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RESEARCH ARTICLE

Dectin-1 isoforms contribute to distinct Th1/Th17 cell activation in mucosal candidiasis

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The recognition of β-glucans by dectin-1 has been shown to mediate cell activation, cytokine production and a variety of antifungal responses. Here, we report that the functional activity of dectin-1 in mucosal immunity to *Candida albicans* is influenced by the genetic background of the host. Dectin-1 was required for the proper control of gastrointestinal and vaginal candidiasis in C57BL/6, but not BALB/c mice; in fact, the latter showed increased resistance in the absence of dectin-1. The susceptibility of dectin-1-deficient C57BL/6 mice to infection was associated with defects in IL-17A and aryl hydrocarbon receptor-dependent IL-22 production and in adaptive Th1 responses. In contrast, the resistance of dectin-1-deficient BALB/c mice was associated with increased IL-17A and IL-22 production and the skewing towards Th1/Treg immune responses that provide immunological memory. Disparate canonical/noncanonical NF-κB signaling pathways downstream of dectin-1 were activated in the two different mouse strains. Thus, the net activity of dectin-1 in antifungal mucosal immunity is dependent on the host's genetic background, which affects both the innate cytokine production and the adaptive Th1/Th17 cell activation upon dectin-1 signaling.
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INTRODUCTION

Pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and C-type lectin receptors, particularly dectin-1, are essential determinants of host antifungal immunity.[1–3] Dectin-1 is a major β-glucan receptor expressed on the surface of a variety of cells, including myeloid[4] and epithelial cells.[5,6] This receptor recognizes β-1,3-glucans that are exposed on particles such as zymosan and many fungi, including species of *Candida*, *Aspergillus* and *Pneumocystis*,[7–9] either alone or in conjunction with other PRRs, most notably TLR2 and the mannose receptor.[10] As the principal non-opsonic receptor involved in fungal uptake,[11] dectin-1 engagement mediates cell activation, cytokine production and a variety of antifungal responses through the spleen tyrosine kinase (Syk)/caspase recruitment domain-containing protein 9-dependent pathway.[12] Recently, dectin-1 was also shown to signal through Raf-1 and both Syk- and Raf-1-dependent pathways, converging at the level of NF-κB activation to control adaptive immunity to fungi.[13]

Although the crucial role of dectin-1 in antifungal immunity is undisputed both in mice and humans, the precise mechanisms by which dectin-1 signaling contributes to innate and adaptive immune resistance to mucosal and systemic candidiasis have not been completely clarified. The recent discovery of a genetic polymorphism in the human *DECTIN1* gene, Y238X, which generates a truncated protein with impaired cell surface expression and decreased ligand-binding ability, points to the important antifungal function of dectin-1 in humans.[14] Indeed, Y238X carriers were more susceptible to mucocutaneous candidiasis[14] and displayed increased frequency of oral and gastrointestinal colonization with *Candida* species when undergoing allogeneic stem cell transplantation.[15] Interestingly, the Y238X polymorphism had no associated risk with systemic candidiasis, likely due to unimpaired phagocytosis and the killing of *C. albicans* by host leukocytes.[15] Thus, in both humans and mice,[16] dectin-1 appears to be crucially involved in the control of mucosal candidiasis, while discrepant results have been observed for its role in systemic infection.

One of the most important mechanisms of dectin-1-mediated immune resistance relies on the activation of Th1 and Th17 cells. Th17 responses are thought to be important in the defense against *C. albicans*, as patients with diseases characterized by defective Th17 responses (e.g., chronic mucocutaneous candidiasis, hyper IgE syndrome and autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy) show increased susceptibility to mucosal candidiasis.[14,17] While murine studies have failed to show an unequivocal role for dectin-1 in IL-17 production during systemic candidiasis,[7,8,12,18] the role of dectin-1 in the Th1/Th17 cell skewing in experimental mucosal candidiasis has never been directly addressed. Ultimately, the contrasting results in the different models may be related to the site-specific requirements of Th17 cells that are central to the control of mucosal, rather than systemic, infection.[19]

In the present study, we have directly assessed the contribution of the dectin-1/Th17 axis in different models of mucosal candidiasis

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using a side-by-side comparison of dectin-1 functional deficiency in genetically unrelated strains of mice. The contribution of dectin-1-mediated mechanisms of antifungal resistance was indeed found to depend to some extent on the genetic background of the host.[5,20] Macrophages from BALB/c and genetically related strains have been reported to express the full-length dectin-1A and the stalkless dectin-1B isoforms at comparable levels, whereas macrophages from the C57BL/6 background and related mice predominantly expressed the smaller isoform.[21] The results of the present study demonstrate that the functional activity of dectin-1 in both gastrointestinal and vaginal candidiasis is contingent upon the host's genetic background and affects both innate cytokine production, as well as the adaptive Th1/Th17 cell activation.

MATERIALS AND METHODS

Mice

Female C57BL/6 and BALB/c mice, 8–10 weeks old, were purchased from Charles River Laboratories (Calco, Italy). Homozygous *Dectin-1*$^{-/-}$ mice in both C57BL/6 and BALB/c backgrounds were kindly provided by Emiko Kazama, University of Tokyo, Japan. All mice were housed in specialized pathogen-free facilities at the Animal Facility of Perugia University, Perugia, Italy, and were used in accordance with protocols approved by Animal Welfare Assurance A-3143-01.

Fungal strains

The wild-type *C. albicans* strain MKY378,[22] and the isogenic strains obtained by mutagenesis of the parental strain 3153A and that were either capable (referred to as virulent Vir^{3}) or not (low-virulence Vir^{-3}) of yeast-to-hyphae transition, as assessed by germ-tube formation *in vitro*,[23] were used. Yeast cells were obtained by harvesting at the end of the exponential growth phase.

Gastrointestinal infection

Unless otherwise stated, gastrointestinal infection was performed by inoculating mice intragastrically (i.g.) with 1×10^{8} Vir^{3} or Vir^{-3} cells in 200 μl of saline using an 18-gauge 4-cm-long plastic catheter. Re-infection was performed 14 days after the primary i.g. infection by intravenous (i.v.) inoculation of 5×10^{5} Vir^{3} cells. Quantification of fungal burden in the stomach, colon and kidneys of infected mice was performed at different days post-infection (dpi) by plating triplicate serial dilutions of homogenized organs in Sabouraud dextrose agar. The results are expressed as colony-forming units (CFUs) per organ (mean±SE). For histology, paraffin-embedded tissue sections (3–4 μm) of the stomach were stained with periodic acid-Schiff (PAS) reagent. Histology sections were observed using a BX51 microscope (Olympus, Milan, Italy), and images were captured using a high-resolution DP71 camera (Olympus).

Vaginal infection

Vaginal infection was performed by inoculating mice intravaginally with 5×10^{6} Vir^{3} cells in 10 μl saline. Seventy-two hours prior to infection, the mice were injected subcutaneously with 0.1 mg estradiol benzoate (Sigma-Aldrich, St Louis, MO, USA) dissolved in 0.1 ml sesame oil. Estrogen treatments were continued at weekly intervals thereafter. Estrogen-treated control mice were treated with estrogen as described above and given saline intravaginally. Re-infection was performed by intravaginal inoculation with 5×10^{6} Vir^{3} cells in intravaginally infected mice 3 weeks after the primary infection. To quantify vaginal fungal burden at different dpi, 100 μl vaginal lavage was directly plated onto Sabouraud dextrose agar plates supplemented with gentamicin (Sigma-Aldrich). After incubation at 37 °C for 48 h, the number of CFUs was calculated, and the results are expressed as the mean±SE.

