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In particular, L-kynurenine, the first breakdown product in the kynurenine pathway, determines resistance to the fungus Candida albicans (Puccetti and Grohmann, 2007). Starvation and a series of endogenous Trp catabolites collectively referred to as "kynurenines" (Puccetti and Grohmann, 2007) are known to modulate the immune response. Indoleamine 2,3-dioxygenase 1 (IDO1), a "metabolic" enzyme that degrades tryptophan (Trp), suppresses inflammation, and is conserved through the past 600 million years of evolution, serves as the host's own pathway of metabolism and immunity through a variety of chemically different metabolites, including amino acid metabolites (Wikoff et al., 2009). In particular, dietary lack of Trp impairs intestinal immunity in mice and alters the gut microbial community (Hashimoto et al., 2012), suggesting that mucosal homeostasis is a multifactorial phenomenon of which Trp metabolism is an important regulatory component. However, the source and nature of any such AhR ligands, any impact of microbial dysbiosis on AhR- and IL-22-driven mucosal reactivity, and whether AhR activation by microbiota-derived metabolites also occurs have all been unclear.

Candida albicans is a commensal of the orogastrointestinal tract, vagina, and skin (Romani, 2011). Owing to microbial dysbiosis or defects in the innate and adaptive immune systems, the fungus shifts from commensalism to parasitism, and it might cause severe infections, such as the oropharyngeal, vulvovaginal, and chronic mucocutaneous forms of human candidiasis. In combination with IL-17A, IL-22 mediates a pivotal innate antifungal resistance in mice (De Luca et al., 2010) and humans (Puel et al., 2010). These cytokines are regulated by the intestinal microbiota (Ivanov et al., 2009; Satoh-Takayama et al., 2008). This could not only explain the high risk of mucosal candidiasis after antibiotic therapy in humans (Sobel, 2007) and gnotobiotic mice (Balish et al., 1993) but also suggest an interplay between Trp catabolism by microbiota, the host’s own pathway of metabolite production, and AhR-mediated mucosal function.

Here, we used Ido1−/− mice and dietary Trp feeding to investigate any links among Trp catabolism in the host, activation of the AhR-IL-22 axis, and the role of microbiota in mucosal immune homeostasis in the gut. By correlating changes in metabolite profiles with microbiota metagenomic composition, we have defined a functional node by which lactobacilli contribute to host-microbial symbiosis and homeostasis at mucosal surfaces.

**RESULTS**

**The AhR-IL-22 Axis Provides Resistance to Mucosal Candidiasis in Ido1−/− Mice**

To examine any effects of host-extrinsic Trp catabolism on AhR-dependent IL-22 production and colonization resistance to Candida, we analyzed Ido1−/− or Ahr−/− mice with or without...
infection. Higher amounts of IL-22 were found in Peyer’s patches (PPs) (Figure 1A), in NKp46+ cells from PPs (Figure 1B), and in the stomachs of naive (0 days postinfection [dpi]) Ido1−/− mice than in their wild-type (WT) counterparts (Figure 1C). After oral infection with C. albicans, the amounts of IL-22 were still higher in the stomach, a target organ in candidiasis, of Ido1−/− mice.
Immunity: Lactobacilli Regulate Mucosal IL-22 via AhR

A-B: Relative abundance of Firmicutes and Bacteroidetes over time in C57BL/6 vs. Ido1−/− mice.

C: Bacteroidetes distribution.

D: Proteobacteria distribution.

E: Lactobacilli, Clostridia, and Bacteroidetes levels in C57BL/6 vs. Ido1−/− mice.

F: Images of C57BL/6 and Ido1−/− mice stomachs.

G: Ct levels of L. reuteri in C57BL/6 and Ido1−/− mice.

H: Pie charts of bacterial diversity at dpi 0 and dpi 3.

I: Log10 copies of L. reuteri in C57BL/6 vs. Ido1−/− mice.

J: Unweighted and weighted analysis of bacteria distribution.

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(Figure 1C) and correlated with the expansion of NKP46+ cells producing IL-22, as revealed by cell number (Figure 1D) and immunofluorescence staining (Figure 1E, 3 dpi). IL-22-producing NKP46+ NK1.1+ cells, expressing lineage-specific transcripts (Figure S1A, available online), also expanded in the PP (Figure S1B). IL-17F and IL-10, but not IL-17A (Figure S1C), were also higher in the stomach (Figure S1C), esophagus (Figure S1D), and ileum (Figure S1E) of Ido1−/− mice. Fewer signs pathogenic for infection were observed in those mice, as revealed by restricted fungal growth (Figure 1F) and low-grade histopathology in the stomach (Figure 1G).

In this setting, dependency of the anticandidal resistance on functional AhR and IL-22 production was demonstrated by the detrimental effects of genetic AhR deficiency (Figures 1F and 1G), which would per se negate IL-22 production (Figure 1C) and ILC3 expansion (Figures 1D and 1E), and by IL-22 neutralization experiments in infected Ido1−/− mice (Figures 1H and 1I and Figure S1F). Overall, these data suggest that sources of AhR ligands—other than host kynurenines—can influence or even enhance mucosal reactivity to Candida.

