Immunity
Article

Dectin-2 Recognition of α-Mannans and Induction of Th17 Cell Differentiation Is Essential for Host Defense against *Candida albicans*

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SUMMARY

Dectin-2 (gene symbol *Clec4n*) is a C-type lectin expressed by dendritic cells (DCs) and macrophages. However, its functional roles and signaling mechanisms remain to be elucidated. Here, we generated *Clec4n*$^{-/-}$ mice and showed that this molecule is important for host defense against *Candida albicans* (*C. albicans*). *Clec4n*$^{-/-}$ DCs had virtually no fungal α-mannan-induced cytokine production. Dectin-2 signaling induced cytokines through an FcRγ chain and Syk-CARD9-NF-κB-dependent signaling pathway without involvement of MAP kinases. The yeast form of *C. albicans* induced interleukin-1β (IL-1β) and IL-23 secretion in a Dectin-2-dependent manner. In contrast, cytokine production induced by the hyphal form was only partially dependent on this lectin. Both yeast and hyphae induced Th17 cell differentiation, in which Dectin-2, but not Dectin-1, was mainly involved. Because IL-17A-deficient mice were highly susceptible to systemic candida infection, this study suggests that Dectin-2 is important in host defense against *C. albicans* by inducing Th17 cell differentiation.

INTRODUCTION

Candida albicans is a member of the dimorphic fungal family and causes pathogenic opportunistic infections in humans. Because immunocompromised people and cortisone-treated mice are highly susceptible (Odds, 1988; Romani, 2004; Saijo et al., 2007), it is thought that acquired immunity is important for defense against this microorganism (Romani, 1999). Neutrophils are also important for the control of systemic fungal infections as shown by the fact that neutrophil-deficient mice have increased susceptibility (Baghian and Lee, 1989). However, the host defense mechanisms against this fungus remain to be fully clarified.

Fungal cell walls are mainly composed of multiple layers of carbohydrates, including mannans, β-glucans, and chitins (Netea et al., 2008). These cell wall components are recognized by pattern-recognition receptors to activate the host innate immune system. Recently, we reported that a C-type lectin, Dectin-1 (gene symbol *Clec7a*), is the specific receptor for β-glucans and induces cytokines through activation of the caspase-recruitment domain family, member 9 (CARD9, gene symbol *Card9*) in dendritic cells (DCs) and macrophages while activating reactive oxygen species in macrophages (Hara et al., 2007; Saijo et al., 2007). However, Dectin-1 was not required for the defense against *C. albicans* in vivo, although it played some role for the defense against *Pneumocystis carinii* (Saijo et al., 2007). This is probably because β-glucans and chitins are located inside of the fungal cell wall (Bowman and Free, 2006).

Because mannosyl residues are exposed on the surface of *C. albicans* (Odds, 1988), one would expect that mannan recognition could represent the initial step of the recognition by the host innate immune system. Several molecules have been reported to recognize *C. albicans* cell wall mannans. Macrophage mannose receptor (MR, CD206, *Mrc1*) recognizes the terminal

α-(1, 2) → (1, 3)-mannose of N-linked mannans, DC-SIGN (SIGNR-1, CD209a) recognizes branched α-mannans, and Galectin-3 recognizes β-(1, 2)-mannans (Netea et al., 2008; Poulain and Jouault, 2004). However, MR-deficient mice show normal host defense during systemic candidiasis and macrophages isolated from MR-deficient mice show normal response to *C. albicans* infection, suggesting that MR is not involved in the host defense against this fungus (Lee et al., 2003). Macrophages from galectin-3-deficient mice show normal binding and endocytosis of *C. albicans,* although TLR-2-dependent TNF production is reduced (Jouault et al., 2006). Whether DC-SIGN-deficient mice are sensitive to *Candida* infection has not been reported yet. Thus, the roles of mannan recognition receptors in the host defense against *C. albicans* remain obscure.

Dectin-2, another C-type lectin that is expressed by DCs and activated macrophages, has also been shown to bind high mannose-type carbohydrates and *C. albicans* hyphae (McGreal et al., 2006; Sato et al., 2006). Recently, Robinson et al. reported that a specific monoclonal antibody (mAb) against Dectin-2 could suppress IL-2, IL-10, and TNF production from bone marrow (BM) DCs (BMDCs) induced by *C. albicans*, as well as the development of candida-specific Th17 cells in cooperation with Dectin-1 during systemic *C. albicans* infection (Robinson et al., 2009). However, the antibody was not able to control systemic candidiasis, leaving the roles of this receptor in the host defense against fungal infection unknown. Although the cytoplasmic region of Dectin-2 has no obvious signaling motif, Sato et al. showed that Fc receptor γ (FcRγ) chain (gene symbol *Fcer1g*) can transduce a Dectin-2 signal and induce TNF and an IL-1 receptor antagonist when FcRγ chain was cotransfected into RAW cells with Dectin-2 (Sato et al., 2006). Robinson et al. showed that FcRγ chain was required for surface expression and signaling of Dectin-2. Syk and MAPK phosphorylation were induced by the treatment of BMDCs with Dectin-2 mAb and induction of IL-2, IL-10, and TNF was Syk and CARD9 dependent (Robinson et al., 2009). However, the importance of MAPK phosphorylation in the Dectin-2-induced cytokine induction remains obscure.

IL-17A is a proinflammatory cytokine produced by Th17 cells (Iwakura et al., 2008; Kastelein et al., 2007). TGF-β and IL-6 are required for the differentiation of Th17 cells in mice, and IL-23 and IL-1 promote their differentiation (Iwakura et al., 2008; McGeachy et al., 2009). IL-17F is also produced by Th17 cells and binds to the same receptors (IL-17RA and IL-17RC) (Toy et al., 2006; Zheng et al., 2008). We and others have shown that IL-17A plays important roles not only in the development of autoimmune diseases, but also in the host defense against bacteria (Mangan et al., 2006; Nakae et al., 2003; Schulz et al., 2008; Shiomi et al., 2008; Umemura et al., 2007; Ye et al., 2001). Recently, we reported that IL-17F, along with IL-17A, is important for host defense against *Staphylococcus aureus* and *Citrobactor rodentium* (Ishigame et al., 2009). IL-17A and IL-17F induce immune cells and epithelial cells to produce various proinflammatory cytokines and antimicrobial peptides that protect the host from bacterial infection. IL-17A, by inducing G-CSF and CXC chemokines, also plays important roles in the generation, recruitment, and activation of neutrophils, which are crucial in the host defense against bacterial and fungal infection (Stark et al., 2005; Ye et al., 2001).

Recently, it was reported that Th17 cells play an important role in the defense against *C. albicans* infection because IL-17RA-deficient mice and Th17 cell (IL-23p19)-deficient mice show increased susceptibility to mucosal candidiasis (Conti et al., 2009). However, because Th17 cells produce both IL-17A and IL-17F, the participation of IL-17A and IL-17F in the host defense mechanisms remains obscure. Furthermore, it was reported that β-glucan-activated DCs promote differentiation of Th17 cells (LeibundGut-Landmann et al., 2007) and that candida mannans induce Th17 cell differentiation through activation of MR (van de Veerdonk et al., 2009). However, the Th17 cell inducers in vivo upon infection with *C. albicans* still remain unclear.

In this report, we have generated Dectin-2-deficient (*Clec4n*$^{-/-}$) mice and examined the roles of Dectin-2 in host defense against systemic *C. albicans* infection. Our data showed that Dectin-2 was the critical DC receptor for candida α-mannans responsible for inducing proinflammatory cytokines and played an important role in host defense against this microorganism. Moreover, Dectin-2 preferentially facilitated differentiation of Th17 cells, and IL-17A, but not IL-17F, is crucial for defense against systemic candidiasis. These findings may be of use to develop new therapeutics against candida infection.

