A). Co-housing did not affect this difference

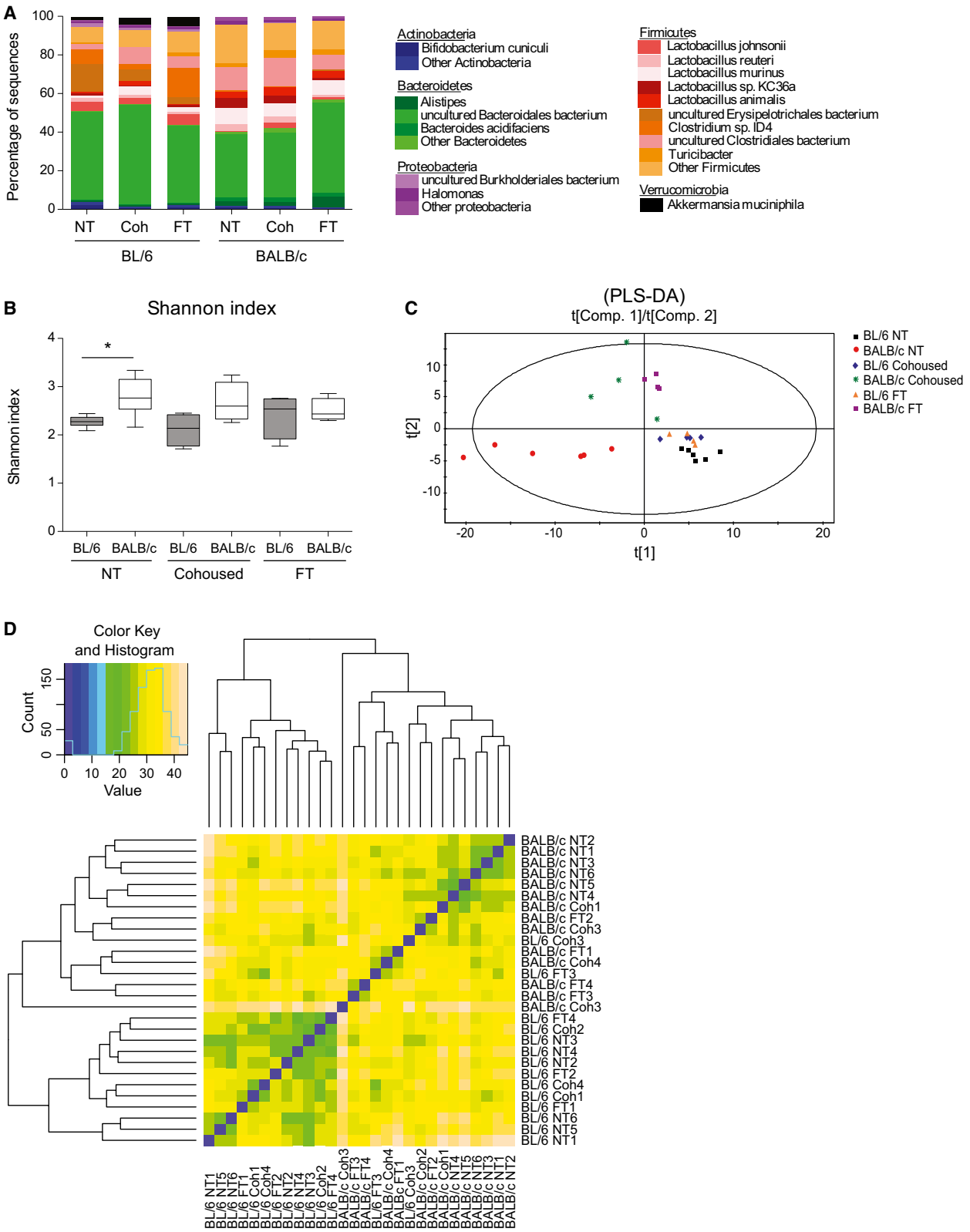

Figure 6. C56BL/6 and BALB/c Mice Harbor a Strain-Specific Microbiota

C57BL/6 and BALB/c mice were housed in separate cages (NT, n = 6) or were co-housed for 8 weeks (Coh, n = 4). The fecal transplant groups (FT, n = 4) received a mix of antibiotics before fecal transplantation with feces from the other mouse strain and were co-housed with untreated mice from the other strain for 4 weeks. Genomic bacterial DNA was isolated from fecal samples and the V5-V6 hypervariable region of 16S rDNA was amplified and sequenced.

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(Figure 6A; Tables S1–S3), but fecal transplantation did reduce the Firmicutes proportion in BALB/c mice, whereas it was increased in C57BL/6 mice (Figure 6A; Tables S4–S6). Moreover, we found significant differences in the abundance of numerous bacterial species between the two mouse strains (Table S7). For several species these differences also remained after co-housing or fecal transplantation.