Cytospin preparations of the lavage fluids were stained with May–Grünwald–Giemsa and analyzed for polymorphonuclear cell recruitment using a BX51 microscope equipped with a high-resolution DP71 camera (Olympus).

In vitro cultures

Peyer's patches (PPs) cells from naive mice were stimulated for 18 h *in vitro* with 10 μg/ml β-glucan (Sigma-Aldrich) prior to assessing *Tnfa*, *p35*, *p19* and *Il10* gene expression by real-time PCR. The expression of *Il22* was assessed following stimulation with β-glucan as above or with 20 nM 6-formylindolo[3,2-b]carbazole (FICZ) (Enzo Life Sciences, Vonci-Biochem, Italy).

Canonical and noncanonical NF-κB signaling in dendritic cells (DCs)

Murine DCs were obtained by culturing bone marrow cells in RPMI medium containing 10% filtered bovine serum, penicillin, streptomycin, 2 mM L-glutamine in the presence of 20 ng/ml mouse recombinant granulocyte–macrophage colony-stimulating factor (PROSPECbio; Prodotti Gianni S.p.A. Milan, Italy) and 10 ng/ml rIL-4 (PROSPECbio) for 7 days to obtain CD11b^{+} DCs. Cells were stimulated with *C. albicans* Vir^{3} (1:1 ratio) for 30 min at 37 °C. We used the ELISA-based TransAM Flexi NF-κB Family Kit (Active Motif) to monitor the activity of NF-κB family members on nuclear extracts (Nuclear Extract Kit, TransAM Flexi NF-κB Family Kit).

Real-time reverse transcription PCR (RT-PCR)

Real-time RT-PCR was performed using the iCycler iQ detection system (Bio-Rad, Milan, Italy) and SYBR Green chemistry (Agilent Technologies, Milan, Italy). Total RNA was extracted from CD4^{+} T cells purified from the mesenteric lymph nodes (MLNs) of infected animals using an RNeasy Mini Kit (Qiagen, Milan, Italy) and was reverse transcribed with Sensiscript Reverse Transcriptase (Qiagen) according to the manufacturer's instructions. PCR primers were as described previously.[23] The sense/antisense primers for *Ahr* were as follows: sense, 5′-TCCATCCTGGAAATTCGAACC-3′, and antisense, 5′-TCTTCATGCGTCAGTGGTCTC-3′. The thermal profile for real-time PCR was 95 °C for 3 min, followed by 45 cycles of denaturation for 1 min at 95 °C, annealing for 1 min at the appropriate temperature and extension for 30 s at 72 °C. Amplification efficiencies were validated and normalized against *Gapdh* expression. Each data point was examined for integrity by analysis of the amplification plot. The mRNA-normalized data are expressed as the fold increase over day zero.

ELISA assay

Cytokine content was determined by enzyme-linked immunosorbent assays (R&D Systems, Milan, Italy) on stomach homogenates or vaginal lavage fluids. The detection limits (pg/ml) of the assays were <10 for IFN-γ, IL-17A, IL-17F and IL-17E and <3.2 for IL-22.

Statistical analysis

Statistical significance was assessed by ANOVA or unpaired Student's *t*-test with Bonferroni's correction using GraphPad Prism software (GraphPad Software, San Diego, CA, USA). *P* values ⩽0.05 were considered statistically significant. Data are representative of at least

two independent experiments or pooled from three to five experiments. The *in vivo* groups consisted of 6–8 mice/group.

RESULTS

The susceptibility of *Dectin-1*$^{-/-}$ mice to gastrointestinal candidiasis depends on the host's genetic background

Given that distinct expression patterns of dectin-1 isoforms have been described among genetically unrelated strains of mice,[21] we assessed whether and how the host's genetic background influenced dectin-1 activity in mucosal candidiasis. For this purpose, we infected dectin-1-deficient mice i.g. in both the C57BL/6 and the BALB/c backgrounds with two different strains of *C. albicans* and assessed the pattern of susceptibility and/or resistance to infection in terms of fungal burden, inflammatory pathology, and innate and adaptive immunity. We found a contradictory role for dectin-1 in antifungal resistance that depended on the host's genetic background. Indeed, the susceptibility to gastrointestinal infection was increased in C57BL/6 *Dectin-1*$^{-/-}$ mice with both strains of *C. albicans*, as judged by the higher fungal burden in the stomach and colon at 4 and 7 dpi, as well as the dissemination to the kidneys (Figure 1a). A similar susceptibility phenotype was observed using a lower inoculum (5×10^6) of the Vir^3 cells or with the low-virulence Vir^{-3} strain (Figure 1b). Both wild-type and *Dectin-1*$^{-/-}$ mice eventually cleared the infection (Figure. 1a). However, histological analysis of wild-type mice revealed a predominantly superficial infection with limited submucosal inflammation and inflammatory cell recruitment in the stomach, while *Dectin-1*$^{-/-}$ mice showed signs of massive inflammatory infiltrates, with extensive tissue invasion and parakeratosis, as well as hyphae that penetrated the mucosal barrier (Figure 1c). In contrast to what was observed in C57BL/6 mice, BALB/c *Dectin-1*$^{-/-}$ mice were more resistant to infection by either strain of *C. albicans* than wild-type mice, as judged by the decreased fungal burden, lack of peripheral dissemination (Figure 2a) and limited signs of inflammatory cell recruitment and mucosal hyperplasia (Figure 2c). Expectedly, BALB/c *Dectin-1*$^{-/-}$ mice were also more resistant to infection with the lower inoculum of the Vir^3 or with the low-virulence Vir^{-3} strain (Figure 2b). These results clearly show that the susceptibility of *Dectin-1*$^{-/-}$ mice to gastrointestinal candidiasis depends largely on the host's genetic background and less on the fungal strains (at least with the fungal strains we have used).

To assess whether the susceptibility phenotypes of *Dectin-1*$^{-/-}$ mice on both the C57BL/6 and BALB/c backgrounds were retained in vaginal candidiasis, we intravaginally infected each strain of mice with Vir^3 *C. albicans* cells. Similarly to what was observed in the gastrointestinal infections, C57BL/6 *Dectin-1*$^{-/-}$ mice were more susceptible than wild-type mice to the infection as judged by the higher fungal burdens in the vagina (Figure 3a) and the prominent inflammatory cell recruitment in the vaginal fluids (Figure 3c, inset) and vagina. BALB/c *Dectin-1*$^{-/-}$ mice were instead more resistant to the infection than their wild-type counterparts, as judged by the decreased fungal burden (Figure 3b) and limited signs of inflammation in the vagina (Figure 3d) or of inflammatory cell recruitment in the vaginal fluid (Figure 3d, inset).

Dectin-1 promotes distinct cytokine profiles in the different mouse strains

The divergent effects of dectin-1 deficiency in C57BL/6 and BALB/c mice on susceptibility to *Candida* infection suggest that distinct cytokine profiles that critically define the phenotypes observed are activated following dectin-1 engagement. We measured the production of proinflammatory cytokines, such as tumor-necrosis factor (TNF)-α and IL-6, and cytokines of the IL-17 family, such as IL-17A, IL-17F and IL-17E, known to be crucial for mucosal antifungal defense,[23,24] in the stomachs of mice with gastrointestinal candidiasis. We found that the levels of TNF-α and IL-6 were higher in C57BL/6 *Dectin-1*$^{-/-}$ mice and lower in BALB/c *Dectin-1*$^{-/-}$ mice (Figure 4a) compared to the respective wild-type control. A different pattern of production was observed with members of the IL-17 family. IL-17A, IL-17F and IL-17E production was greatly reduced in C57BL/6 *Dectin-1*$^{-/-}$ mice and significantly increased in BALB/c *Dectin-1*$^{-/-}$ mice (Figure 4b) compared to the respective wild-type strains. The cytokine profiles in the vaginal fluids mirrored those in the stomach homogenates of gastrointestinally infected mice, as IL-17A, IL-17F and IL-22 were almost completely absent in C57BL/6 *Dectin-1*$^{-/-}$ mice and significantly increased in BALB/c *Dectin-1*$^{-/-}$ mice (Figure 4c) compared to the respective controls. Altogether, these findings point to similar susceptibility phenotypes to both vaginal and gastrointestinal candidiasis in the absence of dectin-1.