RESULTS

Dectin-2 Is the Receptor for α-Mannans and Mediates Cytokine Production from DCs

We generated *Clec4n*$^{-/-}$ mice by replacing exon 1 and exon 2 of the Dectin-2 gene with the neomycin-resistance gene (Figure S1A available online). *Clec4n*$^{-/-}$ mice were born with the expected Mendelian ratio, were fertile, and showed no gross phenotypic abnormalities. Their lymphoid cell populations were also normal. No marked differences between *Clec4n*$^{-/-}$ and wild-type DCs were found in the transcriptional profiles (Figure S1B). The numbers of splenic DCs (CD11c^{hi} cells; wild-type [n = 5]: [0.94 ± 0.023] × 10^6, *Clec4n*$^{-/-}$ [n = 5]: [0.90 ± 0.033] × 10^6) and macrophages (CD11b$^+$F4/80$^+$ cells; wild-type [n = 5]: [1.44 ± 0.014] × 10^6, *Clec4n*$^{-/-}$ [n = 5]: [1.40 ± 0.019] × 10^6) were normal in *Clec4n*$^{-/-}$ mice as well as cell surface markers including CD80, CD86, CD4 class II MHC, and F4/80 (Figures S1C and S1D). Moreover, bone marrow-derived DCs (BMDCs) and bone marrow-derived macrophages (BMDMs) were developed normally and the expression of other C-type lectins such as Dectin-1, DC-SIGN, and MR were also normal in *Clec4n*$^{-/-}$ mice (Figures S1E and S1F).

Mannans from Serotype A *C. albicans* consist of α-1, 6-linked polymannoses attached to Asn residues with α-1, 2-linked mannose side chains (Figure S1G). As it was reported that Dectin-2 can bind high-mannose structures, we examined whether Dectin-2 can function as a receptor for *C. albicans* mannans to induce cellular responses. We found that cytokines, such as IL-12p40, IL-6, TNF, and IL-10, but not IL-1β, were induced from wild-type BMDCs by treatment with *C. albicans* mannans, but not from *Clec4n*$^{-/-}$ BMDCs (Figure 1A). These results demonstrate that Dectin-2 is responsible for the production of cytokines induced by *C. albicans* mannans.

Mannans from Serotype A *C. albicans* also have β-1, 2-linked mannose residues attached to its α-1, 2-linked oligomannose

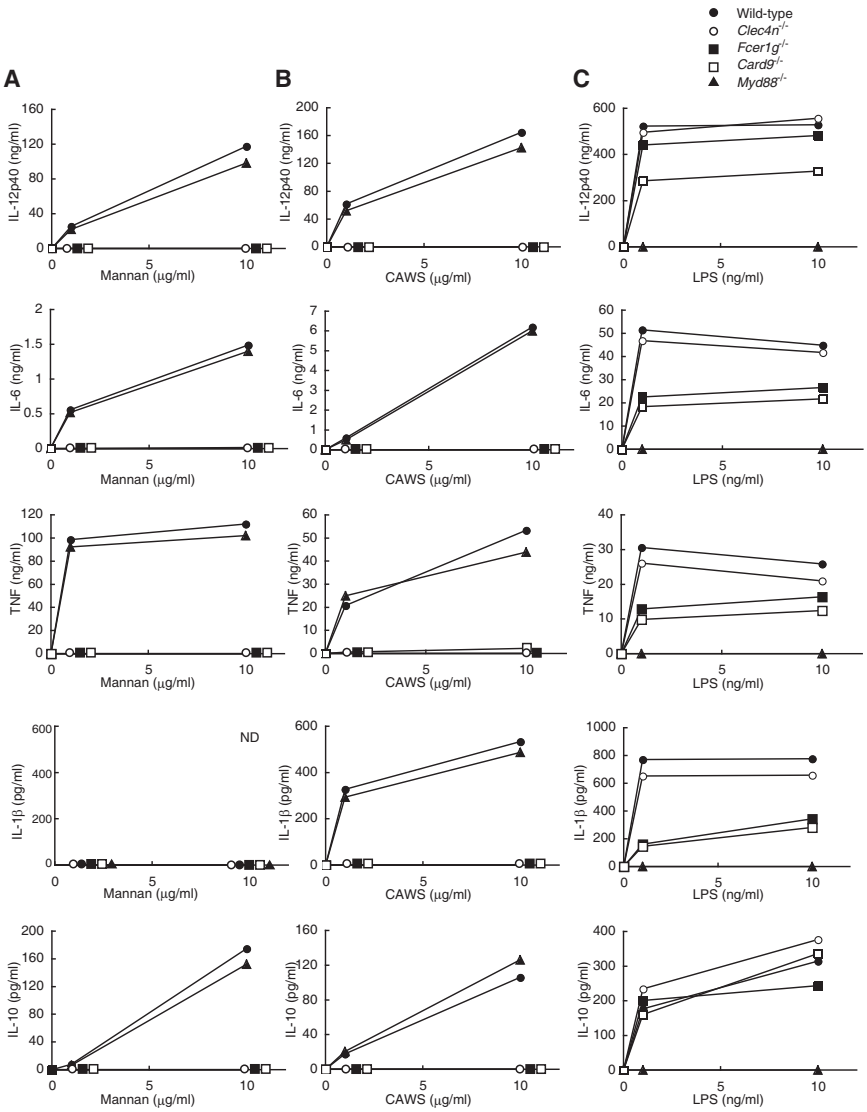

Figure 1. Dectin-2 Is Required for the Cytokine Production Induced by *C. albicans*-Derived Mannans and CAWS

Cytokine concentrations in the supernatants of wild-type (closed circle), *Clec4n*$^{-/-}$ (open circle), *Fcer1g*$^{-/-}$ (closed square), *Card9*$^{-/-}$ (open square), and *Myd88*$^{-/-}$ (closed triangle) BMDCs cultured for 48 hr with CAWS (A), mannans (B), or LPS (C). Data are the means of two wells and were reproducible in at least three independent experiments. ND, not detected.

side chains (Figure S1G; Cutler, 2001; Shibata et al., 2003). These β-1, 2-linked mannose residues are not synthesized when this fungus is cultured in a carbon-limiting low pH medium at a low temperature (Shinohara et al., 2006). Under these conditions, *C. albicans* mannans consist of only α-mannose residues and are secreted into the culture medium as a water-soluble fraction (CAWS). We proceeded to examine the responsiveness of *Clec4n*$^{-/-}$ DCs to CAWS. As shown in Figure 1B, cytokines including IL-1β were induced by CAWS in wild-type BMDCs, whereas the production of these cytokines in *Clec4n*$^{-/-}$ BMDCs was completely abolished. Dectin-2 deficiency did not affect β-glucan-induced cytokine production, and Dectin-1 deficiency did not affect cytokine production after CAWS stimulation, indicating that these receptors function independently (Figures S1H–S1J). MyD88, a downstream TLR adaptor molecule, was also not involved in Dectin-2 signaling, because *Myd88*$^{-/-}$ BMDCs produced normal amount of cytokines upon stimulation with mannans or CAWS (Figure 1; Figures S1H–S1J). These results suggest that Dectin-2 is the sole receptor in BMDCs to induce the production of cytokines in response to *C. albicans* α-mannans.

Dectin-2 Signaling Activates NF-κB through the FcRγ Chain-Syk-CARD9 Signaling Pathway

Upon cotransfection, Dectin-2 forms a complex with FcRγ chain, which has an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain (Sato et al., 2006). However, another group reported that Dectin-2 does not associate with FcRγ chain (Kanazawa et al., 2003), leaving its signal transduction mechanism unresolved. We found that cytokine production from *Fcer1g*$^{-/-}$ BMDCs was completely abolished when cells were stimulated with CAWS or *C. albicans* mannans, clearly indicating that FcRγ chain is the adaptor molecule for Dectin-2 signal transduction (Figures 1A and 1B).