We found that BALB/c mice had more diverse microbial communities than C57BL/6 mice as attested by the Shannon diversity index at species level (Figure 6B). Co-housing had little effect on diversity but FMT slightly increased diversity in C57BL/6 mice and decreased diversity in BALB/c mice (Figure 6B). In addition, BALB/c and C57BL/6 microbiota composition were distinguished in a partial least-squares discriminant analysis (PLS-DA) (Figure 6C). Co-housing or FMT did not seem to significantly alter microbiota composition in C57BL/6 mice; microbiota from the different treatments closely clustered in a PLS-DA analysis (Figures 6B and 6C). However, these treatments did change bacterial communities in BALB/c mice, although they remained different from the corresponding C57BL/6 groups. In particular, FMT of BALB/c mice with C57BL/6 feces rendered the microbiota more similar to but still distinct from that of C57BL/6 mice. This suggests that BALB/c mice can restore their original higher microbial diversity only when they are transplanted with a more diverse microbiota. If BALB/c mice were transplanted with feces from the C57BL/6 mice, they tended to reduce the diversity, presumably because the transplanted feces contain a less diverse microbiota. By contrast, C57BL/6 mice were predisposed to reduce the diversity of the microbiota (Figure 6C). Hierarchical representation of microbiota composition revealed that BALB/c mice continued to cluster together regardless of their treatment (co-housing or FMT, Figure 6D). The same was true for C57BL/6 animals except for two mice (Figure 6D). In the end, the composition of the microbiota of the treated animals was either more similar to that of the original genetic background or was a new microbiota composition different from either donor or recipient. This suggests that it is very difficult in healthy individuals to change the microbiota even after fecal transplantation.

BALB/c and C57BL/6 Germ-free Mice Differ in IgA Abundance and Capacity to Diversify the Microbiota

An important issue that we were unable to control in the previous experiments was that the treated animals had already been exposed to a microbiota and the antigen specificity of the IgAs might impact microbiota selection after its manipulation. In addition, we could not exclude that during antibiotic treatment we were selecting a population of antibiotic-resistant bacteria. To overcome these limitations, we decided to carry out FMT in germ-free naive mice. 5-week-old BALB/c and C57BL/6 germ-free mice were transplanted with feces from SPF BALB/c and C57BL/6 mice. At baseline, 2, 4, 6, and 8 weeks after transplantation, mice were bled for serum IgA analysis and feces were collected for microbiota and IgA assessments. The first observation we did was that BALB/c mice had already a considerable number of innate IgAs in their serum and feces at baseline, suggesting that this was genetically and not microbiota driven (Figures 7A and 7B). Second, we observed that during colonization, the number of IgAs increased in both mouse strains independent of microbiota composition till week 4 in blood and 6 in feces. At these time points the amount of IgA increased more in BALB/c mice when transplanted with BALB/c-derived feces (4-fold compared to BALB/c transplanted with C57BL/6 feces), suggesting that this microbiota can further promote IgA production in this mouse strain. This increase was significant but modest, probably due to the very low abundance of SFB in the inoculum.

By contrast, the capacity to produce IgA in the C57BL/6 background was driven by the microbiota but was independent of the complexity of the transplanted microbiota and remained well below that of BALB/c mice.

Regarding microbiota diversity, we confirmed that C57BL/6 mice had a less diverse microbiota than did BALB/c mice (inoculum, Figure 7C). In BALB/c mice that had already a conspicuous amount of IgA, selection of the microbiota began already at 2 weeks after FMT, generating higher variation in BALB/c mice receiving either BALB/c or C57BL/6 feces. By contrast at the same time point, C57BL/6 mice having low IgA did not diversify microbiota deriving from the same background although they displayed more variability in individual mice when transplanted with BALB/c inoculum. This result was quite unexpected. However, when we tested the amount of IgA-coated bacteria by dot-blot and cytofluorimetry in the inoculum used for FMT, we found that microbiota from BALB/c mice was more intensely bound to IgAs than microbiota from C57BL/6 mice (Figures S7A and S7B). This suggests that when the microbiota is already coated with IgAs, it is immediately selected in mice, creating more variation among individual mice. At 8 weeks, we observed a situation similar to the one observed during FMT in SPF mice, with BALB/c mice receiving BALB/c inoculum having greater diversity as compared to all the other groups (Figure 7C). Consistent with our hypothesis that IgA abundance correlates with increased microbiota diversity, we could observe a correlation between IgA quantities and microbiota diversity. By pooling all the samples, we observed a slight but statistically significant correlation between the alpha diversity (Shannon index) calculated on all operational taxonomic units (OTUs) detected by QIIME pipeline (Palm et al., 2014) and IgA concentration in serum (0.35; p value 3.311×10^{-4}) and feces (0.4; p value 4.559×10^{-5}) (Figure 7D). Of note, differently from what we observed with FMT in SPF mice, germ-free C57BL/6 mice transplanted with BALB/c microbiota clustered closer to BALB/c mice transferred with the same microbiota in a PLS-DA analysis at 8 weeks (Figure 7E). By contrast, BALB/c or C57BL/6 mice transplanted with C57BL/6 microbiota remained separated (Figure 7E). A representation of

(A) The average percentage of reads for the six experimental groups. Phyla and most prevalent bacterial species are indicated.