To further demonstrate that the different isoforms promote distinct cytokine profiles, we assessed cytokine gene expression in PPs following stimulation with β-glucan. We found that *Tnfa* induction was critically dependent on dectin-1, irrespective of the genetic background (Figure 5). In contrast, the expression levels of *IL-12p35*, *IL-23p19* and *Il10* were differentially affected in the absence of dectin-1. The expression of *IL-23p19* was particularly reduced in PP from C57BL/6 compared to BALB/c *Dectin-1*$^{-/-}$ mice, while the expression levels of *IL-12p35* and *Il10* were particularly reduced in BALB/c compared to C57BL/6 *Dectin-1*$^{-/-}$ mice. Altogether, these results indicate that the impact of dectin-1 function on the cytokine response is contingent upon the genetic background and presumably upon distinct dectin-1 isoform expression.

Dectin-1 differentially impacts the Ahr/IL-22 axis in the different mouse strains

IL-22 plays a crucial role in the innate immune defense and mucosal protection from damage in mucosal candidiasis.[23] Produced by NK22 cells expressing the aryl hydrocarbon receptor (AhR), IL-22 directly targets gut epithelial cells to induce signal transducer and activator of transcription 3 phosphorylation and the release of S100A8 and S100A9 peptides (known to have anti-candidal activity and anti-inflammatory effects).[3,23] We looked for *Ahr* expression and IL-22 production *in vivo*, as well as on PP cells comparatively stimulated *in vitro* with either β-glucan or the AhR agonist FICZ. We found that C57BL/6 *Dectin-1*$^{-/-}$ mice failed to upregulate *Ahr* expression and IL-22 production during infection as compared with wild-type controls (Figure 6a). *In vitro*, *Il22* expression was not upregulated in PP from C57BL/6 *Dectin-1*$^{-/-}$ mice upon stimulation with either β-glucan or FICZ (Figure 6b). The opposite pattern of *Ahr* expression and IL-22 production was observed in BALB/c *Dectin-1*$^{-/-}$ mice, which showed a significant increase in *Ahr* expression and concomitant IL-22 production *in vivo* (Figure 6c) and *in vitro* after stimulation with FICZ (Figure 6d). These data suggest that dectin-1 signaling differentially impacts the functional activity of the AhR/IL-22 axis in gastrointestinal candidiasis.

Dectin-1 promotes distinct adaptive memory Th immune responses that are dependent on mouse genetic background

Stimulation of dectin-1 on DCs efficiently generates Th1 and Th17 responses.[25] We evaluated the activation of distinct Th cell subsets in vaccine-induced resistance to *C. albicans*. We subjected i.g. infected mice to i.v. re-infection with the fungus 2 weeks later and evaluated the parameters of fungal growth in the kidneys and activation of CD4$^+$ Th cells in the MLN. We found that C57BL/6 *Dectin-1*$^{-/-}$ mice were