Next, we examined the tyrosine phosphorylation of Syk, which is recruited to the ITAM upon activation of FcRγ chain, and found that Syk was activated in BMDCs upon stimulation with CAWS. We also found that tyrosine phosphorylation of the MAPKs downstream of Syk, such as p38, JNK, and Erk, were markedly increased in wild-type BMDC after stimulation, but this was significantly reduced in *Clec4n*$^{-/-}$ BMDCs (Figure 2A).

We next examined whether CARD9, an adaptor molecule known to mediate Dectin-1 signaling, was involved in the transduction of Dectin-2 signaling. Cytokine production from *Card9*$^{-/-}$ BMDCs was completely abolished, indicating that CARD9 also transduces Dectin-2 signaling (Figures 1A and 1B). CARD9 was also partially involved in TLR signaling, whereas cytokine production was normal upon CD40 antibody or PMA plus ionomycin stimulation (Figure 1C; Figure S2). Consistent with our previous report (Hara et al., 2007), the phosphorylation of MAPKs was not affected in *Card9*$^{-/-}$ BMDCs, indicating that MAPKs are activated in a CARD9-independent manner.

Upon activation, NF-κB complex consisting of the p65-p50 is released and translocates into the nucleus to bind DNA. We found that the amount of the p65 subunit was much lower in the nuclei of *Clec4n*$^{-/-}$ and *Card9*$^{-/-}$ BMDCs (Figure 2B). These

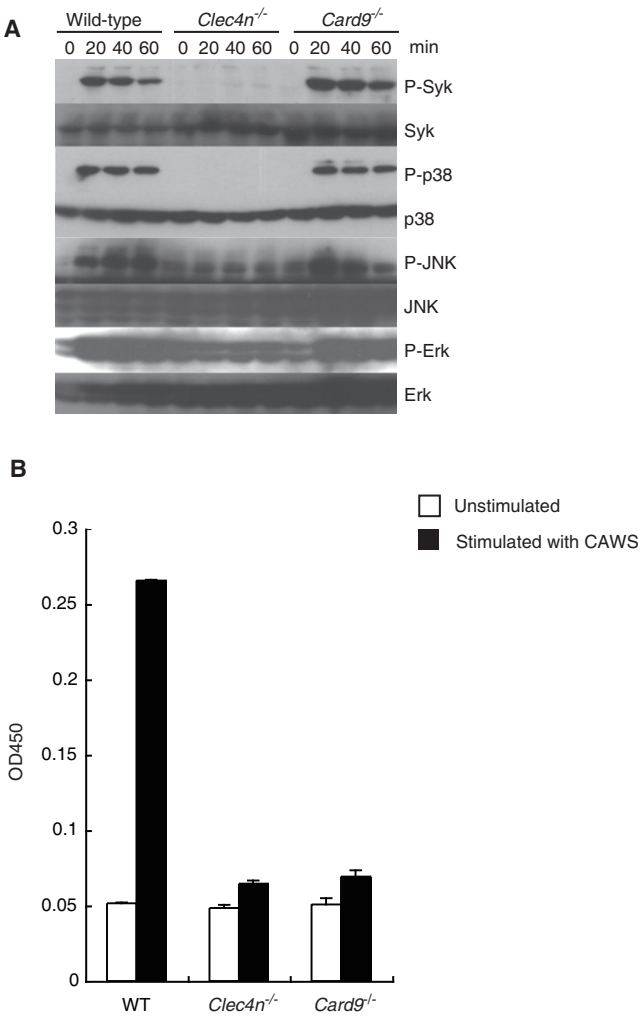

Figure 2. CAWS Stimulation Activates Syk, MAPKs, and NF-κB in a Dectin-2-Dependent Manner

(A) Phosphorylation of Syk, p38, Erk, and JNK was examined by immunoblot analysis with wild-type, *Clec4n*$^{-/-}$, and *Card9*$^{-/-}$ BMDCs 0, 20, 40, and 60 min after stimulation with CAWS. (p-), phosphorylated.

(B) DNA binding assay for NF-κB p65 in nuclear extracts from wild-type, *Clec4n*$^{-/-}$, and *Card9*$^{-/-}$ BMDCs. Data are the means ± SD of triplicates and are representative of two independent experiments. Open bar, unstimulated; closed bar, stimulated with CAWS.

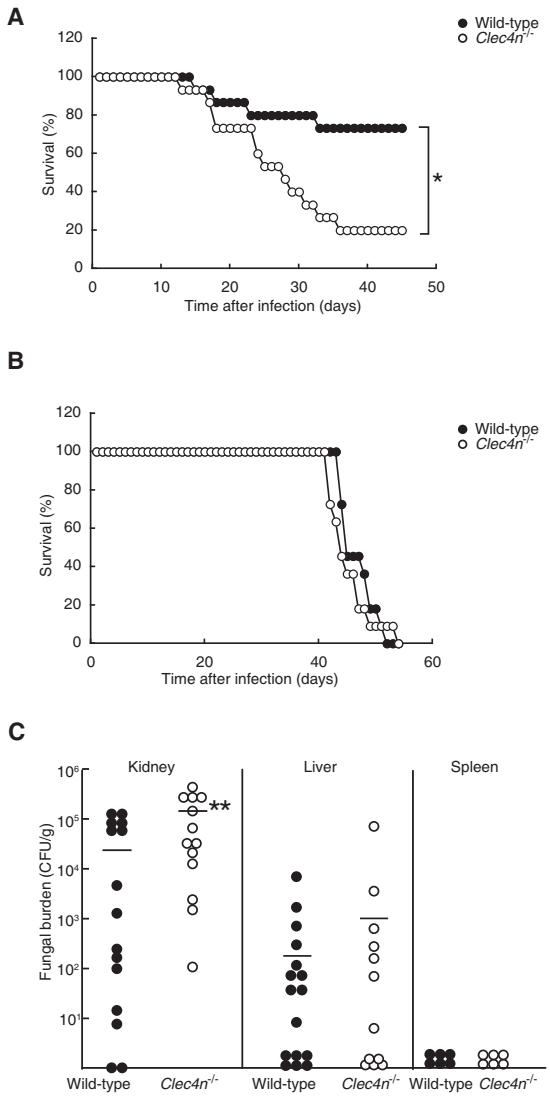

Figure 3. *Clec4n*$^{-/-}$ Mice Show Higher Susceptibility to *C. albicans* Infection

(A) Survival curves of wild-type (n = 15, closed circles) and *Clec4n*$^{-/-}$ (n = 15, open circles) mice after infection i.v. with *C. albicans* NBRC1385 (2 × 10^5 cells/mouse), by means of C57BL/6J background mice. *p = 0.00758 by a logrank test. Similar data were obtained from independent experiments shown in Figure S7.

(B) Survival curves of wild-type (closed circles) and *Clec4n*$^{-/-}$ (open circles) mice after infection i.v. with *C. neofomas* YC-13 (5 × 10^4 cells/mouse) in a BALB/cA background.

(C) CFU in the kidneys, livers, and spleens at 10 days after infection. Data were represented as CFU/weight of the organ (g). **p = 0.033 by a Student's t test.

results suggest that α-mannan binding to Dectin-2 activates FcRγ chain and induces phosphorylation of Syk to activate MAPKs and with downstream activation of CARD9, leading to NF-κB activation resulting in induction of cytokine secretion.

Clec4n$^{-/-}$ Mice Are Susceptible to *C. albicans* Infection

We next examined the susceptibility of *Clec4n*$^{-/-}$ mice to *C. albicans*. The NBRC1385 strain of *C. albicans* (Synonym ATCC18804, isolated from human skin), which was used to prepare the cell wall mannans, was used for infection. *Clec4n*$^{-/-}$ mice had significantly lower survival than wild-type mice when they were infected with a semilethal dose intravenously (i.v.) (Figure 3A). The sensitivity of *Clec4n*$^{-/-}$ mice to *C. albicans* was confirmed with the same strain (NBRC1385; Figure S3A) and also with two other *C. albicans* strains (SC5314 and THK519, isolated from serum of a patient with candidiasis in Japan) (Figures S3B and S3C). In contrast, *Clec4n*$^{-/-}$ mice responded normally to another pathogenic fungus, *Cryptococcus neoformans* (*C. neoformans*) (Figure 3B).