(B) Box-plot representation of the Shannon index of diversity relative to species. Error bars represent SEM. *$p < 0.05$.

(C) Discrimination of mice groups based on microbial composition. Score scatter plot of partial least-squares discriminant analysis (PLS-DA) (predictability parameter [Q2cum] = 0.4, goodness-of-fit parameter [R2Y] = 0.68) of microbial profiles classified as BL/6 NT, BALBc NT, BL/6 Mix, BALB/c Mix, BL/6 FT, and BALB/c FT.

(D) Heatmap of hierarchical clustering (euclidean distance) of microbiota composition of all mice at the species level.

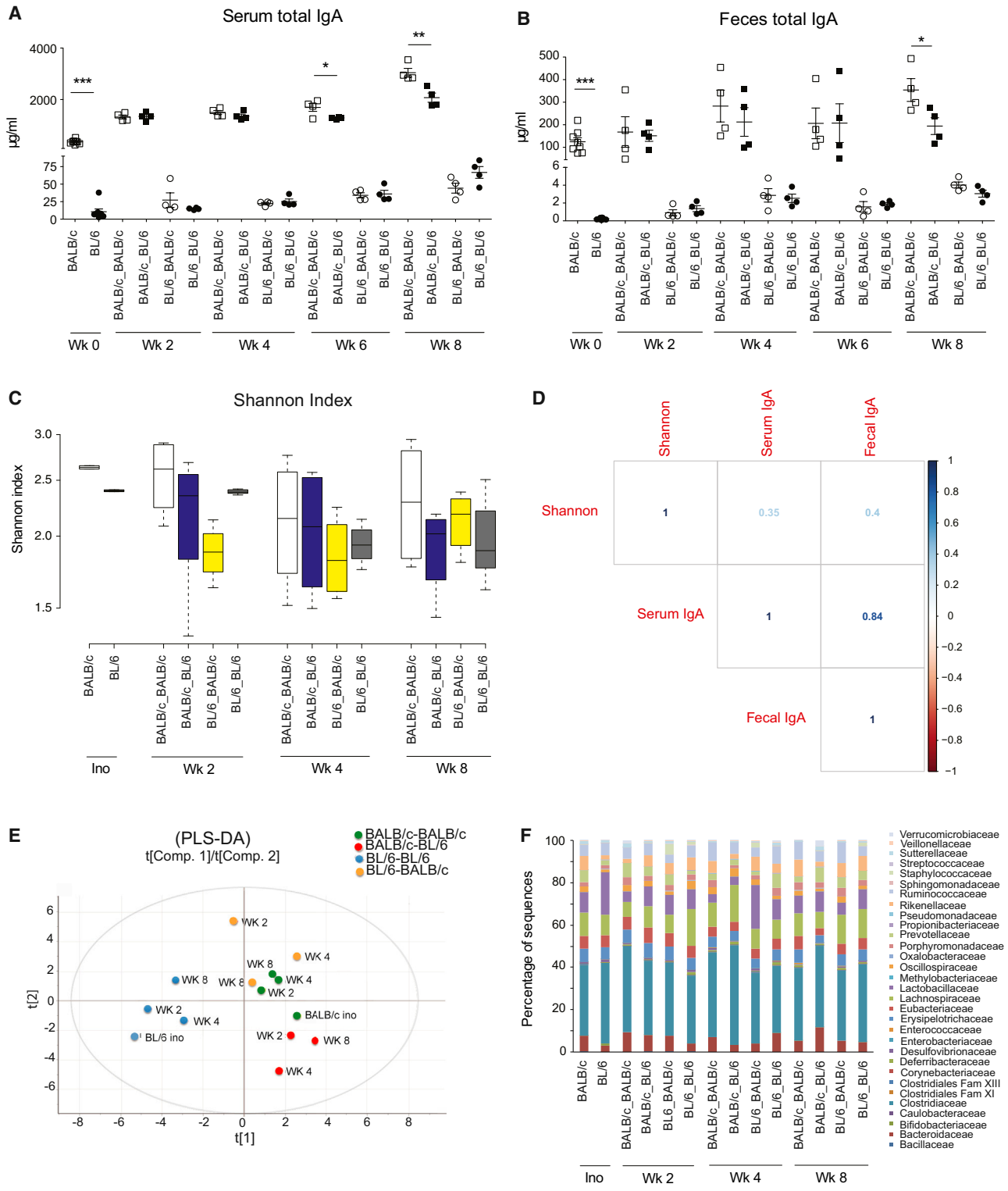

Figure 7. C57BL/6 and BALB/c Germ-free Mice Differ in IgA Amount and Capacity to Diversify the Microbiota

C57BL/6 and BALB/c germ-free mice at 5 weeks of age were orally gavaged for 3 consecutive days with the feces of the same (groups BALB/c_BALB/c and BL/6_BL/6) or the other (groups BALB/c_BL/6 and BL/6_BALB/c) mouse strain (n = 4).