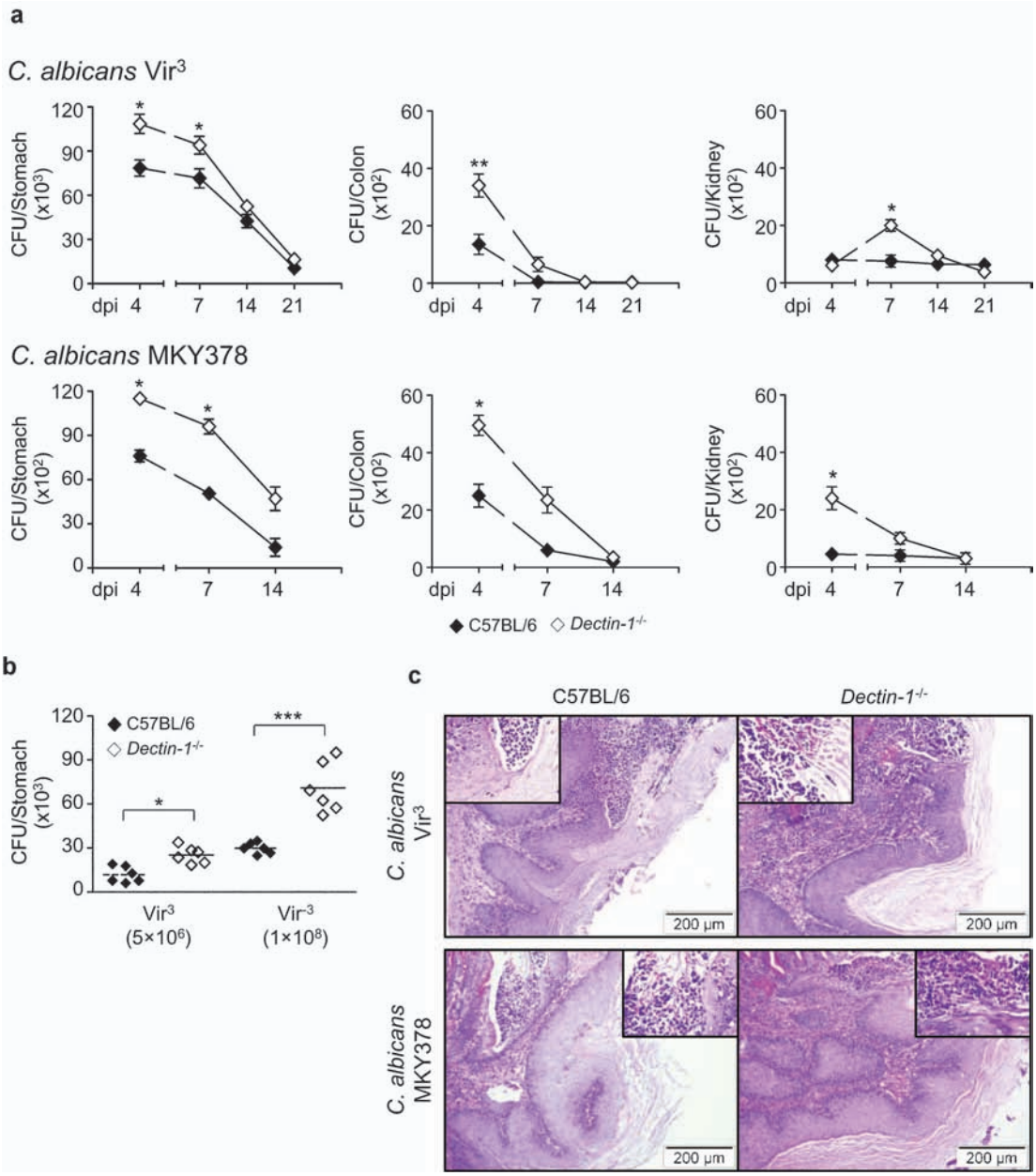

Figure 1 C57BL/6 *Dectin-1*$^{-/-}$ mice are susceptible to gastrointestinal candidiasis. (**a**) Fungal growth (CFU±SE) at 4, 7, 14 and 21 dpi in the stomach, colon and kidneys of wild-type (black diamonds) or *Dectin-1*$^{-/-}$ (white diamonds) mice infected i.g. with 1×10^8 cells of Vir^3 or MKY378 strains of *C. albicans*. Data are representative of 2–4 independent experiments. (**b**) Fungal growth (CFU±SE) at 4 dpi in the stomach of wild-type (black diamonds) or *Dectin-1*$^{-/-}$ (white diamonds) mice infected i.g. with 5×10^6 Vir^3 cells or 1×10^8 low-virulence Vir^{-3} cells. Data are representative of 2–4 independent experiments. (**c**) Histological analysis of stomach tissue from wild-type or *Dectin-1*$^{-/-}$ mice infected i.g. with 1×10^8 *C. albicans* Vir^3 or MKY378 cells. PAS-stained stomach sections (at 4 dpi) showing fungal elements penetrating the mucosal barrier and inflammatory cell recruitment in *Dectin-1*$^{-/-}$ mice. Representative images of 2–4 independent experiments are shown. *$P\leqslant0.05$, **$P\leqslant0.01$ and ***$P\leqslant0.001$, wild-type C57BL/6 *vs. Dectin-1*$^{-/-}$ mice. *C. albicans*; *Candida albicans*; CFU, colony-forming unit; dpi, days post-infection; i.g., intragastrically; PAS, periodic acid-Schiff.

unable to resist re-infection, while wild-type C57BL/6 mice were able to resist re-infection (Figure 7a). In contrast, both wild-type and *Dectin-1*$^{-/-}$ BALB/c mice resisted the re-infection (Figure 7b). Indeed, resistance to re-infection occurred despite the high susceptibility to the primary disseminated infection exhibited by BALB/c *Dectin-1*$^{-/-}$ mice; this finding points to the ability of these mice to mount strong protective memory responses to the fungus. Similar to the above findings, intravaginally infected C57BL/6 *Dectin-1*$^{-/-}$ mice were unable to resist re-infection, in contrast to BALB/c *Dectin-1*$^{-/-}$ mice (Figure 7c and d). On assessing the quality of the Th cell responses, we found a decreased expression of *Ifng* and *Il10* and the corresponding transcription factors *Tbet* and *Foxp3* in the MLNs of C57BL/6 *Dectin-1*$^{-/-}$ mice compared to wild-type mice (Figure 7e). This reduction was associated with an increased expression of *Il17a* and *Rorc* (Figure 7e), suggesting that Th1 cell activation, more than Th17, is dependent on dectin-1 in C57BL/6 mice. In BALB/c *Dectin-1*$^{-/-}$ mice, resistance to re-infection was instead associated with a robust Th1/Treg response and was associated with levels of *Il17a/*

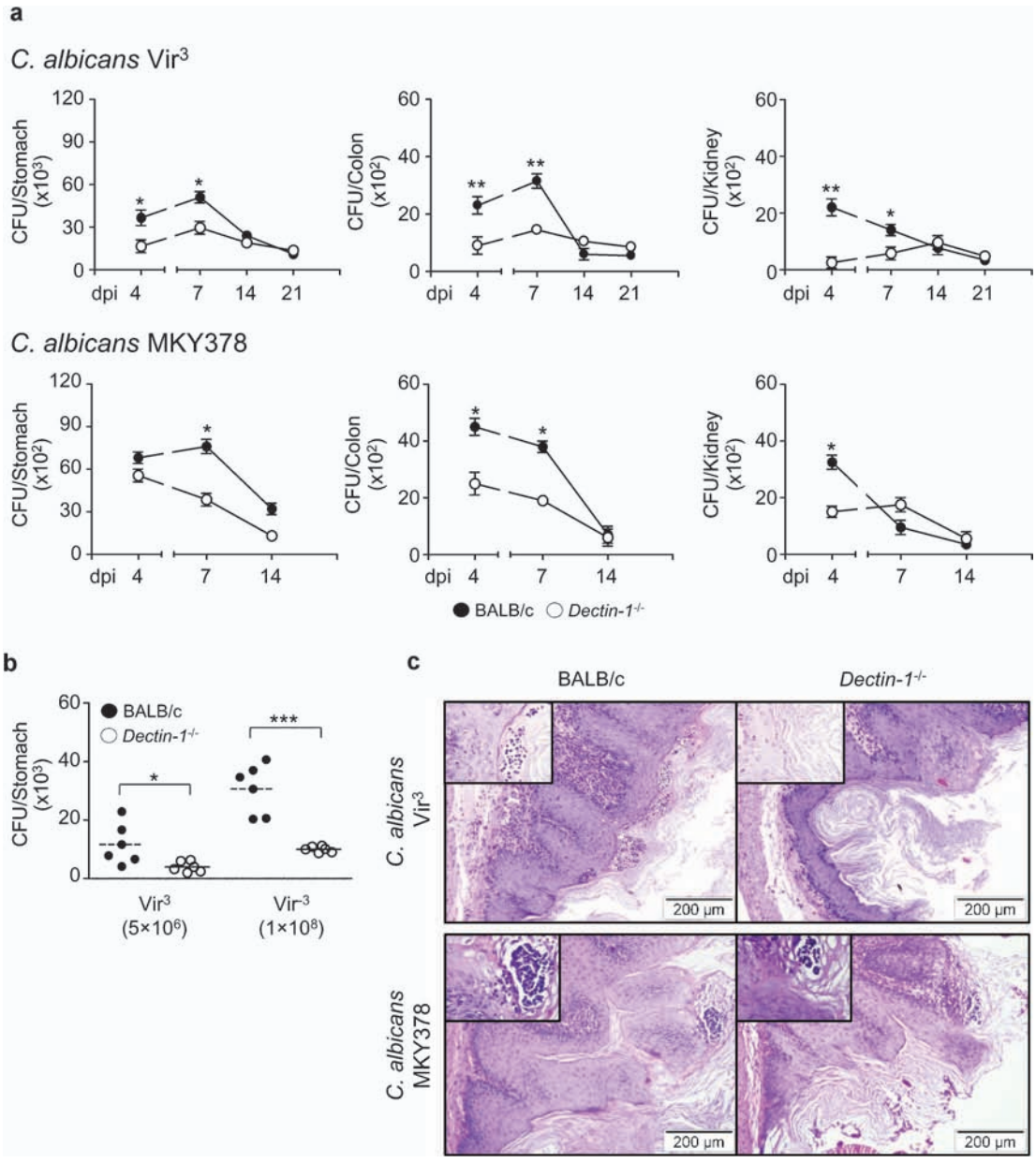

Figure 2 BALB/c *Dectin-1*$^{-/-}$ mice are resistant to gastrointestinal candidiasis. (**a**) Fungal growth (CFU±SE) at 4, 7, 14 and 21 dpi in the stomach, colon and kidneys of wild-type (black dots) or *Dectin-1*$^{-/-}$ (white dots) mice infected i.g. with 1×10^8 cells of Vir^3 or MKY378 strains of *C. albicans*. Data are representative of 2–4 independent experiments. (**b**) Fungal growth at 4 dpi in the stomach of wild-type (black dots) or *Dectin-1*$^{-/-}$ (white dots) mice infected i.g. with 5×10^6 Vir^3 cells or 1×10^8 low-virulence Vir^{-3}cells. Data are representative of 2–4 independent experiments. (**c**) Histological analysis of stomach tissue from wild-type or *Dectin-1*$^{-/-}$ mice infected i.g. with 1×10^8 cells of *C. albicans* Vir^3 or MKY378. PAS-stained stomach sections (at 4 dpi) showing absence of mucosal damage and inflammatory cell recruitment in *Dectin-1*$^{-/-}$ mice. Representative images of 2–4 independent experiments are shown. *$P\leqslant0.05$, **$P\leqslant0.01$ and ***$P\leqslant0.001$, wild-type BALB/c *vs.* *Dectin-1*$^{-/-}$ mice. *C. albicans*; *Candida albicans*; CFU, colony-forming unit; dpi, days post-infection; i.g., intragastrically; PAS, periodic acid-Schiff.