**Lactobacilli Expand in the Stomachs of Ido1-Deficient Mice**

Of the dietary Trp that is not used in protein synthesis, 99% is metabolized in mammals by IDO1 (Puccetti and Grohmann, 2007). Thus, increased Trp availability in the gut of Ido1−/− mice could alter both the qualitative composition and the metabolic pathways in the local mixed microbial community. We searched for qualitative and/or quantitative changes in microbial communities from fecal samples and the stomach—whose metagenomic profile is far less characterized than that of feces—by barcoded pyrosequencing of 16S rRNA. Upon analyzing a total of 620,369 reads with a median length of 365.4 bp, we found that WT and Ido1−/− mice, either uninfected or infected with Candida, displayed similar relative abundances of major bacterial phyla in the stomach (Firmicutes predominated, and Bacteroidetes and Proteobacteria were the next most abundant) (Figure 2A). Both types of mice shared most taxa at the family levels within Firmicutes (e.g., Lactobacillaceae, Clostridiaceae, Ruminococcaceae, Lachnospiraceae, and Erysipelotrichaceae) (Figure 2B), Bacteroidetes (e.g., uncultured Bacteroidetes, Prevotellaceae, Rikenellaceae, Bacteroidaceae, Porphyromonaceae, and others) (Figure 2C), and Proteobacteria (e.g., Enterobacteriaceae, Suterellaceae, Desulfovibrioaceae, Rhodocyclaceae, Alcaligenaceae, and uncultured Proteobacteria) (Figure 2D) (Tables S1–S4). However, closer examination of the relative abundance of taxa in Firmicutes revealed noticeable differences between Ido1−/− and WT mice. Lactobacilli predominated over Clostridia in Ido1−/− mice, whether naive or after infection (Figures 2B and 2E). Scanning electron microscopy (Figure 2F) and fluorescence in situ hybridization (FISH) (Figure 2G) confirmed that Lactobacillus morotypes were abundant in the stomach, and lactobacilli could be identified in gastric fluids from Ido1−/− mice. Lactobacilli were also expanded by infection in the esophagus (10^7/g versus 10^5/g; Figure S2A) and ileum (10^5/g versus 10^7/g; Figure S2B) of Ido1−/− mice.

Distinct species of the Lactobacillus genus are present in the murine intestinal tract and are grouped in several taxa on the basis of, among other parameters, resistance or susceptibility to vancomycin (Peña et al., 2004). We found striking differences at the level of lactobacilli species between Ido1−/− and WT mice, either naive or carrying infection. L. johnsonii, a member of the vancomycin-sensitive acidophilus group, found as a commensal in humans (Pridmore et al., 2004) and abundantly present in rodents (Jeng et al., 2012), was most abundant in both types of mice, either naive or infected (Figure 2H and Figure S2C). In contrast, vancomycin-resistant L. reuteri—a model gut symbiont in vertebrates as a result of host-driven evolution (Walter et al., 2011)—was poorly represented in naive WT mice but expanded upon infection to a degree comparable to that seen in naive Ido1−/− mice, in which a further striking increase was observed after infection (Figure 2H and Figure S2C). FISH analysis with a L. reuteri-specific fluorescent probe (Figure 2G), unreactive to other Lactobacillus species in gastric fluids (Figure S2D), and quantitative PCR on amplifying 16S rRNA genes (Figure 2I) confirmed the expansion of L. reuteri in the stomachs of Ido1−/− mice. In addition, FISH analysis showed copresence of L. johnsonii, but not L. acidophilus (Figure S2E). Microbial profiling of fecal samples showed that L. reuteri was also highly represented in those mice (Figure S2F).

Differences within lactobacilli in the stomach (Figure 2J) and feces (Figure S2G) of Ido1−/− and WT mice were confirmed by unweighted and weighted UniFrac distance analysis, which takes into account the presence or absence and evolutionary relatedness of operational taxonomic units (OTUs), and by subsequent principal-coordinate analysis, the first principal coordinate (P1) of which explained most of the variance in each
Lactobacilli Regulate Mucosal IL-22 via AhR

A. Tryptophan metabolism diagram:

- TrpH (tryptophan hydroxylase)
- TDQ (tryptophan dioxygenase)
- IDO (indoleamine 2,3-dioxygenase)
- TMO (tryptophan monooxygenase)
- TrpD (tryptophan decarboxylase)
- IAM (indole-3-acetamide)
- IpA (indole-3-pyruvate)
- ILA (indole-3-lactic acid)
- IET (tryptophol)
- TRP, Trp
- TNA, Tryptophanase
- TrpA