(A and B) Total IgA concentrations in the serum (A) and feces (B) of the mice was assessed by ELISA at baseline, 2, 4, 6, and 8 weeks after transfer. Metagenomic analysis on the two pooled inoculi (BL/6 and BALB/c) and on samples at 2, 4, and 8 weeks on V5-V6 hypervariable region of 16S rDNA was performed with the Illumina MiSeq platform.

(legend continued on next page)

the composition of microbiota at family level of mice transplanted with the different microbiota is shown in Figure 7F.

Altogether, these results suggest that C57BL/6 mice are genetically driven to have a reduced amount of IgAs. This correlates with a reduced diversification of the microbiota, independent of the microbiota to which they are exposed. By contrast, BALB/c mice have a greater capacity to diversify their microbiota, which, however, is limited by the microbiota they are exposed to. Hence, if the microbiota is more diverse, they have an increased Shannon index; if it is less diverse, they cannot diversify it more.

DISCUSSION

The inter-relationship between IgAs and the microbiota has been an open question. Even though recent publications have shown that IgAs can participate to microbiota diversification (Kawamoto et al., 2014) and that the microbiota can drive IgA production in a T-cell-independent manner (Macpherson et al., 2000), it was still unclear how the two were interacting and influencing each other. Here we have shown that genetically distinct mouse strains have different predisposition to diversify the microbiota and that this depends on the abundance and repertoire diversity of innate IgAs. Germ-free naive BALB/c mice already have a conspicuous amount of innate IgAs independent of microbiota colonization. This indicates that there are genetic drivers that regulate IgA production independent of microbial triggers. The finding that BALB/c mice have more abundant B1 B cells, particularly of the B1a subtype, might be responsible for this basal production. We also demonstrated that innate IgAs are required to induce an IgA response to non-invasive bacteria and that this correlated with microbiota diversity. Indeed, we found that IgA coating allowed bacterial penetration in the PPs, which is a prerequisite to induce IgA responses to non-invasive bacteria (Martinoli et al., 2007). This might explain why PPs are absolutely required to induce an IgA response to non-invasive *E. coli* as recently reported (Lécuyer et al., 2014). We have also shown that the presence of bacteria-binding IgAs (be they innate or antigen-driven) favors the induction of antigen-specific IgAs through a positive feedback loop.

Indeed, IgA-coated bacteria penetrated the PPs and mice immunized with an invasive strain of *Salmonella*—that have *Salmonella*-specific IgAs in the feces—could mount an IgA response to antigens expressed only by non-invasive bacteria during a second immunization. By contrast, if C57BL/6 mice were not preimmunized, three administrations of non-invasive bacteria at short time intervals did not elicit an antigen-specific IgA response.

However, mice on a different genetic background (BALB/c), having higher abundance of polyreactive innate IgAs, could induce an IgA response also with the short time interval schedule of vaccination in response to non-invasive pathogenic and commensal bacteria.

Gut mononuclear phagocytes can extend protrusions across the intestinal lumen for direct bacterial uptake (Farache et al., 2013; Niess et al., 2005; Rescigno et al., 2001). $CX3CR1^+$ macrophages have been shown to be absolutely required for the internalization of non-invasive bacteria (Hapfelmeier et al., 2008). Indeed, we found that these cells contributed to bacterial internalization in mice that lacked IgAs cross-reactive to *Salmonella*, because in the absence of $CX3CR1^+$ cell protrusions, non-invasive *Salmonella* could not reach the PPs in C57BL/6 mice. However, these cells were not required for the internalization of IgA-coated bacteria nor for the initiation of an IgA response. Rather, in their absence the IgA response was higher, indicating a negative role of these cells in IgA induction. Indeed, we previously described that $CX3CR1^+$ cells after antigen uptake deliver their cargo to $CD103^+$ dendritic cells that then migrate to mLNs to induce Treg cell differentiation (Mazzini et al., 2014).

Therefore, we are suggesting that when the bacteria are coated with IgAs (either polyreactive during the first stages of life or delivered by the mother during lactation), the bacteria enter through receptor-mediated endocytosis via M cells and are delivered to the right type of antigen-presenting cells, capable of inducing IgA responses. However, we excluded that IgA-coated bacterial entrance is dependent on Dectin-1, a receptor shown to be involved in the reverse trancytosis of IgA-antigen complexes (Rochereau et al., 2013).