Rorc expression that were actually lower than those of wild-type mice (Figure 7f), suggesting that Th17 cell activation, more than Th1, is dependent on dectin-1 in BALB/c mice. These results indicate that the role of Dectin-1 in shaping antifungal memory Th cell responses also depends on the genetic background of the host.

NF-κB signaling pathways are affected differently by dectin-1 deficiency, depending on the mouse strain

In addition to the classical Syk-dependent pathway leading to canonical NF-κB p65 and c-Rel subunit activation and TNF-α/IL-10 production,[26] the Raf-1-dependent pathway, which inhibits the expression of the noncanonical NF-κB RelB subunits and crucially promotes *Il12b* transcription,[13] has also been described. We assessed whether either or both of these pathways were altered in the absence of dectin-1 in DCs from C57BL/6 and BALB/c mice exposed to *C. albicans*. We found that the nuclear translocation of c-Rel was impaired to a greater degree in *Dectin-1*$^{-/-}$ mice in the C57BL/6 background than in the BALB/c background in response to the fungus (Figure 8a). In contrast, the nuclear translocation of RelB was increased to a higher degree in *Dectin-1*$^{-/-}$ mice in the BALB/c background than in the

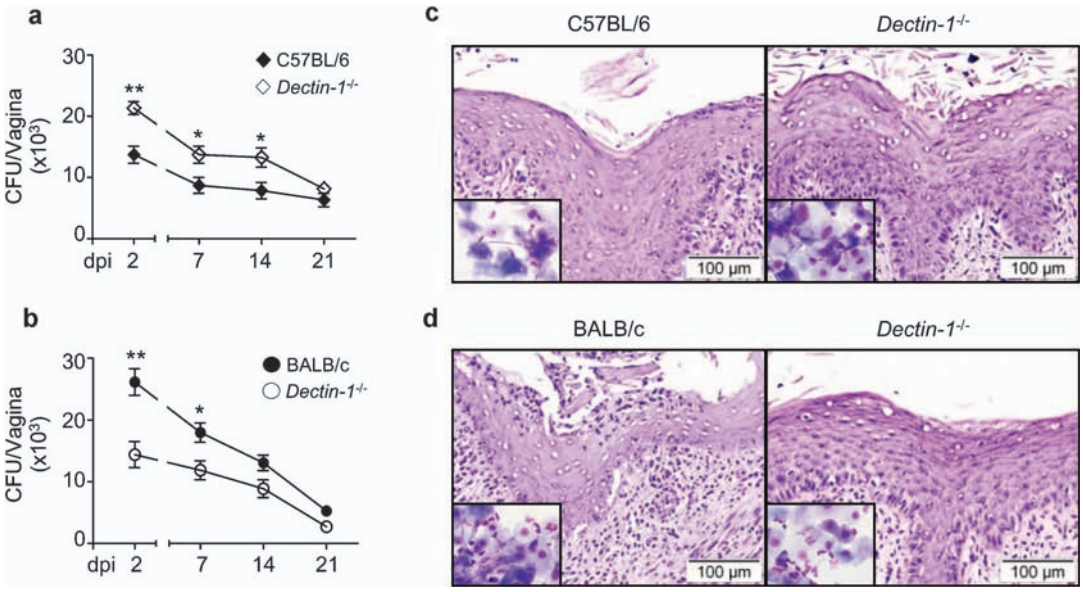

Figure 3 Dectin-1 deficiency affects susceptibility to vaginal candidiasis. Fungal growth (CFU ± SE) at 2, 7, 14 and 21 dpi in the vagina of (**a**) C57BL/6 wild-type (black diamonds) or *Dectin-1* $^{-/-}$ (white diamonds) and (**b**) BALB/c wild-type (black dots) or *Dectin-1* $^{-/-}$ (white dots) mice infected intravaginally with 5×10^6 cells of *C. albicans* Vir[3]. Data are representative of 2–4 independent experiments. Histological analysis of the vagina from (**c**) C57BL/6 or *D,* BALB/c wild-type or *Dectin-1*$^{-/-}$ mice, infected intravaginally with 5×10^6 cells of *C. albicans* Vir[3], as indicated. PAS-stained vaginal fluids (at 2 dpi) showing hyphal growth and inflammatory cell recruitment in C57BL/6 *Dectin-1*$^{-/-}$ mice but not in BALB/c *Dectin-1*$^{-/-}$ mice. Representative images of 2–4 independent experiments are shown. $*P\leqslant0.05$, $**P\leqslant0.01$, wild-type *vs. Dectin-1*$^{-/-}$ mice in either background, as indicated. *C. albicans*; *Candida albicans*; CFU, colony-forming unit; dpi, days post-infection; PAS, periodic acid-Schiff.

C57BL/6 background (Figure 8b). These findings suggest that the Syk and the Raf-1 pathways are affected differently in the absence of dectin-1 signaling in the two mouse strains.

DISCUSSION

Although the dectin-1/inflammasome host immune pathway drives protective Th17 responses and distinguishes between colonization and tissue invasion by *C. albicans*,[27] the present study shows that the function of dectin-1 in mucosal antifungal immunity extends beyond Th17 cell activation and is critically dependent on the genetic background of the host. This phenomenon has been previously demonstrated for infections with *A. fumigatus*[5] and *Coccidioides* spp.,[20] in which the mechanisms of antifungal resistance were to some extent determined by the host's genetics. It has been suggested that dectin-1 responses may be dependent on the fungal strains[1] and on the physical status of the β-glucans (soluble or particulate).[28] Although we have obtained similar findings using two distinct *C. albicans* strains, we cannot rule out that these responses are also contingent upon the fungal strains. The present study is the first to show a side-by-side comparison of dectin-1 functional deficiency in mucosal candidiasis driven by a given fungal strain in genetically unrelated strains of mice. Consistent with the distinct downstream signaling pathways that are activated to regulate immunity to fungi upon dectin-1 engagement,[13] we found that dectin-1 signaling is either required or dispensable in mucosal candidiasis, depending on mouse genetics. In C57BL/6 mice, dectin-1 was required for the control of fungal colonization at mucosal surfaces, both in the gastrointestinal and vaginal tracts, and was required for the production of IL-17A, IL-17F and IL-22 at sites of infection. In contrast, dectin-1 was dispensable in BALB/c mice, in which resistance to infection was associated with the production of IL-17A, IL-17F and IL-22. With respect to adaptive memory Th responses, dectin-1 was apparently required for the activation of Th1/Treg memory responses in C57BL/6 mice and for Th17 memory responses in BALB/c mice. It has been shown that dectin-1 may influence cytokine production in DCs, leading to Th1/Th17 cell activation and affecting the balance between canonical/noncanonical NF-κB activation in DCs.[13] Although we found a different pattern of canonical/noncanonical NF-κB activation in DCs from each type of mouse, the exact molecular pathways linking DC activation to Th skewing during infection in the absence of dectin-1 in the different genetic backgrounds need further evaluation. Additional studies aimed at selectively inhibiting the Syk or Raf-1 pathway in the two strains of mice are required to provide the causal association between signaling pathways activated *in vitro* and susceptibility/resistance to infection *in vivo*.