Ten days after infection i.v. with the *C. albicans* NBRC1385 strain, the fungal burden in the kidneys was significantly higher in the *Clec4n*$^{-/-}$ mice than in wild-type mice. No differences in fungal burden were observed in the liver, and the microbe was

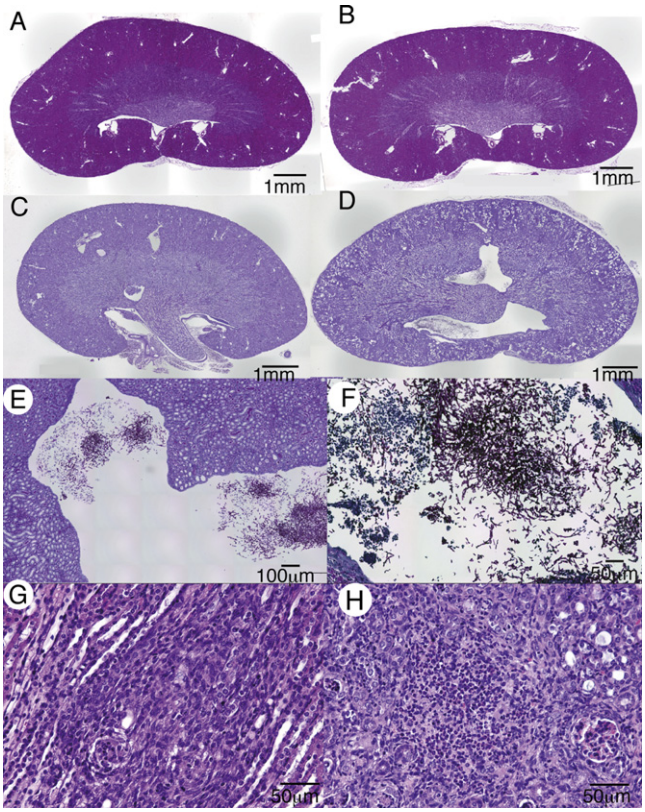

Figure 4. The Histopathology of the *Clec4n*$^{-/-}$ Mouse Kidney after *C. albicans* Infection

Kidneys were removed from uninfected mice (A, B) or 10 days after infection i.v. (C–H) of wild-type (A, C, G) and *Clec4n*$^{-/-}$ (B, D, E, F, H) mice. The organs were stained with hematoxylin and eosin (A, B, G, H) or PAS (C, D, E, F). *Clec4n*$^{-/-}$ mouse kidney is normal before infection (A, B). Extensive enlargement of pyelic cavity and tubular dilatation in the cortex are observed after infection in the kidney of *Clec4n*$^{-/-}$ mouse (D), but these changes are only mild in wild-type mouse kidney (C). Fungal growth of mycelial form is also seen in the *Clec4n*$^{-/-}$ kidney (E, F). There are several nodules of interstitial inflammation associated with mononuclear cell infiltration in the wild-type kidney (G), while neutrophil infiltration is still seen in such nodular lesions of the *Clec4n*$^{-/-}$ kidney (H).

not detected in the spleen of either *Clec4n*$^{-/-}$ or wild-type mice (Figure 3C). The kidneys of *Clec4n*$^{-/-}$ mice developed hydronephrosis, in which scatterred foci of abscess consisting of both neutrophils and lymphocytes were formed in the interstitium (Figures 4A–4D). Fungal growth was detectable in the pelvises of *Clec4n*$^{-/-}$ mice, indicating that *Clec4n*$^{-/-}$ mice were unable to eradicate *C. albicans* from their kidneys (Figures 4E and 4F). Nodules of interstitial inflammation associated with mononuclear cell infiltration were observed in wild-type kidneys (Figure 4G), whereas neutrophil infiltration was rather accelerated in such nodular lesions of the *Clec4n*$^{-/-}$ kidney (Figure 4H). Thus, Dectin-2 is critically important for host defense against *C. albicans*.

Cytokine-Inducing Activity Differs between the Yeast and Hyphal Forms of *C. albicans*

We next assessed the cytokine-inducing ability of the yeast and hyphal forms of *C. albicans* in vitro. *Clec4n*$^{-/-}$ and wild-type BMDCs were stimulated with either heat-killed yeast or hyphae, and cytokine concentrations in the culture medium were measured. The production of IL-6, TNF, IL-1β, IL-10, IL-23, and IL-12p70 induced by the yeast form completely disappeared in *Clec4n*$^{-/-}$ BMDCs, indicating that only Dectin-2 is responsible for the cytokine induction by this form of candida (Figure 5A). Consistent with these observations, cytokine production by the yeast form of *C. albicans* in *Fcer1g*$^{-/-}$ and *Card9*$^{-/-}$ BMDCs was also completely abolished (Figure S4A). Interestingly, the cytokine-inducing activity of the hyphal form was much stronger than that of the yeast form. Although cytokine production including IL-6, IL-1β, and IL-23, which are important in inducing Th17 cell, was markedly affected by the deficiency of Dectin-2, considerably high concentrations of cytokines were still detected in the culture medium (Figure 5B). The *Fcer1g*$^{-/-}$ BMDC cultures had cytokine concentrations that were similar to those of *Clec4n*$^{-/-}$ BMDCs (Figure S4). In contrast, cytokine production from *Card9*$^{-/-}$ BMDCs was completely absent, indicating that CARD9 is also involved in signaling pathways other than Dectin-2 (Figure S4B). On the other hand, cytokine production from Dectin-1-deficient BMDCs induced by either yeast form or hyphal form of *C. albicans* was similar to wild-type BMDCs (Figures 5A and 5B), suggesting that Dectin-2 plays a more important role than Dectin-1 for cytokine induction by this fungus.

Dectin-2 Signaling Preferentially Promotes Th17 Cell Differentiation

Because Dectin-2 signaling induced IL-6, IL-1β, and IL-23, we next examined whether Dectin-2 signaling in BMDCs could stimulate the differentiation of Th17 cells. Wild-type and *Clec4n*$^{-/-}$ BMDCs were cultured in the presence of the yeast or hyphal forms of *C. albicans* for 48 hr, then freshly isolated naive CD4^{+} T cells were cultured with the conditioned medium in the presence of plate-coated anti-CD3 and anti-CD28. Both types of conditioned media from yeast- or hyphae-treated culture greatly enhanced the differentiation of naive CD4^{+} T cells into Th17 cells, although Th1 cell differentiation was also weakly induced (Figures 6A and 6B). In contrast, the induction of Th17 cell differentiation was significantly reduced in the conditioned medium obtained from *Clec4n*$^{-/-}$ BMDCs. Th1 cell differentiation was also inhibited in CD4^{+} T cells treated with *Clec4n*$^{-/-}$ BMDC-derived conditioned medium when BMDCs were treated with yeast form candida. These data indicate that both the yeast form and hyphal forms of *C. albicans* induce differentiation of both Th17 cells and Th1 cells through activation of Dectin-2 signaling. In contrast, no significant difference in T cell differentiation was observed in the presence of conditioned medium from Dectin-1-deficient BMDCs (Figures 6A and 6B).