B. Culture broths

- L. reuteri
- L. johnsonii
- IAM
- IAA
- IAd

C. Stomach supernatants

- IAM
- IAA
- IAd

D. Gastric fluids

- Naive
- Infected
- IAM
- IAA
- IAd

E. IL-22 levels

- C57BL/6
- L. reuteri
- L. johnsonii
- IAM

F. IL-22 levels

- C57BL/6
- ido1−/−

G. L. reuteri 100-23

- IAM
- IAA

H. IL-22 levels

- L. reuteri 100-23
- ΔLeu4905

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sample between WT and Id01−/− mice and the second principal coordinate (P2) of which showed a high degree of similarity among all samples. In accordance with the ability of lactobacilli to inhibit autochthonous uncultivable segment filamentous bacteria (SFB) (Fuentes et al., 2008), SFB, abundantly present in the distal ilea of naive Id01−/− mice, were markedly decreased in these mice by Candida infection (Figures S3A and S3B). The microbial composition and lactobacilli species in the stomachs of C57BL/6 mice bred at Charles River Laboratories (Calco) was similar to that of C57BL/6 mice bred at The Jackson Laboratory (Bar Harbor) (Figure S3C), thus ruling out the existence of variation from colony to colony. Therefore, lactobacilli are the dominant microbiota in the mouse stomach (Tannock et al., 1982), and IDO1 deficiency is permissive for L. reuteri to establish a niche there.

**L. reuteri Is Selectively Expanded as a Result of Unrestricted Availability of Trp**

Lactobacilli are nutritionally fastidious anaerobes and rely on the availability of easy fermentable sugars, amino acids, vitamins, and nucleotides. In vivo expression technology has shown that lactobacilli might undergo gut-specific gene expression in order to adapt (Frese et al., 2011). Both L. johnsonii (Denou et al., 2007) and L. reuteri (Frese et al., 2011; Walter et al., 2011) are very active transcriptionally in the stomach, where distinct nutritional adaptations provide niche differentiation that allows cohabitation by the two strains in the mouse stomach (Tannock et al., 2012). Because lactobacilli are typically auxotrophic for several amino acids and are capable, under carbohydrate-starvation conditions, of catabolizing l-Trp to bioactive indole derivatives (Gumnallaa and Broadbent, 1999), we reasoned that under conditions of high levels of Trp, lactobacilli might switch from sugar to Trp as an energy source. We measured Trp levels in Id01−/− and WT mice and assessed the consequences of exposure to Trp, over that range, on the growth and metabolic activity of L. reuteri and L. johnsonii. We found that Trp levels were much higher in Id01−/− mice than in WT mice and that levels of l-kynurenines were considerably lower (Figure S4A). Exposure to concentrations of Trp in the range of Id01−/− mice (0.4–0.6 mM) greatly promoted growth of L. reuteri, but not of L. johnsonii, whose growth was, in fact, inhibited (Figure S4B). Growth of L. reuteri was also promoted in the epithelial cells in which Id01 expression had been inhibited by siRNA technology (Figure S4C), suggesting that an increased Trp availability in Id01−/− mice would selectively expand L. reuteri while promoting alternate pathways of Trp degradation in the population being expanded.

**Lactobacilli Produce Indole-3-Aldehyde, a Trp-Indole Derivative**

A variety of indole derivatives act as AhR ligands (Chung and Gadupudi, 2011), which are generated through catabolism of dietary Trp along the “indole pathway” (Figure 3A) by mostly anaerobic intestinal bacteria with tryptophanase activity (Wilkoff et al., 2009). We employed targeted metabolomics to detect indole derivatives both in vivo during infection and in vitro in conditioned media (CM) from L. reuteri and L. johnsonii, which, recovered from the stomach of Id01−/− mice, were grown in the presence of 0.4–0.6 mM Trp with (MRS broth) or without (peptone-tryptone water) carbohydrates. Metabolic profiling revealed that, of the different putative metabolites, indole-3-aldehyde (IAld), a molecule with AhR ligand activity (Figure S4D), was abundantly produced by L. reuteri and only poorly by L. johnsonii in the presence of Trp under carbohydrate-starvation conditions (Figure 3B). IAld was also detected in ex vivo cultures of the stomachs exposed to L. reuteri, but not L. johnsonii (Figure 3C). In infection, IAld was detected in the gastric fluids and urine, where excretion of specific metabolites reflects pathways in Trp metabolism (Figure 3D), and it was maximally produced in Id01−/− mice (Figure 3D insets). IAld was not produced by Clostridia or indole-positive bacteria with tryptophanase activity (Figure S4E), nor were indole levels higher in Id01−/− mice than in WT mice (Figure S4F). Overall, the bulk of these data demonstrate that IAld-producing lactobacilli (rather than tryptophanase–competent organisms or Clostridia) are mostly expanded and utilize Trp in Id01−/− mice.