Our results support the hypothesis that microbiota diversity is genetically and environmentally driven and depends on the amount and diversity of innate IgAs present at birth, which relates to genetic predisposition, lactation, and the type of microbiota that is inherited. The observation that BALB/c mice have an increase in B1a B cells would support the innate nature of IgAs, and the recent finding that also IgAs derived from B1a B cells undergo somatic hypermutation (Roy et al., 2013) would support the generation of a more diverse repertoire of innate IgAs. We also observed an increase in T follicular helper cells in BALB/c mice as compared to C57BL/6 mice. T follicular helper cells together with T follicular regulatory cells are fundamental to control the quality of the IgA response and microbial diversity (Kawamoto et al., 2014). Along the same lines, it has been shown that during lactation, mother-derived IgAs shape the composition of offspring microbiota (Rogier et al., 2014). Offspring that receive milk from mothers that cannot release IgAs have a different microbiota and are more susceptible to experimental colitis as adults, suggesting that early exposure to affinity-matured IgAs is fundamental for selecting a healthy and protective microbiota.

We decided to swap the microbiota either via co-housing the animals or after antibiotic depletion of the microbiota and subsequent fecal transplantation with the microbiota of the other

(C) Box plot representing the Shannon diversity index relative to species.

(D) Matrix representation of the observed correlation between the alpha diversity (Shannon index) calculated on all OTUs detected by QIIME and IgA concentration in serum (0.35; p value 3.311×10^{-4}) and feces (0.4; p value 4.559×10^{-5}).

(E) Discrimination of mice groups based on microbial composition. Score scatter plot of partial least-squares discriminant analysis (PLS-DA) (predictability parameter [Q2cum] = 0.3, goodness-of-fit parameter [R2Y] = 0.56)

(F) Dynamics of the relative contribution of different microbial groups (family level) to the total microbiota in the feces of BALB/c and BL/6 mice at different time points after conventionalization.

See also Figure S7.

strain. We found in both cases only a minor effect on IgA abundance. When we analyzed the composition of the microbiota after manipulation, we found that the recipient mice did not display a microbiota of the donor, but rather their microbiota was a new and different microbiota that was, however, more similar to their own original microbiota. This indicates that in healthy mice the microbiota composition is quite stable and manipulations will end up with a microbiota that is more similar to the original one. Hence, caution should be taken because co-housing or fecal transplantation does not necessarily mean uniformity of the microbiota. This is probably due to an already defined repertoire of IgAs that controls the colonization and composition of the microbiota and that restores the original microbiota after manipulation. This is supported by a recent report showing that there is a memory pool of IgAs that is restored after plasma cell elimination (Lindner et al., 2012). In addition, we found that C57BL/6 germ-free mice transplanted with a BALB/c microbiota clustered more closely in a PLS-DA analysis to BALB/c mice transplanted with the same microbiota than with C57BL/6 microbiota. This is different from what happens in SPF mice and is probably related to the absence of preformed IgAs that might reselect the original microbiota, or it might also be due to the higher amount of IgA-coated bacteria in the BALB/c microbiota. Indeed, germ-free C57BL/6 mice transplanted with BALB/c microbiota showed already at 2 weeks an increased variability in diversity among the individual mice as compared to mice transplanted with C57BL/6 microbiota. This is in line with the finding that there is very little overlap of the polyclonal IgA repertoires in individual mice (Lindner et al., 2012), confirming that when bacteria are coated with IgAs, they drive a feedback loop of IgA production and this might be stochastically induced in individual mice.

The bacterial species that were different in abundance between C57BL/6 and BALB/c mice and also remained different after co-housing or fecal transplantation mainly belonged to groups of bacteria known to interact closely with the host, such as *Akkermansia*, *Lactobacilli*, and *Bifidobacteria*. These results suggest that host genetics play a role in determining which bacteria colonize the gut and this might impact the induction of an immune response (Gaboriau-Routhiau et al., 2009). Other studies have also suggested that host genetics affect microbiota composition (Spor et al., 2011). Another study reports that bacteria belonging to Sutterella are involved in the degradation of the secretory component and the stability of IgAs (Moon et al., 2015). We observed that C57BL/6 mice inoculated with microbiota from either BALB/c or C57BL/6 mice displayed an increase in the genus Parasutterella, which might contribute to reduce IgA abundance in C57BL/6 mice.

The finding that IgA coating can specify colitogenic bacteria isolated from colitis-prone mice or inflammatory bowel disease patients (Palm et al., 2014) suggests that these bacteria might have a higher probability to colonize the gut and might therefore set the bases for inflammation in predisposed individuals. It would be interesting to know the effect of colonizing mice with IgA-coated bacteria from healthy individuals.

In conclusion, our results indicate a correlation between microbiota diversity and the abundance of polyreactive IgAs (whose number is genetically driven). Higher abundance of IgAs would increase the probability of having a cross-reactive antibody that will allow bacterial internalization in the PPs and the loop of microbiota-specific IgA amplification. This might explain difficulties in colonizing the gut of a healthy adult with probiotics.