IL-17A, but Not IL-17F, Is Required for the Host Defense against *C. albicans*

Because Dectin-2 induced differentiation of Th17 cells, we examined the roles of IL-17A and IL-17F in the host defense against *C. albicans* NBRC1385 infection. Wild-type and *Clec4n*$^{-/-}$ mice were infected with the fungi i.v. and their survival was monitored. IL-17A-deficient mice had significantly lower survival than wild-type mice after infection (Figure 6C), indicating that IL-17A is critically important for the host defense

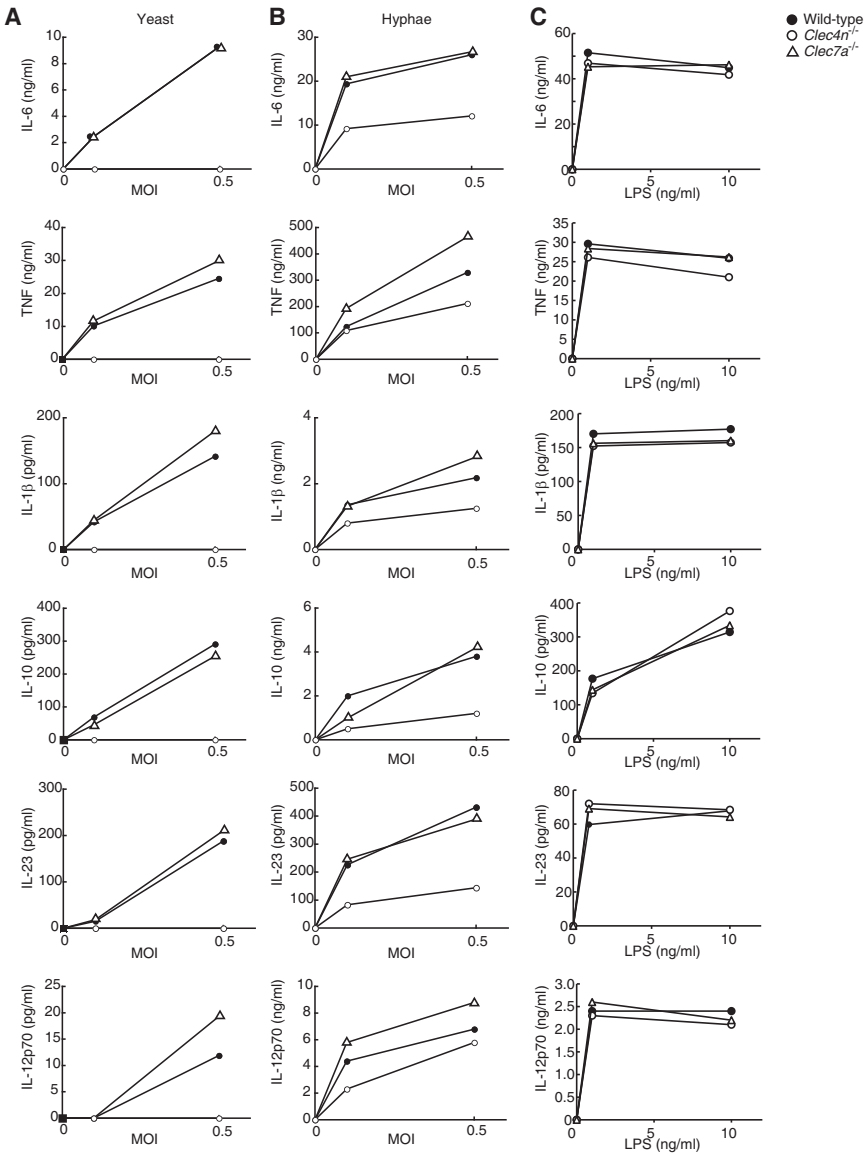

Figure 5. Cytokine Production from BMDCs Stimulated with *C. albicans*

ELISAs for cytokines in the supernatants from cultures of wild-type (closed circle), *Clec4n*$^{-/-}$ (open circle), and *Clec7a*$^{-/-}$ (closed square) BMDCs. Cytokine concentrations in the supernatants of BMDCs cultured with the (A) yeast, (B) hyphal forms of *C. albicans*, or (C) LPS. Data are the means of two wells and were reproducible in at least three independent experiments.

against *C. albicans*. In contrast, deficiency in IL-17F did not affect survival.

DISCUSSION

In this report, we showed that Dectin-2 plays an important role in the host defense against *C. albicans* infection via *Clec4n*$^{-/-}$ mice. The survival was clearly decreased in *Clec4n*$^{-/-}$ mice, and fungal burden in the kindney was significantly increased, resulting in the development of hydronephrosis. This was confirmed with three different strains, NBRC1385, SC5314, and THK519. However, the susceptibility against *C. neoformans* infection did not change, suggesting that the host defense mechanisms are different between these fungi. These observations make a clear contrast to our previous observation that Dectin-1-deficient mice show normal susceptibility to *C. albicans* (Saijo et al., 2007), suggesting that Dectin-2 is more important than Dectin-1 for the defense against *C. albicans* infection, at least in systemic infection with these fungi. On the other hand, Taylor et al. reported the involvement of Dectin-1 in the defense against this fungus via SC5314 (Taylor et al., 2007). In this report, we have shown that *Clec4n*$^{-/-}$ mice also became more susceptible to this strain of candida, indicating that Dectin-2 is also important for the host defense against this strain. Although Robinson et al. did not observe any difference of kidney fungal burdens in mice treated with Dectin-2 antibody at 7 days after systemic infection of *C. albicans*, this was probably because the observation period was too short to observe the difference as the authors suggested (Robinson et al., 2009). Indeed, we did not detect any difference in survival between *Clec4n*$^{-/-}$ and wild-type mice before 10 days after infection with NBRC1385 strain.

We showed that cytokine production from BMDCs induced by mannans or CAWS from *C. albicans* was completely abolished in *Clec4n*$^{-/-}$ BMDCs, indicating that Dectin-2 is an essential receptor for candida cell wall-derived α-mannans. It remains to be elucidated, however, what kinds of structures, such as the terminal mannose residues, branched termini, or inner tri-mannose structures, are recognized by Dectin-2.

Previously, Sato et al. reported that FcRγ chain can function as an adaptor molecule for Dectin-2 when both FcRγ chain and Dectin-2 were cotransfected and activated with Dectin-2 antibodies (Sato et al., 2006). In this report, we have demonstrated that cytokine production induced by CAWS or *C. albicans* mannans disappeared completely in *Fcer1g*$^{-/-}$ BMDCs, indicating that FcRγ chain is the only adaptor molecule for Dectin-2 in BMDCs. Consistent with this observation, surface expression of Dection-2 and NFAT activation by Zymosan is suppressed by a mutation in FcRγ chain (Robinson et al., 2009). On the other hand, MyD88 deficiency did not affect the cytokine induction by CAWS, suggesting that mannan-induced signaling was independent of TLR signaling. Upon activation, Syk was phosphorylated, suggesting ITAM association of the