IAld is a metabolite derived from Trp via several pathways (Figure 3A). The indole pyruvate (IPyA) route is one of the main pathways for IAld synthesis from Trp (Trp→IPyA→IAld) and is catalyzed by the aromatic amino acid aminotransferase (ArAT), a key enzyme phylogenetically conserved in many bacterial species, including lactobacilli, but not Clostridia spp., belonging in the clusters IV and XIVa, which are dominant in the murine gut (Atarashi et al., 2011) (Figure S4G). Although transamination of Trp belongs in minor pathways of amino acid metabolism, ArAT plays a major role in the conversion of aromatic amino acids to aroma compounds by lactobacilli (Rijnen et al., 1999). We assessed whether any enzyme with ArAT-type activity is involved in IAld production by L. reuteri. We found that Lreuz3DRAFT_4905, encoding an ArAT-related aminotransferase (Figure S3A) (http://jgi.doe.gov/), was maximally expressed in L. reuteri under conditions of high Trp levels and low carbohydrate levels (Figure S4B). We were also able to confirm the crucial role of this aminotransferase in Trp utilization...
by *L. reuteri* by generating an araT mutant (referred to as ΔLeu4905) of *L. reuteri* 100-23, a highly adaptive strain from the murine gut (Walter et al., 2011). The mutant was still capable of growing in the presence of externally added Trp (Figure S5C) yet was unable to produce IAld (Figure 3G). Importantly, at variance with *L. reuteri*, araT in *L. johnsonii* FI9785 (National Center for Biotechnology Information [NCBI] GI 268319536) (53% identity, $p = 1 \times 10^{-15}$, by a translated nucleotide query in BLASTx [http://www.blast.ncbi.nlm.nih.gov/]) was not upregulated in high-Trp growing conditions (Figure S5B). In line with the defective growth of *L. johnsonii* under high Trp levels (Figure S4B), the lack of the pyruvate dehydrogenase complex and other enzymes required for conversion of pyruvate to acetdehyde in this strain (Boekhorst et al., 2004), and the role of sugar, more than amino acid, digestion in affecting its gut persistence (Denou et al., 2008), this finding suggests that *L. reuteri*, more than *L. johnsonii*, is equipped with the appropriate metabolic machinery for Trp utilization in the stomach.

**Lactobacilli Induce IL-22 via IAld**

To investigate the role of IAld in AhR-dependent IL-22 production in infection, we measured IL-22 production in ex vivo cytokine secretion testing. We found that ampicillin and chronic restriction of Trp impact gut ecology (Hashimoto et al., 2012) and, specifically, that vancomycin-resistant but ampicillin-sensitive *L. reuteri* 100-23 fully restored antifungal resistance in GF mice, an effect only minimally afforded by the murine *L. reuteri* mutant, and murine *L. johnsonii*. Although equally capable of colonizing mice (Figure S7A), *L. reuteri* 100-23-1 fully restored antifungal resistance in GF mice, an effect only minimally afforded by the *L. reuteri* mutant or *L. johnsonii*, as indicated by the restrained fungal growth (Figure 5A), high levels of IAld in the stomach (Figure 5B) and urine (ranges: 0.4–0.5 μg/ml in naive GF mice, 1.1–1.5 μg/ml in infected GF mice, 5.8–8.7 μg/ml in GF mice + *L. reuteri* 100-23, 1.2–1.7 μg/ml in GF mice + ΔLeu4905 mutant, and 0.9–1.4 μg/ml in GF mice + *L. johnsonii*), IL-22 production (Figure 5C), increased numbers of IL-22+ NKP46+ cells (Figure S7B), and a visible reduction of the enlarged cecum (Figure S7C). *L. reuteri* 100-23, but much less its mutant, similarly provided antifungal resistance (Figure 5D) and mucosal protection (Figure S7D) in antibiotic-treated mice, an effect that was dependent on IL-22 and AhR. As in the in vitro setting, the efficacy of lactobacilli in vivo was independent of IL-17A and adaptive immunity (Figure S7E). These findings indicate that lactobacilli promote anticalendal resistance in vivo via host innate mechanisms converging on the AhR-IL-22 axis, although alternative and/or additional mechanisms of protection are also plausible (Table S5).

**Dietary Trp Affects Amounts of IAld and IL-22 in the Stomach**

To investigate whether lactobacilli expand and produce IAld under physiologically relevant conditions, we evaluated resistance to candidiasis in conventional mice fed a Trp-enriched (Trp+) diet or Trp-low (Trp-) diet for 4 weeks and treated with ampicillin that promotes a long-lasting depletion of lactobacilli, among others, in the ileum and cecum (Jeng et al., 2012; Ubeda et al., 2010) (minimum inhibitory concentration [MIC] ranges of *L. reuteri* and *L. johnsonii* isolated from the murine stomach were 0.15–2.50 and 0.25–1.00 μg/ml, respectively, on susceptibility testing). We found that ampicillin and chronic restriction of Trp both decreased antifungal resistance in WT and *Ido1*−/− mice, whereas in WT mice, Trp feeding increased antifungal resistance, which was negated by ampicillin (Figure 4A) (incidentally, psoriatic-like lesions were observed in Trp-fed *Ido1*−/− mice; Figure S6A). The degree of resistance correlated with the local expression of *Leu23DRAFT_4905* (Figure 4B), IAld (Figure 4C), IL-22 (Figure 4D), and IL-22-producing ILC3s (Figure 4E) in the stomach; all were higher in Trp-fed WT mice and lower in mice treated with ampicillin or on a low-Trp diet. Scanning electron microscopy (Figure 4F) and FISH (Figure 4G) analyses confirmed a decreased lactobacilli content in the stomach after ampicillin treatment and chronic restriction of Trp and the selective expansion of *L. reuteri* upon Trp feeding. Ampicillin did not expand other commensals, such as Clostridia, in the stomach (Figure S6B), nor did it eliminate SFB from the ileum (Figure S6C). Accordingly, treatment with vancomycin, which inhibits Th17-inducing SFB in the ileum (Ivanov et al., 2009), as well as Bacteroidetes from the cecum (Ubeda et al., 2010), slightly decreased antifungal resistance in WT, but not in *Ido1*−/− mice (Figure S6D), a finding pointing to a minor role for SFB and Bacteroidetes in providing antifungal resistance under conditions of high amounts of Trp. We could confirm that, within the species-dependent susceptibility of lactobacilli to vancomycin (Peña et al., 2004), *L. johnsonii* from the mouse stomach was susceptible (MIC range of 1.50–3.00 μg/ml) and *L. reuteri* was resistant (MIC > 256 μg/ml). Thus, these data support the observation that gut amounts of Trp impact gut ecology (Hashimoto et al., 2012) and, specifically, that vancomycin-resistant but ampicillin-sensitive *L. reuteri* expand in the stomach upon Trp feeding and affect local immune homeostasis.