EXPERIMENTAL PROCEDURES

Mice

C57BL6/J and BALB/c mice were purchased from Harlan. Germ-free C57BL6/J and BALB/c mice were purchased from Clean Mouse Facility, Department of Clinical Research, University of Bern (Switzerland). Other strain sources are listed in the Supplemental Experimental Procedures. All experiments were performed in accordance with the guidelines established in the Principles of Laboratory Animal Care (directive 86/609/EEC).

Flow Cytometry

Cells were incubated with anti-FcR antibody (clone 24G2, BD Biosciences) for 15 min on ice. Next, cells were incubated with the antibodies of interest for 30 min on ice. DAPI was added before acquisition to exclude dead cells. Samples were acquired with the FACSCanto II or FACSCalibur (BD Biosciences) and analyzed with FlowJo (Treestar). Antibodies used are listed in the Supplemental Experimental Procedures.

454 Sequencing of IgA-Immunoglobulin and Sequence Analysis

Murine IgA repertoire was obtained as described previously (Lindner et al., 2012). For diversity estimation, Shannon indexes were calculated as described in the Supplemental Experimental Procedures.

Immunization and Infection Experiments

All *Salmonella* Typhimurium strains were on a SL1344 background and have been described previously (Martinoli et al., 2007). For infection experiments, 10^6–10^7 CFU wild-type SL1344 were administered. For immunization experiments, 10^9 CFU metabolically defective *S.* Typhimurium (aroA) was administered or 10^9 CFU metabolically defective and non-invasive *S.* Typhimurium (aroAinvA). *Clec7a*-deficient mice were infected with 10^{10} CFUs of non-invasive *S.* Typhimurium.

In some experiments the following strains were used: aroAinvA *Salmonella*-expressing HEL on the cell surface or OVA under transcriptional control of the promoter of the *lacZ* gene and *E. coli* DH5α-expressing OVA (10^9 CFU).

Specific IgA and IgG in serum and feces were detected with ELISA assays as described in the Supplemental Experimental Procedures.

For in vivo Dectin-1 neutralization, BALB/c mice were intraduodenally injected with 200 μg of rat anti-mouse Dectin-1 (clone 2A11, AbD Serotec) or rat IgG2b (Isotype Control, AbD Serotec) and infected with 10^9 CFU *S.* Typhimurium aroAinvA. After 14 hr, PPs and mLNs were collected for gentamicin protection assay. In vivo binding of anti-Dectin-1 antibody to cells derived from PPs compared to isotype control was measured by ELISA (data not shown).

Gentamicin Protection Assay

To determine CFUs in organs, cells were incubated 30 min at 37°C with 50 μg/ml gentamicin to kill extracellular bacteria. Next, cells were lysed with 0.5% sodium deoxycholate and plated on TB-agar plates containing appropriate antibiotics to exclude bacteria other than *Salmonella*.

Microbiota Analysis

C57BL/6 and BALB/c mice were housed in separated cages or were co-housed for 8 weeks. The fecal transplant groups were treated with antibiotic mix and then received transplantation with feces from the other mouse strain or the same mouse strain as a control. BALB/c and C57BL/6 germ-free mice received fecal transplantation for 3 consecutive days with feces from SPF-raised mice of the other mouse strain or the same mouse strain as a control.

For metagenomic analysis, feces were collected and genomic bacterial DNA was isolated with the G'NOME kit (BIO101) as previously described (Furet et al., 2009). The V5-V6 hypervariable region of 16S rRNA gene was sequenced with the Illumina MiSeq platform as described (Manzari et al., 2014). For detailed description of the fecal transplantation and bioinformatic analysis, see the Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes seven figures, seven table, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2015.08.011.

AUTHOR CONTRIBUTIONS

F.F., G. Penna, and M.R. conceived the experiments and wrote the paper. F.F. performed IgA-coating experiments and E.Z. and E.M. performed experiments on germ-free mice. A.C. initiated the study. C.M. and A.M.D. performed 16S metagenomic sequencing. B.F., S.E.A., M.M., and G. Pesole performed bioinformatic analysis. S.M. and O.P. evaluated IgA repertoire diversity. S.M. and L.R. experimented on *Clec7a*$^{-/-}$ mice.

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REFERENCES

Brandtzaeg, P. (2003). Role of secretory antibodies in the defence against infections. Int. J. Med. Microbiol. *293*, 3–15.

Buhnik-Rosenblau, K., Danin-Poleg, Y., and Kashi, Y. (2011). Predominant effect of host genetics on levels of *Lactobacillus johnsonii* bacteria in the mouse gut. Appl. Environ. Microbiol. *77*, 6531–6538.

Cerutti, A., and Rescigno, M. (2008). The biology of intestinal immunoglobulin A responses. Immunity *28*, 740–750.