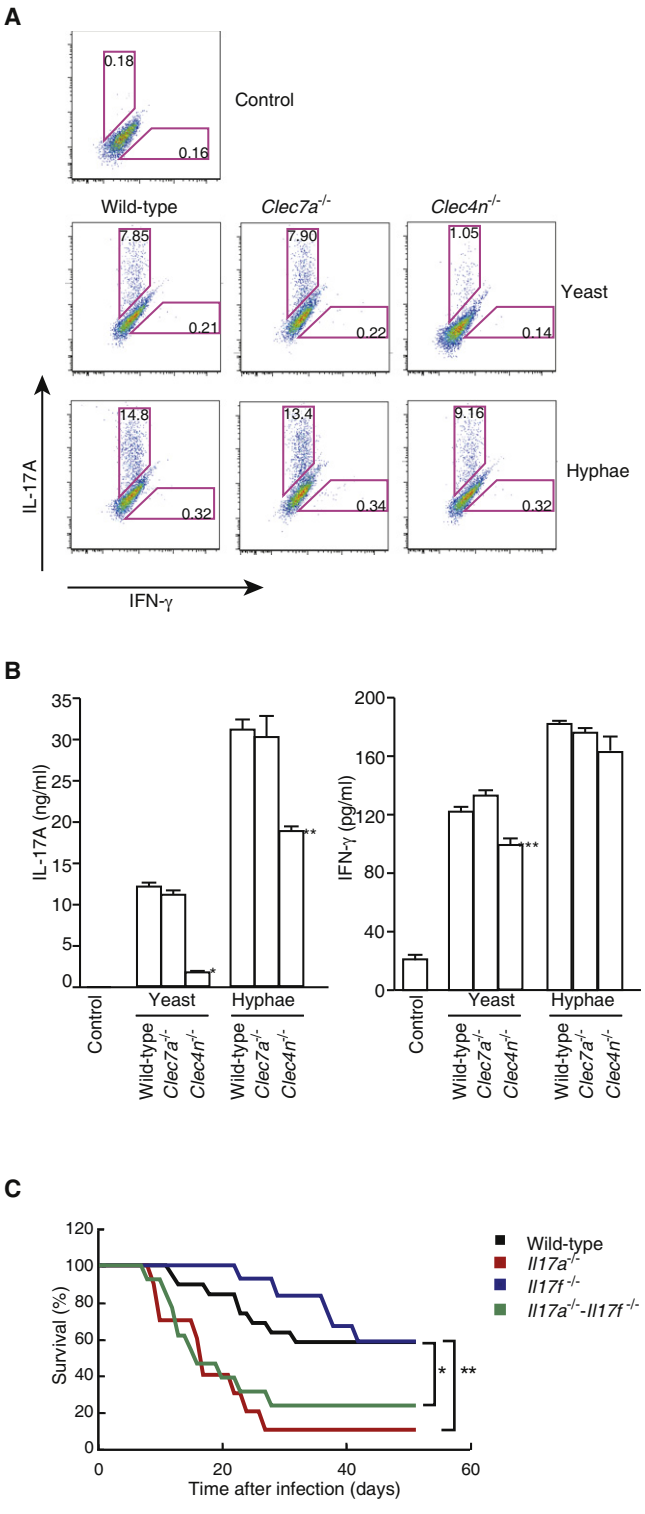

Figure 6. Dectin-2 Is Required for the Th17 Cell Differentiation Induced by *C. albicans* Stimulation

(A) Purified wild-type naive CD4$^+$ T cells were stimulated with anti-CD3 and anti-CD28 for 5 days in the presence of conditioned medium from BMDCs cultured with the yeast or hyphal forms of *C. albicans*, then analyzed by flow cytometry for expression of intracellular cytokines. Data are representative of at least two independent experiments.

(B) Concentrations of IL-17A and IFN-γ in the cultures were determined. *p = 0.000006, **p = 0.0003, ***p = 0.002, by a Student's t test (wild-type versus *Clec4n*$^{-/-}$). Data are the mean and ± SD of triplicates and are representative of two independent experiments.

(C) *Il17a*$^{-/-}$ mice are more susceptible to *C. albicans* infection. Survival curves of wild-type (n = 19, black), *Il17a*$^{-/-}$ (n = 10, red), *Il17f*$^{-/-}$ (n = 12, blue), and *Il17a*$^{-/-}$-*Il17f*$^{-/-}$ (n = 13, green) mice after infection i.v. with NBRC1385 *C. albicans* (2 × 10^5 cells/ mouse). *p = 0.048, **p = 0.0015 by a logrank test. Data are representative of two independent experiments.

FcRγ chain, and activated NF-κB and MAPKs. Furthermore, we showed that CARD9 is crucial for the cytokine induction by Dectin-2 and that NF-κB, but not MAPKs, is activated downstream of CARD9. In this regard, it was reported recently that ERK, p38, and JNK are activated in BMDCs upon stimulation with Dectin-2 mAb, suggesting that MAPK activation is important for the induction of cytokines by Dectin-2 (Robinson et al., 2009). However, we showed that this MAPK activation is not involved in the induction of cytokines, because MAPK activation was normally observed in the absence of CARD9 upon stimulation with CAWS, in which no cytokine induction was observed. We showed that NF-κB activation is also dependent on CARD9, suggesting that this activation is important for the cytokine induction. We reported similar NF-κB dependency of cytokine induction in Dectin-1-induced cytokine production (Hara et al., 2007).

The importance of IL-17A in host defense against *C. albicans* has been suggested with IL-17RA-deficient mice and also IL-23p19-deficient mice (Conti et al., 2009; Huang et al., 2004). However, because IL-17RA binds both IL-17A and IL-17F, and IL-23 is involved in the differentiation of Th17 cells that produce both IL-17A and IL-17F, it remained to be elucidated whether IL-17A or IL-17F or both are involved in this defense mechanism. We showed that IL-17A, but not IL-17F, is important for the defense against this fungal infection. This finding provides a contrast to the results obtained in *C. rodentium* and *S. aureus* infection, in which both IL-17A and IL-17F play important roles in the host defense (Ishigame et al., 2009). Because we showed that IL-17F is mainly involved in mucosal infection, it is possible that IL-17F is involved in the host defense only against mucosal or epithelial fungal infection, but not systemic infection. We are now examining this possibility.

Both the yeast form and the hyphal form of *C. albicans* induced the production of various proinflammatory cytokines from wild-type BMDCs, including IL-6, IL-23, and IL-1β, all of which are involved in Th17 cell differentiation, as well as IL-12, which is involved in Th1 cell differentiation. The cytokine-inducing activity was much higher in the hyphal form, consistent with the observation that recombinant soluble Dectin-2 preferentially binds the hyphal form (Sato et al., 2006). Interestingly, however, the production of these cytokines by the yeast was almost completely abolished in *Clec4n*$^{-/-}$ BMDCs. In contrast, only small decreases, although they were significant, were observed in *Clec4n*$^{-/-}$ BMDCs stimulated with the hyphal form. These observations suggest that the yeast form expressed only mannans on the surface of the cell wall as a functional ligand, while the density is much lower compared to the hyphal form. On the other hand, in addition to mannans, the hyphal form expressed other ligands that can induce cytokines through activation of other receptors than Dectin-1 or Dectin-2 in a

CARD9-dependent manner. In contrast to our results, IL-10 and TNF induction were not inhibited significantly by Dectin-2 mAb or Dectin-2 shRNA upon stimulation with the heat-killed yeast form of *C. albicans* (Robinson et al., 2009). The difference of candida stains or incompleteness of the inhibitory activity of the reagents may be the cause for the discrepancy.

We found that Th17 cell differentiation was strongly induced in naive $CD4^+$ T cells cultured with *C. albicans*-stimulated BMDC-conditioned medium. Th17 cell differentiation was observed with both yeast and hyphal forms. However, Th17 cell differentiation was greatly reduced with conditioned medium from *Clec4n*$^{-/-}$ BMDCs, indicating that Dectin-2 plays a major role for the differentiation of Th17 cells. Although it was reported that Dectin-1 signaling can induce Th17 cell differentiation (LeibundGut-Landmann et al., 2007), we did not observe significant reduction in Th17 cell differentiation with conditioned medium obtained from Dectin-1-deficient BMDCs when cells were treated with the heat-killed yeast or hyphal form of *C. albicans*. β-glucans in this candida strain (NBRC1385) may not be accessible to Dectin-1 in the cell wall structure, because cytokine production from Dectin-1-deficient BMDC was not altered compared with wild-type BMDCs upon stimulation with CAWS or *C. albicans*. Consistent with our results, Robinson et al. recently reported that IL-17 production by splenocytes upon recall response against *C. albicans* was greatly reduced in mice treated with Dectin-2 mAb (Robinson et al., 2009).