**Lactobacilli Exert Species-Specific Probiotic Effects in Candidiasis**

We next assessed the species-specific probiotic effects of lactobacilli in vivo by employing several experimental approaches that did not intentionally include lactobacilli-free mice, because these animals also lack SFB (Tannock and Archibald, 1984). We assessed antifungal resistance in germ-free (GF) mice, whose stomach is a target organ in candidiasis (Bailish et al., 1993), and conventional mice after gut decontamination by prolonged antibiotic treatment. We reconstituted mice with *L. reuteri* 100-23, its ΔLeu4905 mutant, and murine *L. johnsonii*. Although equally capable of colonizing mice (Figure S7A), *L. reuteri* 100-23 fully restored antifungal resistance in GF mice, an effect only minimally afforded by the *L. reuteri* mutant or *L. johnsonii*, as indicated by the restrained fungal growth (Figure 5A), high levels of IAld in the stomach (Figure 5B) and urine (ranges: 0.4–0.5 μg/ml in naive GF mice, 1.1–1.5 μg/ml in infected GF mice, 5.8–8.7 μg/ml in GF mice + *L. reuteri* 100-23, 1.2–1.7 μg/ml in GF mice + ΔLeu4905 mutant, and 0.9–1.4 μg/ml in GF mice + *L. johnsonii*), IL-22 production (Figure 5C), increased numbers of IL-22+ NKP46+ cells (Figure S7B), and a visible reduction of the enlarged cecum (Figure S7C). *L. reuteri* 100-23, but much less its mutant, similarly provided antifungal resistance (Figure 5D) and mucosal protection (Figure S7D) in antibiotic-treated mice, an effect that was dependent on IL-22 and AhR. As in the in vitro setting, the efficacy of lactobacilli in vivo was independent of IL-17A and adaptive immunity (Figure S7E). These findings indicate that lactobacilli promote anticalendal resistance in vivo via host innate mechanisms converging on the AhR-IL-22 axis, although alternative and/or additional mechanisms of protection are also plausible (Table S5).
Lactobacilli Exert Organ-Specific Probiotic Effects in Candidiasis

“Normal” vaginal flora typically shows a predominance of several Lactobacillus species, which are believed to promote a healthy vaginal milieu by providing numerical dominance and by other mechanisms (Lamont et al., 2011). We assessed whether and which Lactobacillus species would act through the Ahr-IL-22 axis involving IAld in murine vaginal candidiasis. Lactobacilli and in particular L. acidophilus, a constituent of the human vaginal microbiota, were present in the vagina (Figure 6A) (about 10^5 to 10^7 cfu/g of vagina, Ido1^−/− versus WT mice, by quantitative cultures in selected media), as detected by FISH (Figure 6A) and 16S rRNA PCR (Figure 6B). L. acidophilus, recovered from the vagina of Ido1^−/− mice, induced IL-22 (Figure 6C) and produced IAld in vitro (Figure 6D) and in vivo upon administration to GF mice (Figure 6E). IAld was also detected in the vaginal fluids of conventional mice after the infection (Figure 6F) (ranges: 12.3–15.7 ng/ml in naive WT mice, 17.6–22.5 ng/ml in naive Ido1^−/− mice, 38.4–45.7 ng/ml in infected WT mice, and 59.6–71.4 ng/ml in infected Ido1^−/− mice), resistance to which involved expression of antimicrobial-peptide-encoding genes (Figure 6G), occurred via IL-22 and AhR (Figure 6H), and was negated in Ahr^−/− mice or by IL-22 blockade (Figure 6I). Therefore, L. acidophilus exploits the IL-22-AhR axis to provide local resistance to Candida in the vagina, but not in the stomach, thus explaining why healthy women remain resistant to this infection.

entirely asymptomatic despite being colonized by Candida spp. (Sobel, 2007).