Chieppa, M., Rescigno, M., Huang, A.Y., and Germain, R.N. (2006). Dynamic imaging of dendritic cell extension into the small bowel lumen in response to epithelial cell TLR engagement. J. Exp. Med. *203*, 2841–2852.

Corthésy, B. (2010). Role of secretory immunoglobulin A and secretory component in the protection of mucosal surfaces. Future Microbiol. *5*, 817–829.

Corthésy, B. (2013). Multi-faceted functions of secretory IgA at mucosal surfaces. Front. Immunol. *4*, 185.

Farache, J., Koren, I., Milo, I., Gurevich, I., Kim, K.W., Zigmond, E., Furtado, G.C., Lira, S.A., and Shakhar, G. (2013). Luminal bacteria recruit CD103+ dendritic cells into the intestinal epithelium to sample bacterial antigens for presentation. Immunity *38*, 581–595.

Furet, J.P., Firmesse, O., Gourmelon, M., Bridonneau, C., Tap, J., Mondot, S., Doré, J., and Corthier, G. (2009). Comparative assessment of human and farm animal faecal microbiota using real-time quantitative PCR. FEMS Microbiol. Ecol. *68*, 351–362.

Gaboriau-Routhiau, V., Rakotobe, S., Lécuyer, E., Mulder, I., Lan, A., Bridonneau, C., Rochet, V., Pisi, A., De Paepe, M., Brandi, G., et al. (2009). The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. Immunity *31*, 677–689.

Hapfelmeier, S., Müller, A.J., Stecher, B., Kaiser, P., Barthel, M., Endt, K., Eberhard, M., Robbiani, R., Jacobi, C.A., Heikenwalder, M., et al. (2008). Microbe sampling by mucosal dendritic cells is a discrete, MyD88-independent step in DeltainvG *S.* Typhimurium colitis. J. Exp. Med. *205*, 437–450.

Hase, K., Kawano, K., Nochi, T., Pontes, G.S., Fukuda, S., Ebisawa, M., Kadokura, K., Tobe, T., Fujimura, Y., Kawano, S., et al. (2009). Uptake through glycoprotein 2 of FimH(+) bacteria by M cells initiates mucosal immune response. Nature *462*, 226–230.

Ivanov, I.I., Atarashi, K., Manel, N., Brodie, E.L., Shima, T., Karaoz, U., Wei, D., Goldfarb, K.C., Santee, C.A., Lynch, S.V., et al. (2009). Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell *139*, 485–498.

Kadaoui, K.A., and Corthésy, B. (2007). Secretory IgA mediates bacterial translocation to dendritic cells in mouse Peyer's patches with restriction to mucosal compartment. J. Immunol. *179*, 7751–7757.

Kawamoto, S., Maruya, M., Kato, L.M., Suda, W., Atarashi, K., Doi, Y., Tsutsui, Y., Qin, H., Honda, K., Okada, T., et al. (2014). Foxp3(+) T cells regulate immunoglobulin a selection and facilitate diversification of bacterial species responsible for immune homeostasis. Immunity *41*, 152–165.

Lécuyer, E., Rakotobe, S., Lengliné-Garnier, H., Lebreton, C., Picard, M., Juste, C., Fritzen, R., Eberl, G., McCoy, K.D., Macpherson, A.J., et al. (2014). Segmented filamentous bacterium uses secondary and tertiary lymphoid tissues to induce gut IgA and specific T helper 17 cell responses. Immunity *40*, 608–620.

Lelouard, H., Fallet, M., de Bovis, B., Méresse, S., and Gorvel, J.P. (2012). Peyer's patch dendritic cells sample antigens by extending dendrites through M cell-specific transcellular pores. Gastroenterology *142*, 592–601.e3.

Lindner, C., Wahl, B., Föhse, L., Suerbaum, S., Macpherson, A.J., Prinz, I., and Pabst, O. (2012). Age, microbiota, and T cells shape diverse individual IgA repertoires in the intestine. J. Exp. Med. *209*, 365–377.

Lostroh, C.P., and Lee, C.A. (2001). The *Salmonella* pathogenicity island-1 type III secretion system. Microbes Infect. *3*, 1281–1291.

Macpherson, A.J., Gatto, D., Sainsbury, E., Harriman, G.R., Hengartner, H., and Zinkernagel, R.M. (2000). A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. Science *288*, 2222–2226.

Mantis, N.J., Cheung, M.C., Chintalacharuvu, K.R., Rey, J., Corthésy, B., and Neutra, M.R. (2002). Selective adherence of IgA to murine Peyer's patch M cells: evidence for a novel IgA receptor. J. Immunol. *169*, 1844–1851.

Manzari, C., Chiara, M., Costanza, A., Leoni, C., Volpicella, M., Picardi, E., D'Erchia, A.M., Placido, A., Trotta, M., Horner, D.S., et al. (2014). Draft genome sequence of *Sphingobium* sp. strain ba1, resistant to kanamycin and nickel ions. FEMS Microbiol. Lett. Published online October 7, 2014. http://dx.doi.org/10.1111/1574-6968.12618.