It is possible that receptors other than Dectin-2 are also involved in the induction of Th17 cells in the mouse upon infection with the hyphal form of *C. albicans*, because a significant percentage of Th17 cells was still differentiated by the conditioned medium of *Clec4n*$^{-/-}$ mouse BMDCs. Indeed, there are several molecules that may be involved in this process, including MR and DC-SIGN, which are coexpressed in DCs and macrophages. Because it was reported that MR-deficient mice show normal host defense against systemic candidiasis, this receptor may not be the major receptor for the induction of innate host defense mechanisms in mice. However, in cooperation with Dectin-2 or under some conditions, such as in animals with Dectin-2 deficiency, these mannose receptors may function as supportive mechanisms. It remains to be elucidated, however, whether or not Dectin-2 plays the same role in the antifungal mechanisms in humans. With regard to this, it was reported that Th17 cells are also important for the defense against fungal infection in humans (Milner et al., 2008). van de Veerdonk et al. reported that MR is involved in the Th17 cell differentiation of human PBMC induced by *C. albicans* (van de Veerdonk et al., 2009). Furthermore, it was recently reported that Dectin-1 is important for the defense against mucocutaneous fungal infection (Ferwerda et al., 2009). Also, it was reported that patients with a homozygous CARD9 mutation become susceptible to fungal infection (Glocker et al., 2009). Clearly, further studies are required in the human system.

The mechanisms of how IL-17 controls fungal infection have not been elucidated completely. It was reported that IL-17 is important for the generation and recruitment of neutrophils to the inflammatory sites (Ye et al., 2001). Because neutrophils are important for the protection against fungal infection, we examined neutrophil infiltration into kidneys after infection in the *Clec4n*$^{-/-}$ mice. However, the infiltration was rather accelerated in these animals, reflecting the severity of the lesion. Therefore, in this case, neutrophil recruitment was not impaired in these *Clec4n*$^{-/-}$ mice, although Th17 cell differentiation was impaired in these mice. In another possibility, we and others reported that IL-17A and IL-17F is important for the induction of β-defensins in epithelial cells (Ishigame et al., 2009; Kao et al., 2004). This mechanism may be important for the mucosal infection by candida. But, the significance of β-defensins in the systemic infection is not clear at present. Thus, the activity of IL-17A to activate humoral and cellular immune responses by inducing cytokines, chemokines, and cell adhesion molecules may be important for the protection against *C. albicans* (Iwakura et al., 2008).

It should be noted that IL-12p35-deficient mice show increased susceptibility to *C. albicans* because of the defect in Th1 cell differentiation and that the IL-23-Th17 cell pathway rather promotes inflammation and impairs antifungal immune resistance when mice are infected gastrointestinally (Zelante et al., 2007). Thus, these observations suggest that both Th17 cell and Th1 cell are important for the host defense against this microbe by recruiting neutrophils and activating macrophages, respectively. Because Th17 cells and Th1 cells differentiate in a mutually exclusive way (Iwakura et al., 2008), and the balance between Th17 cells and Th1 cells may change depending on the infection conditions, excess Th17 cell differentiation may cause pathogenic rather than protective effects under some conditions (Zelante et al., 2007).

In conclusion, we have shown that Dectin-2 is the critical receptor for the α-mannans on *C. albicans* and plays an important role in the host defense against this fungal infection by inducing Th17 cell differentiation in mice. Apparently paradoxical observations in the literatures regarding the roles of C-type lectins and Th17 cells suggest that the host defense mechanisms against *C. albicans* are complex and that different defense mechanisms consisting of multiple layers are used in response to different fungal strains, infectious routes, disease stages, organs, and hosts. Our observations should be useful for developing new therapeutics against candida infection.

EXPERIMENTAL PROCEDURES

Mice

Clec4n$^{-/-}$ mice were generated by homologous recombination by means of the embryonic stem cell line E14.1 (Supplemental Experimental Procedures). *Clec4n*$^{-/-}$, *Clec7a*$^{-/-}$, *Il17a*$^{-/-}$, *Il17f*$^{-/-}$, and *IL17a*$^{-/-}$-*Il17f*$^{-/-}$ mice were backcrossed for eight generations to C57BL/6J (Nihon SLC, Shizuoka, Japan). *Card9*$^{-/-}$ mice and *Myd88*$^{-/-}$ mice were backcrossed for more than seven generations and eight generations to C57BL/6J mice, respectively. *Fcer1g*$^{-/-}$ mice were purchased from Taconic (New York, NY) and were backcrossed for eight generations to C57BL/6J. 6- to 10-week-old male and female mice were used for experiments and wild-type C57BL/6J mice were used as controls. All mice, except *Card9*$^{-/-}$ mice, were kept in specific pathogen-free conditions at the Center for Experimental Medicine, The Institute of Medical Science, The University of Tokyo. *Card9*$^{-/-}$ mice were kept in specific pathogen-free conditions at RIKEN RCAI. Experiments were done according to institutional ethical and recombinant guidelines and were approved by the institutional committees.

Cell Preparation and Ligand Sensitivity Analysis

BM cells were removed from the femurs and tibiae of 6- to 8-week-old male mice and BMDCs were prepared as described (Fujikado et al., 2008). CAWS (1 μg/ml or 10 μg/ml), mannan (1 μg/ml or 10 μg/ml), or LPS (1 ng/ml or

10 ng/ml) was added on day 10 after cultivation with granulocyte-macrophage colony-stimulating factor. On day 12, the culture supernatants were collected for cytokine titrations.

Cytokine Titrations

Concentrations of TNF, IL-1β, IL-6, IL-10, IL-12p40, IL-12p70, and IL-23 were determined with commercially available OptiEIA kits (BD Biosciences, Franklin Lakes, NJ) or Ready-Set-Go cytokine kits (eBioscience, San Diego, CA) according to the manufacturer's instructions. Concentrations of IL-17A and IFN-γ were determined with commercially available Cytometric Beads Array set (BD Biosciences, San Jose, CA).

Preparation of CAWS and *C. albicans* Mannan

CAWS was prepared from the *C. albicans* strain NBRC1385 in accordance with conventional methods (Tada et al., 2008). In brief, *C. albicans* strain NBRC1385 was cultured with 5 l of C-limiting medium in a glass incubator and cultured for 2 days at 27°C with air supplied at a rate of 5 l/min, rotating at 400 rpm. After this culture, an equal volume of ethanol was added and, after the mixture was allowed to stand overnight, the precipitate was collected. The precipitate was dissolved in 250 ml of distilled water. After centrifugation to separate cell debris, ethanol was added to the supernatant and the mixture was left to stand overnight. The precipitate was collected and dried with acetone to obtain CAWS. This CAWS preparation contained 78% ± 6.6% carbohydrates and 15% ± 6.3% proteins, and the mannan/glucan ratio was 6.3 ± 1.3.

Mannans were prepared from *C. albicans* (NBRC1385) as described (Kobayashi et al., 1989). In brief, *C. albicans* was grown as follows: 4 l of YPD medium were added to a fermenter and the yeast was cultured for 2 days at 27°C with air supplied at a rate of 5 l/min. Then, an equal volume of ethanol was added to kill the yeast. The cells were extensively washed with distilled water and acetone-dried. The acetone-dried cells were further delipidated with $CHCl_3$-MeOH (2:1, v/v; 600 ml) for 2 hr under reflux. The residual cells were then washed with EtOH (600 ml) for 2 hr under reflux to remove low-molecular-weight organic compounds. The washed cells were suspended in 1 l of distilled water and then autoclaved for 4 hr at 128°C. This suspension was then allowed to cool to room temperature (RT). After centrifugation, the supernatant was carefully collected. The residual sediment was re-extracted twice via the method described above. The combined supernatant was concentrated at 40°C in vacuo to a volume of about 250 ml. After centrifuging to remove the traces of insoluble material, the water extract was dialyzed against distilled water. Then, the mannans were purified by Fehling precipitation. Proteins were not detected in this mannan preparation (N content: less than 0.3%).

C. albicans Infections

C. albicans (NBRC1385, SC5314, and THK519) was grown for 36 hr at 30°C on potato dextrose agar plates (Eiken Kizai, Tokyo, Japan). For survival analysis, wild-type and *Clec4n*$^{-/-}$ mice were infected intravenously with 2 × 10^5 cells and then monitored daily. For the analysis of cytokine production and T cell differentiation assays, *C. albicans* were harvested and diluted with RPMI medium (Wako Pure Chemical Industries, Osaka, Japan) containing 10% FCS into two replicate 24-well plates. One plate was kept on ice, while the other was incubated for 2 hr at 37°C to form hyphae. Then, both plates were incubated for 2 hr at 65°C to kill both the yeast and hyphae, and then they were cooled on ice. Next, BMDCs were added at a concentration of 2 × 10^6 cells per well and cultured for 2 days. Culture supernatants were used for cytokine titrations and the T cell differentiation assays.