It is plausible to hypothesize that these two extremely divergent phenotypes may rely on the differential expression of dectin-1 isoforms. Indeed, these alternatively spliced isoforms have been found to lead to the production of different levels of TNF upon zymosan recognition,[21] suggesting that the structure of the receptor and its ability to form dimers through the stalk region may influence cytokine production. It is therefore not surprising that dectin-1 deficiency in C57BL/6 and BALB/c mice leads to disparate cytokine profiles upon mucosal infection with *C. albicans*. In addition and despite that no functional consequences have been observed upon zymosan recognition, nonsynonymous single nucleotide polymorphisms have been identified in C57BL/6 mice by genetic comparison with the BALB/c background,[21] suggesting additional functional variability of dectin-1 between these strains. In this regard, as cooperative signaling between dectin-1 and other PRRs is crucial for efficient recognition of *C. albicans* cells,[10] the differences in TLR2 and mannose receptor expression observed among different inbred strains of mice[29,30] may offer an additional plausible explanation.

One interesting observation of the present study is the intriguing relationship between dectin-1 and AhR. Originally recognized as

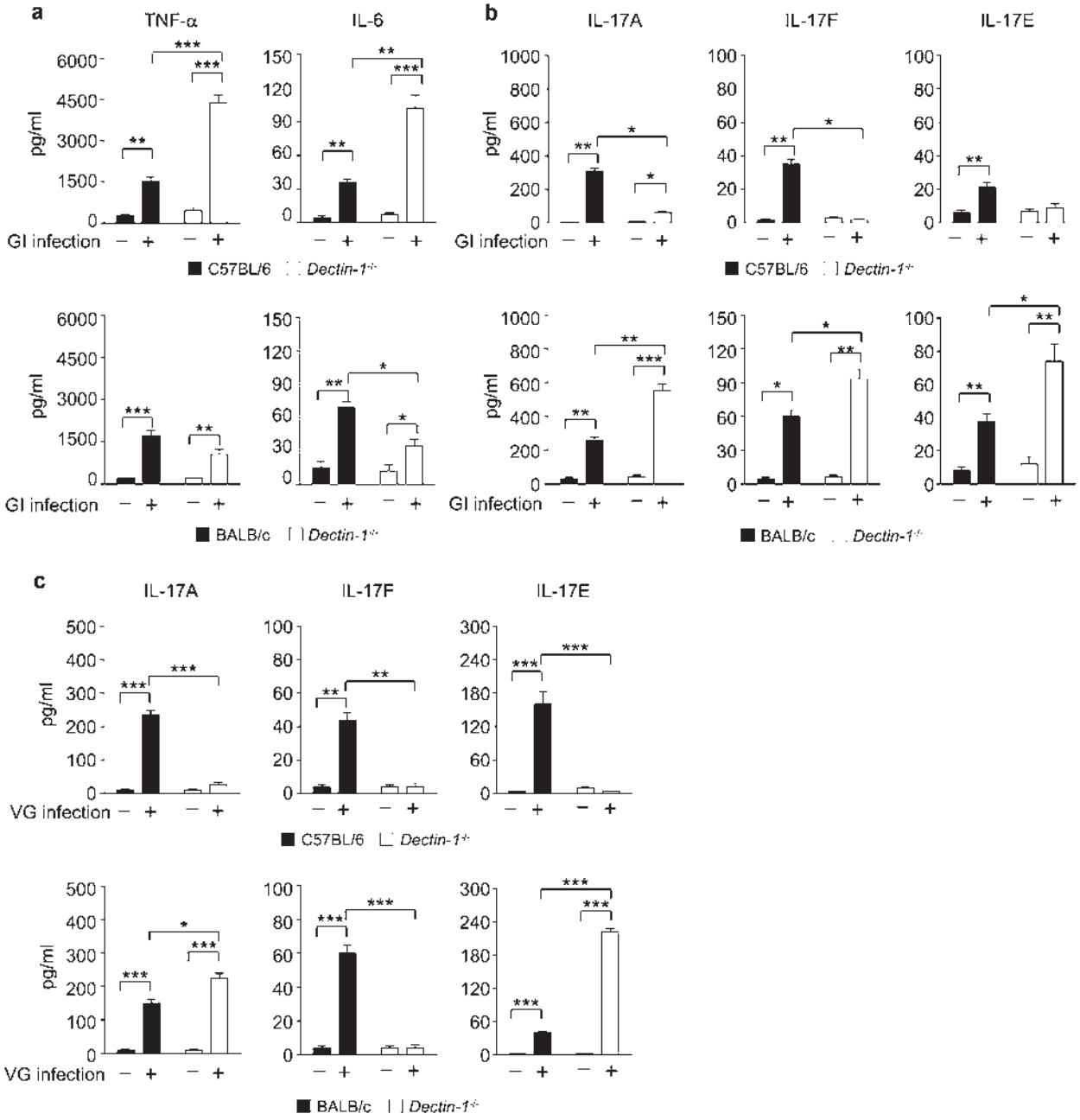

Figure 4 Dectin-1 promotes distinct cytokine profiles in the different mouse strains. Production of (**a**) TNF-α and IL-6 and (**b**) IL-17A, IL-17F and IL-17E at 4 dpi in the stomach of wild-type (black bars) and *Dectin-1*$^{-/-}$ (white bars) mice from a C57BL/6 or BALB/c background infected i.g. with 1×10^8 cells of *C. albicans* Vir[3]. (**c**) Production of IL-17A, IL-17F and IL-22 at 2 dpi in the vaginal fluid of wild-type (black bars) or *Dectin-1*$^{-/-}$ (white bars) mice from a C57BL/6 or BALB/c background infected intravaginally with 5×10^6 cells of *C. albicans* Vir[3]. Data represent the mean±SE of three independent experiments. $^*P\leqslant0.05$, $^{**}P\leqslant0.01$ and $^{***}P\leqslant0.001$, uninfected *vs.* infected mice or infected wild-type *vs.* infected *Dectin-1*$^{-/-}$ mice in either background, as indicated. *C. albicans*; *Candida albicans*; dpi, days post-infection; i.g., intragastrically; TNF, tumor-necrosis factor.

causing immunosuppression after binding dioxin, mammalian AhR is now known to crucially affect IL-22 production[31]and the balance of T-cell differentiation into Th1/Treg *vs.* Th17 cells.[32] In this regard, IL-22 was recently found to be produced in response to *A. fumigatus* by a dectin-1-dependent mechanism.[33] In combination with IL-17A, IL-22 has been found to be crucially involved in the control of *Candida* growth in the gastrointestinal tract in conditions of Th1 and Th17 deficiency.[23]Moreover, vaginal epithelial cells also produced S100A8 and S100A9 following interaction with *Candida*,[34] suggesting the possible involvement of IL-22 in vaginal candidiasis. Thus, IL-22$^+$ cells, employing ancient effector mechanisms of immunity, may represent a primitive mechanism of resistance against the fungus under conditions of limited inflammation. The finding that the AhR/IL-22 axis was impaired in C57BL/6 *Dectin-1*$^{-/-}$ mice but not in BALB/c *Dectin-1*$^{-/-}$ mice indicates that dectin-1 receptor cooperativity may go beyond PRRs to include receptors involved in the cell cycle and metabolism.[35]