IAld Administration Provides Antifungal Resistance and Mucosal Protection

The ability of IAld to act as an AhR ligand and to promote IL-22 production led us to assess whether IAld could be exploited to provide antifungal resistance and mucosal protection from inflammation. We administered IAld to WT and Ahr^/-^/C0 mice with mucosal candidiasis or with dextran sodium sulfate (DSS)-induced colitis, a model in which AhR signaling inhibits inflammation via IL-22 (Monteleone et al., 2011). IAld restored antifungal resistance (Figure 7A) and IL-22 production (Figure 7B) upon infection, ameliorated colitis (Figures 7C and 7D), and induced IL-22 production by colonic NKp46^+^ cells (Figure 7E) in WT mice. These effects were not seen in Ahr^/-^/C0 mice (Figures 7A–7E). Considering that defective Th1 and Treg cell adaptive immune responses—required for clearing the fungus and preventing infection-associated chronic inflammation (De Luca et al., 2010)—are observed in Ido1^/-^/C0 mice (Figure S8), these data clearly indicate that the AhR agonistic activity of IAld could be exploited to provide homeostasis and microbial symbiosis at mucosal surfaces in conditions of impaired adaptive immunity.

DISCUSSION

Our study discloses a signaling pathway that links the bacterial-fungal population dynamics with the mammalian host at mucosal surfaces. A microbial Trp metabolic pathway appeared to be evolved to preserve immune physiology at mucosal surfaces via recruitment of host cells competent for AhR-regulated Il22 transcription. The IL-22-regulated mucosal response allows for survival of mixed microbial communities yet provides colonization resistance to C. albicans. Infections caused by opportunistic fungi have traditionally been viewed as the gross result of a pathogenic automatism, which makes a weakened host more vulnerable to microbial insults. Our study reveals that fungal interaction with the host is more complex than previously appreciated because it includes a triad interaction with indigenous bacteria.

It is known that lactobacilli promote specific immune and metabolic processes in a strain-specific manner (Tannock, 2004; van Baarlen et al., 2011) and that "metabolic products of the lactobacillus population in the stomach could influence the physiology of the adjacent tissues of the host" (Tannock, 2004). The stomach favors the colonization of acid-resistant lactobacilli and is a normal habitat of various fungal taxa in rodents and humans, where yeasts are associated with the mucin layer covering the secreting epithelial cells (Karczewska et al., 2009; Scupham et al., 2006; Tannock and Savage, 1974). Within the stomach, lactobacilli are known to promote resistance to colonization by the fungus (Noverr and Huffnagle, 2004; Savage, 1969) and to be antagonized by C. albicans during postantibiotic recolonization and gastritis (Mason et al., 2012). Decreased lactobacilli content is indeed associated with sustained gastric colonization by the fungus in wasting nude mice (Brown and Ballish, 1978) or mice fed a purified diet (Yamaguchi et al., 2005). Thus, a functional interplay between C. albicans and lactobacilli does occur, a disturbance of which might affect microbial symbiosis and C. albicans mutualism. Although the mechanisms of protection might include the production of other mediators (Vollan et al., 2008) and direct microbial antagonism by lactobacilli (Noverr and Huffnagle, 2004) and IAld (Table S5), both affecting
Candida morphology, our study might help explain how the interaction between commensal fungi and the host’s immune system via local microbiota determines their position from commensals to pathogens and why this position can change continuously (Romani, 2011).

Through the activation of local metabolic pathways involving AhR and converging on IL-22, lactobacilli mediated mucosal antifungal resistance that was contingent upon the host metabolic environment, involved microbial adaptation, and was modulated by diet. In mice, autochthonous SFB are responsible for Th17 accumulation and IL-22 production by Th17 cells in the gut (Ivanov et al., 2009), but it has been unclear whether and which signals from the microbiota selectively direct the production of IL-22 by ILC3s (Lee et al., 2012). A substantial reduction in IL-22"NKP46" pool size was observed in the gut upon depletion of lactobacilli, whereas monoassociation with L. reuteri increased IL-22 production by NKP46" cells in GF mice, thus revealing that certain Lactobacillus species stimulate ILC3s for IL-22 production, an effect carried out in a strain-specific manner, as typically occurs (Tannock, 2004; van Baarlen et al., 2011).

Although lactobacilli share with Gram-positive bacteria the ability to stimulate IL-22 production via TLR2, the metabolic pathway of IL-22 production via AhR stimulation by IAlD...
Lactobacilli are phylogenetically closely related by their small genomes and common metabolic pathways for sugar fermentation and lactic acid production, and they occupy a diverse set of ecological niches as a result of considerable genetic adaptation (Frese et al., 2011). Both L. johnsonii (Denou et al., 2007) and L. reuteri (Walter et al., 2011) are transcriptionally very active in the mouse forestomach, where carbohydrate partitioning provides niche differentiation that allows cohabitation by the two strains (Tannock et al., 2012). We found in this study that, in addition to sugar, amino acid partitioning might also crucially affect cohabitation of the strains in the stomach, where amino acid digestion is crucial for L. reuteri expansion. Accordingly, L. reuteri not only expanded but also expressed Lreu23-IAld, encoding ArAT, in the presence of Trp. Thus, L. reuteri, more than L. johnsonii, is competent for Trp utilization in the stomach. Similar to L. reuteri, L. acidophilus is competent for Trp utilization in the vagina, and together these findings highlight the organ-specific dependency of the host-microbiota interaction at the mucosal surfaces.