Marlovits, T.C., Kubori, T., Sukhan, A., Thomas, D.R., Galán, J.E., and Unger, V.M. (2004). Structural insights into the assembly of the type III secretion needle complex. Science *306*, 1040–1042.

Martinoli, C., Chiavelli, A., and Rescigno, M. (2007). Entry route of *Salmonella* typhimurium directs the type of induced immune response. Immunity *27*, 975–984.

Mazzini, E., Massimiliano, L., Penna, G., and Rescigno, M. (2014). Oral tolerance can be established via gap junction transfer of fed antigens from CX3CR1^{+} macrophages to CD103^{+} dendritic cells. Immunity *40*, 248–261.

Mestecky, J., Russell, M.W., and Elson, C.O. (1999). Intestinal IgA: novel views on its function in the defence of the largest mucosal surface. Gut *44*, 2–5.

Moon, C., Baldridge, M.T., Wallace, M.A., Burnham, C.A., Virgin, H.W., and Stappenbeck, T.S. (2015). Vertically transmitted faecal IgA levels determine extra-chromosomal phenotypic variation. Nature *521*, 90–93.

Niess, J.H., Brand, S., Gu, X., Landsman, L., Jung, S., McCormick, B.A., Vyas, J.M., Boes, M., Ploegh, H.L., Fox, J.G., et al. (2005). CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. Science *307*, 254–258.

Palm, N.W., de Zoete, M.R., Cullen, T.W., Barry, N.A., Stefanowski, J., Hao, L., Degnan, P.H., Hu, J., Peter, I., Zhang, W., et al. (2014). Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. Cell *158*, 1000–1010.

Peterson, D.A., McNulty, N.P., Guruge, J.L., and Gordon, J.I. (2007). IgA response to symbiotic bacteria as a mediator of gut homeostasis. Cell Host Microbe *2*, 328–339.

Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., Granucci, F., Kraehenbuhl, J.P., and Ricciardi-Castagnoli, P. (2001). Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat. Immunol. *2*, 361–367.

Rochereau, N., Drocourt, D., Perouzel, E., Pavot, V., Redelinghuys, P., Brown, G.D., Tiraby, G., Roblin, X., Verrier, B., Genin, C., et al. (2013). Dectin-1 is essential for reverse transcytosis of glycosylated SIgA-antigen complexes by intestinal M cells. PLoS Biol. *11*, e1001658.

Rogier, E.W., Frantz, A.L., Bruno, M.E., Wedlund, L., Cohen, D.A., Stromberg, A.J., and Kaetzel, C.S. (2014). Secretory antibodies in breast milk promote long-term intestinal homeostasis by regulating the gut microbiota and host gene expression. Proc. Natl. Acad. Sci. USA *111*, 3074–3079.

Round, J.L., and Mazmanian, S.K. (2009). The gut microbiota shapes intestinal immune responses during health and disease. Nat. Rev. Immunol. *9*, 313–323.

Roy, B., Agarwal, S., Brennecke, A.M., Krey, M., Pabst, O., Düber, S., and Weiss, S. (2013). B-1-cell subpopulations contribute differently to gut immunity. Eur. J. Immunol. *43*, 2023–2032.

Spor, A., Koren, O., and Ley, R. (2011). Unravelling the effects of the environment and host genotype on the gut microbiome. Nat. Rev. Microbiol. *9*, 279–290.

Suzuki, K., Meek, B., Doi, Y., Muramatsu, M., Chiba, T., Honjo, T., and Fagarasan, S. (2004). Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. Proc. Natl. Acad. Sci. USA *101*, 1981–1986.

Talham, G.L., Jiang, H.Q., Bos, N.A., and Cebra, J.J. (1999). Segmented filamentous bacteria are potent stimuli of a physiologically normal state of the murine gut mucosal immune system. Infect. Immun. *67*, 1992–2000.

Umesaki, Y., Setoyama, H., Matsumoto, S., Imaoka, A., and Itoh, K. (1999). Differential roles of segmented filamentous bacteria and clostridia in development of the intestinal immune system. Infect. Immun. *67*, 3504–3511.

Vazquez-Torres, A., Jones-Carson, J., Bäumler, A.J., Falkow, S., Valdivia, R., Brown, W., Le, M., Berggren, R., Parks, W.T., and Fang, F.C. (1999). Extraintestinal dissemination of Salmonella by CD18-expressing phagocytes. Nature *401*, 804–808.

Wijburg, O.L., Uren, T.K., Simpfendorfer, K., Johansen, F.E., Brandtzaeg, P., and Strugnell, R.A. (2006). Innate secretory antibodies protect against natural *Salmonella* typhimurium infection. J. Exp. Med. *203*, 21–26.