Regardless of the mechanisms of this cooperative signaling, our study clearly shows that dectin-1 crucially contributes to the balance of Th1/

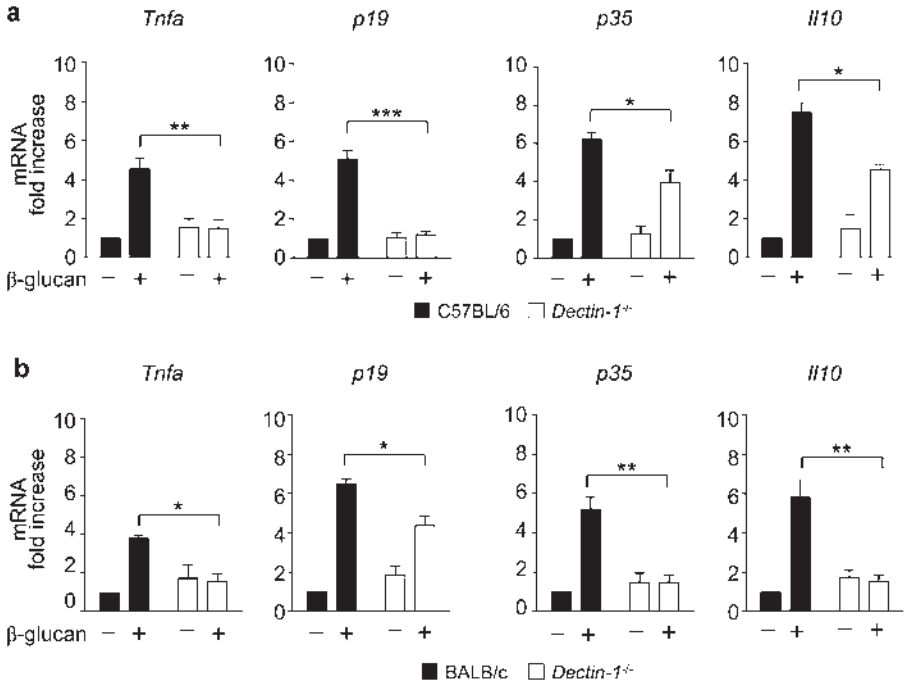

Figure 5 Dectin-1 deficiency differentially affects cytokine induction in response to β-glucan stimulation *in vitro*. Expression of *Tnfa*, *Il12p35*, *Il23p19* and *Il10* in *ex vivo* Peyer's patches from naive wild-type and *Dectin-1*$^{-/-}$ mice from (**a**) C57BL/6 or (**b**) BALB/c backgrounds, either unstimulated (−) or stimulated (+) *in vitro* with β-glucan for 18 h. *$P \leqslant 0.05$, **$P \leqslant 0.01$ and ***$P \leqslant 0.001$, unstimulated *vs.* stimulated Peyer's patches from either background, as indicated.

Th17/Treg CD4$^+$ T-cell populations during infection. In contrast with what was observed in murine aspergillosis,[36] dectin-1 deficiency disproportionally increases both Th1/Treg (in BALB/c mice) and Th17 (in C57BL/6 mice) cell responses after *C. albicans* infection, a finding showing that dectin-1 signaling can be involved in either Th1 or Th17 cell differentiation. This result may explain the relative ability of *Dectin-1*$^{-/-}$

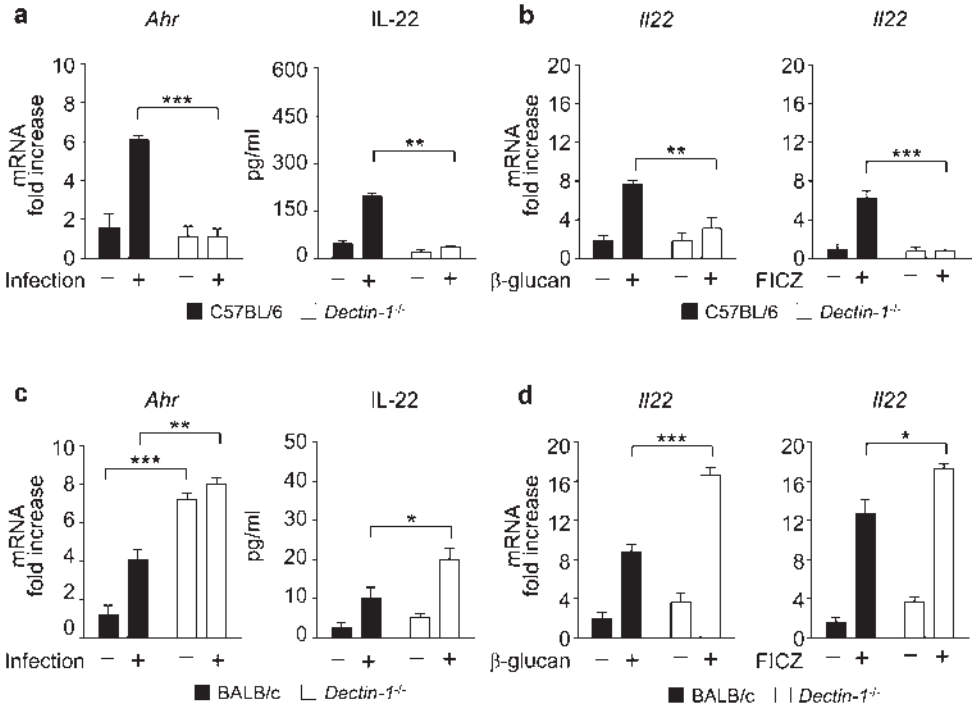

Figure 6 Dectin-1 differentially impacts the AhR/IL-22 axis in the different mouse strains. (**a**, **c**) *Ahr* expression and IL-22 production *in vivo* or (**b**, **d**) *in vitro* in wild-type and *Dectin-1*$^{-/-}$ mice from the (**a**, **b**) C57BL/6 or (**c**, **d**) BALB/c mice infected i.g. with *C. albicans* Vir^3 at 4 dpi. *Ahr* and *Il22* expression (real-time RT-PCR) and IL-22 production (ELISA) were performed on *ex vivo* Peyer's patches cells from naive (−) or infected (+) mice or in cells from naive mice stimulated *in vitro* with β-glucan or FICZ for 18 h. *$P \leqslant 0.05$, **$P \leqslant 0.01$ and ***$P \leqslant 0.001$, wild-type *vs. Dectin-1*$^{-/-}$ mice in either background, as indicated. *C. albicans*; *Candida albicans*; dpi, days post-infection; FICZ, 6-formylindolo[3,2-b]carbazole; i.g., intragastrically; RT-PCR, reverse transcriptase PCR.