The species-specific effects of lactobacilli on C. albicans colonization might offer a plausible explanation for the organ tropism of mucosal candidiasis in mice and humans, for the susceptibility to infection in specific clinical settings, and for the variable and inconsistent effects of probiotic administration in human candidiasis. Because lactobacilli are greatly reduced in the neonatal period (Sjögren et al., 2009) and by stress (Lutgendorf et al., 2008; Tannock and Savage, 1974), our data are consistent with the occurrence of neonatal and vaginal candidiasis, clinical conditions under which the empirical use of lactobacilli as probiotics to prevent infection has long been advocated (Falagas et al., 2006; Manzoni et al., 2006) but never mechanistically explained. Moreover, the very high diversity of Lactobacillus spp. in the human vagina (Human Microbiome Project Consortium, 2012) might offer a plausible explanation for the idiopathic recurrence of vaginal candidiasis in the relative absence of recognized risk factors (Sobel, 2007). We also anticipate that responsiveness to the different lactobacilli probiotic species is determined not only by the characteristics of the consumed strains but also by microbial adaptation, metabolic, and nutritional status of the host. On a translational level, considering that probiotic L. reuteri of human origin also produced IAld upon growing on Trp (data not shown), these findings imply that Trp supplementation in the context of antibiotic coverage might optimize antifungal and probiotic therapy and, most likely, immune physiology (Li et al., 2011). We also found that IAld could substitute for probiotics in protecting and maintaining mucosal integrity during infectious or chemical damage, a finding pointing to the possible use of IAld as a supportive therapy during flora manipulation and intestinal dysbiosis. In this regard, loss of overall diversity and expansion of lactobacilli has been described in murine and human recipients of kynurenine production, by regulating the local amino acid levels and consequently the size and metabolic activity of gut microbiota, IDO1 might be a key molecule in directing the host-microbiota symbiotic relationship and its integration within the innate and adaptive immune systems of vertebrate hosts.
allogeneic bone marrow transplantation, whereas reintroducing the predominant species of Lactobacillus induced significant protection against graft-versus-host disease (Jeng et al., 2012). Considering the reciprocal influence between the fungal community in the gut and local levels of inflammation (Iliev et al., 2012), IAld might represent a prototypical candidate capable of multitasking effectively in transplanted recipients and patients with mucosal inflammatory diseases. The flipside will be the generation of potentially harmful proinflammatory effects of IL-22, such as psoriasis in susceptible hosts.

In conclusion, although the enzyme Trp 2,3-dioxygenase (Opitz et al., 2011), mainly expressed in the liver, regulates Trp concentrations after nutritional Trp uptake under normal circumstances, the high amounts of IDO1 expression at mucosal sites during immune activation (Dai and Zhu, 2010) point to IDO1 as the dominant enzyme regulating the local amino acid nutrient levels, the size and metabolic activity of gut microbiota, and, owing to the host’s own immunomodulatory activity via L-kynurenine production, mucosal immune reactivity. Thus, these data qualify IDO1 as a key molecule in dictating host-microbiota symbiotic relationships and their integration within the adaptive immunity of vertebrate hosts (Figure 7F).

**EXPERIMENTAL PROCEDURES**

A detailed description of the experimental procedures used in this paper can be found in the Supplemental Information.

**Mice, Infections, Colitis, and Treatments**

C57BL/6 mice, 8–10 weeks old, were purchased from The Jackson Laboratory and Charles River Laboratories. Breeder pairs of Ido1<−/−> mice were purchased from The Jackson Laboratory. Homozygous SCID mice on a BALB/c background and Tr2<−/−>, Myd88<−/−>, Ahr<−/−>, Il17a<−/−> mice on a C57BL/6 background were bred under specific pathogen-free conditions at the Animal Facility of Perugia University. Mice were housed in specific pathogen-free barrier facilities at the Animal Facility of Perugia University. Female 8-week-old GF C3H/Orl mice were purchased from The Jackson Laboratory and housed in isolators and fed an irradiated standard rodent chow diet (Mucedola). For infections and colitis, see Supplemental Information. C57BL/6 mice were purchased from The Jackson Laboratory and Charles River Laboratories. Breeder pairs of Ido1<−/−> mice were purchased from The Jackson Laboratory. Homozygous SCID mice on a BALB/c background and Tr2<−/−>, Myd88<−/−>, Ahr<−/−>, Il17a<−/−> mice on a C57BL/6 background were bred under specific pathogen-free conditions at the Animal Facility of Perugia University. Mice were housed in specific pathogen-free barrier facilities at the Animal Facility of Perugia University. Female 8-week-old GF C3H/Orl mice were purchased from The Jackson Laboratory and housed in isolators and fed an irradiated standard rodent chow diet (Mucedola). For infections and colitis, see Supplemental Information. GF mice were monoassociated with a single gavage (gastrointestinal candidiasis) or intravaginal injection (vaginal candidiasis) of 10^5 cfu of lactobacilli 1 day before infection. For Trp supplementation, mice were fed the control diet supplemented with 1g/kg Trp. The composition of the diets and their final Trp levels are listed in Table S6. For metagenomic analysis, littermates from Ido1<−/−> × Ido1<−/−> crosses, kept in the same cage, were compared. Control littermates were included in individual experiments so that nonspecific effects of each genetic manipulation could be checked for, but none were found. See also Supplemental Information.