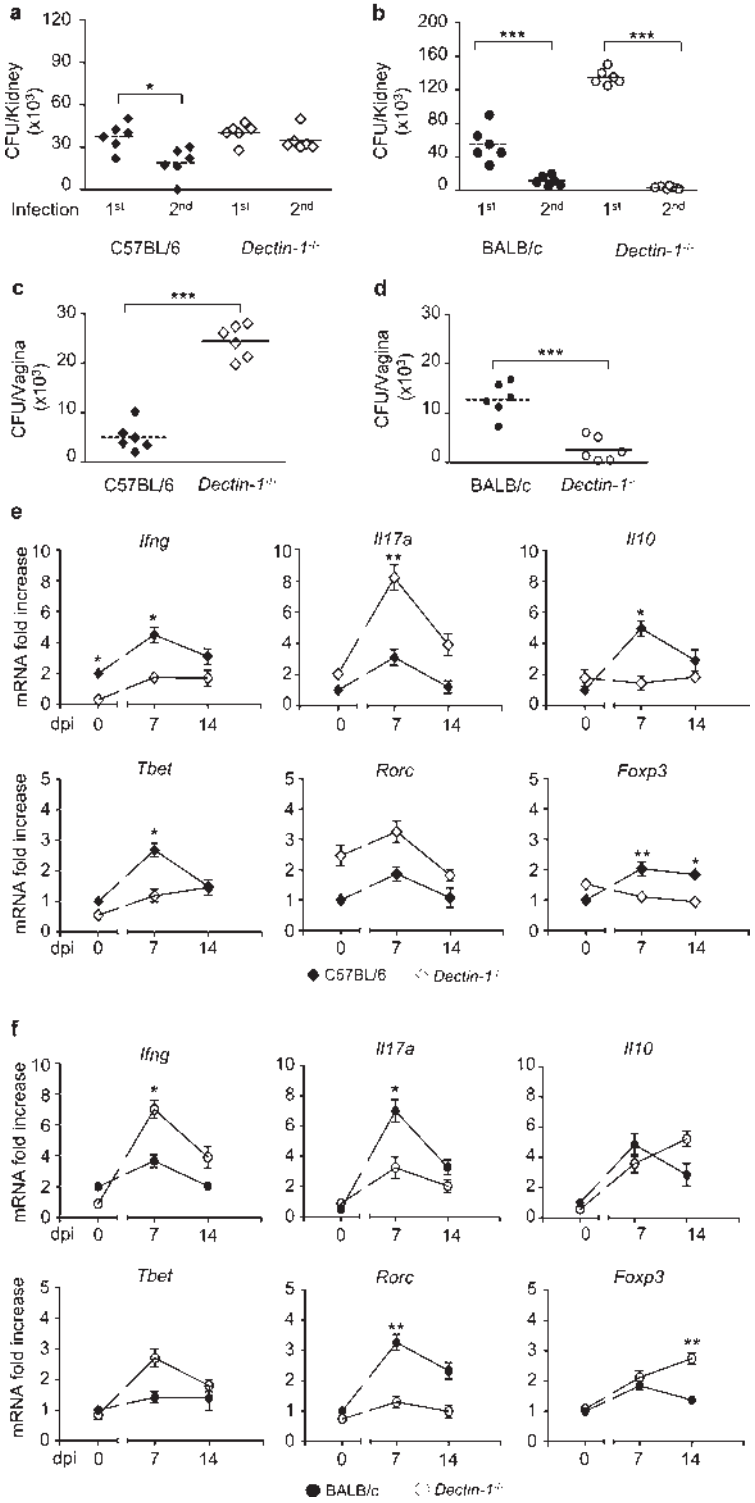

Figure 7 Dectin-1 deficiency associates with distinct adaptive Th responses. Fungal growth (CFU±SE) in the kidneys of (**a**) C57BL/6 wild-type (black diamonds) or *Dectin-1*$^{-/-}$ (white diamonds) and (**b**) BALB/c wild-type (black dots) or *Dectin-1*$^{-/-}$ (white dots) mice subjected to an i.v. rechallenge (second) with 5×10^6 cells of *C. albicans* Vir^3 14 days after the primary i.g. infection. Control mice were subjected to a primary i.v. infection (first) under the same conditions. Data are representative of 2–4 independent experiments. *$P\leqslant0.05$ and ***$P\leqslant0.001$, wild-type control *vs.* rechallenged mice, as indicated. Fungal growth (CFU±SE) in the vagina of (**c**) C57BL/6 wild-type (black diamonds) or *Dectin-1*$^{-/-}$ (white diamonds) and (**d**) BALB/c wild-type (black dots) or *Dectin-1*$^{-/-}$ (white dots) mice subjected to an intravaginal rechallenge with 5×10^6 cells of *C. albicans* Vir^3 at 14 days following the primary infection. Data are representative of two to four independent experiments. Gene expression of *Ifng*, *Il17a*, *Il10*, *Tbet*, *Rorc* and *Foxp3* at 0, 7 and 14 dpi in MLN from (**e**) C57BL/6 wild-type (black diamonds) or *Dectin-1*$^{-/-}$ (white diamonds) and (**f**) BALB/c wild-type (black dots) or *Dectin-1*$^{-/-}$ (white dots) mice infected i.g. with 1×10^8 cells of *C. albicans* Vir^3. Data are representative of 2–4 independent experiments. *$P\leqslant0.05$ and **$P\leqslant0.01$, wild-type *vs. Dectin-1*$^{-/-}$ mice in either background, as indicated. *C. albicans*; *Candida albicans*; CFU, colony-forming unit; dpi, days post-infection; i.g., intragastrically; i.v., intravenous.

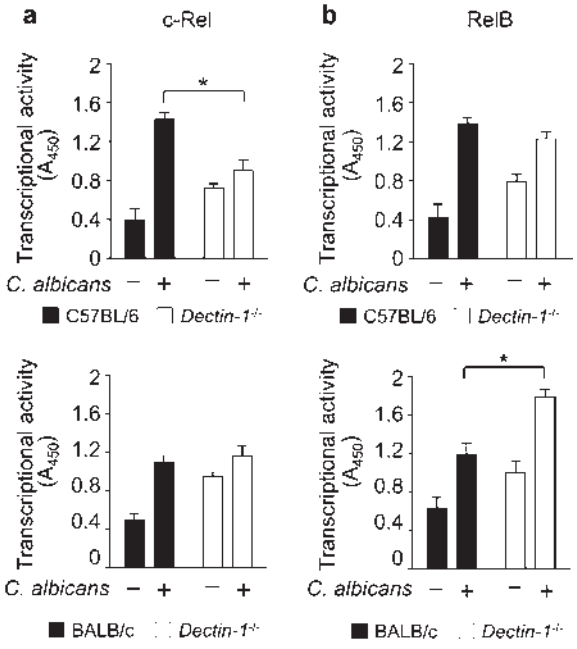

Figure 8 Dectin-1 deficiency is associated with distinct activation of NF-κB subunits. The activation of (**a**) c-Rel or (**b**) RelB subunits of NF-κB was assessed by ELISA on dendritic cells from wild-type or *Dectin-1*$^{-/-}$ mice that were either untreated (–) or stimulated (+) with *C. albicans* Vir^3 (1:1 ratio) for 30 min. The results are expressed as transcriptional activity levels of NF-κB determined by measuring absorbance at 450 nm (A_{450}). Data represent the mean±SE of three independent experiments. *P<0.05, wild-type *vs. Dectin-1*$^{-/-}$ mice in either background, as indicated. *C. albicans*; *Candida albicans*.

mice in either background to eventually control the infection. However, just as BALB/c mice, more than C57BL/6 mice, showed resistance to re-infection, Th1 cells, more than Th17 cells, are endowed with long-term immune protection to the fungus, as has already been shown.[23] In the early phase of the infection, however, IL-17A (and likely IL-17F) production is clearly associated with a better control of the infection at both the gastrointestinal and vaginal sites. The production of both is influenced by dectin-1. In candidiasis, the mechanisms by which dectin-1 regulates IL-17A/IL-17F production and in which innate immune cells this occurs is not known, but it is of interest that both neutrophils[37] and γ-δT cells[38] produce IL-17A *via* dectin-1.

Altogether, our data indicate that the net activity of dectin-1 in antifungal mucosal immunity is dependent on the host's genetic background and affects both the innate production of IL-17A, IL-17F and IL-22 and the regulation of the Th1/Th17/Treg balance in adaptive immunity.

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