**Microbiota Analysis by 16S rRNA Sequencing**

Bacterial DNA from gastric fluids and feces of eight mice from each group was extracted with the QIAamp DNA Mini Kit (Qiagen). For each sample, 16S rRNA genes were amplified with a composite forward primer and a reverse primer containing a unique 12-base barcode used for tagging each PCR product. For each sample, five replicate PCR products were combined and purified with Ample XP magnetic purification beads (AgenCourt Bioscience), and products were quantified with the Quant-IT PicoGreen dsDNA Assay Kit (Invitrogen). All libraries were subjected to quality control with the DNA 1000 Agilent Bioanalyzer (Agilent Technologies). Two master DNA libraries were generated from the purified products in equimolar ratios to final concentrations of 35.6 and 33.9 ng/μl. The pooled products were sequenced with a Roche 454 GS Titanium pyrosequencer at the BMR Genomics genome sequencing center at the University of Padova.

**Data Analysis and Sequence Classification**

A total of 620,369 reads with a median length of 365.4 bp were obtained for all samples in both libraries. For making phylogenetic assignments, reads were clustered against a high-quality seed library with the use of CD-HIT-454 with a 97% similarity cutoff. Species names were assigned to OTUs with Ribosomal Database Project SeqMatch (http://rdp.cme.msu.edu/seqmatch/seqmatch.jsp). Weighted and unweighted UniFrac distances were calculated and principal-coordinate analyses were performed with QiIME software.

**Quantification of Gut Microbiota by Culture Method and by Quantitative and Qualitative PCR**

See Supplemental Information.

**Bacterial Strains and Cultures**

Lactobacilli were isolated from the stomach of Ido1<−/−> infected mice, as previously described (Roach et al., 1977). The isolates were identified as lactobacilli on the basis of Gram-stain morphology and by the API 50 CHG system (BioMérieux Italia). For obtaining lactobacilli from the murine stomach, gastric fluids were plated on Man-Rogosa-Sharpe (MRS) agar plates and were incubated anaerobically at 37 °C for at least 3 days, after which visible colonies were selected and cultured anaerobically in MRS broth at 37 °C. DNA was extracted from isolated colonies as described in the Supplemental Information. The obtained sequences were subjected to BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST/) for the identification of Lactobacillus species. Murine L. intestinalis, mnnirus, gasser, animals, helveticus, and crispatus were purchased from Leibniz Institut DSMZ-German Collection of Microorganisms and Cell Cultures (Brunswick). E. coli, Clostridia, Enterococci, and Bacteroides were obtained from mice specimens derived from the gastrointestinal tract and were isolated with the use of selective media.

**Generation of the L. reuteri Lreu4905 Mutant**

The aromatic amino acid aminotransferase nucleotide sequences of L. reuteri DSM 20016 (NCBI GI 14853277, Lreu_0044) and JCM 1112 (NCBI GI 183223999, LAR_0041) were used for identifying a homologous gene in the genome of L. reuteri 100-23 (http://www.jgi.dow.gov/). The locus of the gene encoding the putative aromatic amino acid aminotransferase and flanking nucleotide sequences in L. reuteri 100-23 were analyzed with the BLASTx program against the NCBI databases (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The gene coding for aminotransferase class I was referred to as Lreu4905 in L. reuteri 100-23 was truncated with the use of plRS233 according to a deletion strategy described earlier (see Supplemental Information for references). Transforms were grown in mMRS-erythromycin broth at 42–44 °C for 80 generations for the selection of single-crossover mutants.

**Immunohistochemistry and FISH**

Organs were removed and fixed in 10% phosphate-buffered formalin, embedded in paraffin, and sectioned at 5 μm. Sections were then rehydrated, and after antigen retrieval in Citrate Buffer (10 mM, pH 6), sections were blocked with 5% BSA in PBS and stained with PE anti–IL-22 and FITC-anti–NKP46 (eBioscience). mAbs were incubated overnight at 4 °C. Images were acquired with a fluorescence microscope (BX51 Olympus) with a 40x objective and the analySIS image processing software (Olympus). DAPI (Molecular Probes) was used for counterstaining tissues and detecting nuclei. FISH analysis with fluorescently labeled specific probes was done in gastric and vaginal fluids (see Supplemental Information). Images were acquired with a fluorescence microscope (BX51 Olympus) and the analySIS image processing software (Olympus).

**HPLC-HRMS Analysis**

For metabolomic analysis, HPLC-HRMS (high-performance liquid chromatography-high-resolution mass spectrometry) was performed with both the 1290 Infinity HPLC coupled to a 6540 Q-ToF mass spectrometer equipped with a Jet Stream ESI interface (Agilent Technologies) and the Ultimate 3000 HPLC coupled to a LTQ-Orbitrap mass spectrometer through an ESI interface (Thermo Scientific) (see Supplemental Information).

Statistical Analysis

Data are expressed as mean ± SD. Horizontal bars indicate the means. For multiple comparisons, p values were calculated by a one-way ANOVA (Bonferroni’s post hoc test). For single comparison, p values were calculated by a two-tailed Student’s t test. The data reported are either from one representative experiment out of three to five independent experiments or compiled from three to five experiments.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, eight figures, and eight tables and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2013.08.003.

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