Histological Analysis

Wild-type (n = 10) and *Clec4n*$^{-/-}$ (n = 10) mice were infected with *C. albicans* NBRC1385 (2 × 10^5 cells/mouse). After 10 days, their kidneys, livers, and spleens were removed, fixed with 10% neutral formalin, embedded in paraffin, sectioned in 5 μm slices, and stained with PAS or Hematoxylin and eosin.

C. neoformans Infection

C. neoformans (YC-13) was grown on potato dextrose agar plates (Eiken Kagaku, Tokyo, Japan). Wild-type (n = 10) and *Clec4n*$^{-/-}$ (n = 10) mice were infected i.v. with *C. neoformans* (5 × 10^4/mouse) and then monitored daily for 60 days.

Immunoassays and NF-κB Binding to DNA

BMDCs were stimulated with CAWS (10 μg/ml) or LPS (10 ng/ml). After incubation at 37°C for various periods of time, the cells were lysed in ice-cold lysis buffer (Cell Signaling, Danvers, MA) supplemented with a phosphatase inhibitor cocktail (Sigma Aldrich, St. Louis, MO). The cell lysates were separated by SDS-PAGE and transferred onto polyvinyldifluoride membranes (BioRad Laboratories, Shinagawa, Japan). The membranes were incubated with antibodies to Syk, phosphorylated Syk, ERK, phosphorylated ERK, p38, phosphorylated p38, JNK, or phosphorylated JNK (Cell Signaling Technology, Danvers, MA) and then with horseradish peroxidase-conjugated secondary antibodies, followed by development with an ECL detection system (GE Healthcare, Buckinghamshire, UK).

For analysis of NF-κB activity, BMDCs were stimulated for 2 hr with CAWS (10 μg/ml) or LPS (10 ng/ml). Nuclear extracts were prepared with a Transfactor Extraction kit (Clontech, Mountain View, CA), and the binding activity of the NF-κB p65 subunit in the extracts was determined with Transfactor Colorimetric kits according to the manufacturer's protocols.

T Cell Differentiation Assays

Naive $CD4^+$ T cells were purified with a combination of cell sorting techniques. In brief, single-cell suspensions were prepared from the lymph nodes of wild-type C57BL/6J mice, and $CD4^+$ T cells were purified with biotin-conjugated anti-mouse B220, anti-mouse CD8a, anti-mouse CD11b, anti-mouse DX5, and anti-mouse Ter119 antibodies (BD Biosciences, San Jose, CA) and an AutoMacs (Milteny Biotec GmbH, Bergisch, Germany). Then naive $CD4^+$ T cells were sorted with APC-conjugated anti-mouse CD4, pacific blue-conjugated anti-mouse CD62L (BD Biosciences, San Jose CA), and PE-conjugated anti-mouse CD25 antibodies (eBiosciences, San Diego, CA) and FACSAria (BD Biosciences, San Jose, CA). Naive $CD4^+$ T cells were stimulated with plate-coated anti-mouse CD3 (11C11) and CD28 antibodies (eBiosciences, San Diego, CA) for 5 days and supplemented with conditioned media that were obtained from BMDC cultures with the yeast or hyphal forms of *C. albicans*. Then, the cells were collected and stained with APC-conjugated mouse CD4 antibody followed by intracellular staining for IL-17 and IFN-γ with PE-conjugated anti-mouse IL-17 (BD Biosciences, San Jose CA) and FITC-conjugated anti-mouse IFN-γ antibodies (eBiosciences, San Diego, CA). The cells were analyzed with a FACS Canto II (BD Biosciences, San Jose, CA) and FlowJo software (TreeStar Inc., Ashland, OR).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at doi:10.1016/j.immuni.2010.05.001.

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REFERENCES

Baghian, A., and Lee, K.W. (1989). Systemic candidosis in beige mice. J. Med. Vet. Mycol. *27*, 51–55.

Bowman, S.M., and Free, S.J. (2006). The structure and synthesis of the fungal cell wall. Bioessays *28*, 799–808.

Conti, H.R., Shen, F., Nayyar, N., Stocum, E., Sun, J.N., Lindemann, M.J., Ho, A.W., Hai, J.H., Yu, J.J., Jung, J.W., et al. (2009). Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. J. Exp. Med. *206*, 299–311.

Cutler, J.E. (2001). N-glycosylation of yeast, with emphasis on *Candida albicans*. Med. Mycol. *39* (*Suppl 1*), 75–86.

Ferwerda, B., Ferwerda, G., Plantinga, T.S., Willment, J.A., van Spriel, A.B., Venselaar, H., Elbers, C.C., Johnson, M.D., Cambi, A., Huysamen, C., et al. (2009). Human dectin-1 deficiency and mucocutaneous fungal infections. N. Engl. J. Med. *361*, 1760–1767.

Fujikado, N., Saijo, S., Yonezawa, T., Shimamori, K., Ishii, A., Sugai, S., Kotaki, H., Sudo, K., Nose, M., and Iwakura, Y. (2008). Dcir deficiency causes development of autoimmune diseases in mice due to excess expansion of dendritic cells. Nat. Med. *14*, 176–180.

Glocker, E.O., Hennigs, A., Nabavi, M., Schäffer, A.A., Woellner, C., Salzer, U., Pfeifer, D., Veelken, H., Warnatz, K., Tahami, F., et al. (2009). A homozygous CARD9 mutation in a family with susceptibility to fungal infections. N. Engl. J. Med. *361*, 1727–1735.

Hara, H., Ishihara, C., Takeuchi, A., Imanishi, T., Xue, L., Morris, S.W., Inui, M., Takai, T., Shibuya, A., Saijo, S., et al. (2007). The adaptor protein CARD9 is essential for the activation of myeloid cells through ITAM-associated and Toll-like receptors. Nat. Immunol. *8*, 619–629.

Huang, W., Na, L., Fidel, P.L., and Schwarzenberger, P. (2004). Requirement of interleukin-17A for systemic anti-*Candida albicans* host defense in mice. J. Infect. Dis. *190*, 624–631.

Ishigame, H., Kakuta, S., Nagai, T., Kadoki, M., Nambu, A., Komiyama, Y., Fujikado, N., Tanahashi, Y., Akitsu, A., Kotaki, H., et al. (2009). Differential roles of interleukin-17A and -17F in host defense against mucoepithelial bacterial infection and allergic responses. Immunity *30*, 108–119.

Iwakura, Y., Nakae, S., Saijo, S., and Ishigame, H. (2008). The roles of IL-17A in inflammatory immune responses and host defense against pathogens. Immunol. Rev. *226*, 57–79.

Jouault, T., El Abed-El Behi, M., Martínez-Esparza, M., Breuilh, L., Trinel, P.A., Chamaillard, M., Trottein, F., and Poulain, D. (2006). Specific recognition of *Candida albicans* by macrophages requires galectin-3 to discriminate *Saccharomyces cerevisiae* and needs association with TLR2 for signaling. J. Immunol. *177*, 4679–4687.

Kanazawa, N., Tashiro, K., Inaba, K., and Miyachi, Y. (2003). Dendritic cell immunoactivating receptor, a novel C-type lectin immunoreceptor, acts as an activating receptor through association with Fc receptor gamma chain. J. Biol. Chem. *278*, 32645–32652.

Kao, C.Y., Chen, Y., Thai, P., Wachi, S., Huang, F., Kim, C., Harper, R.W., and Wu, R. (2004). IL-17 markedly up-regulates beta-defensin-2 expression in human airway epithelium via JAK and NF-kappaB signaling pathways. J. Immunol. *173*, 3482–3491.

Kastelein, R.A., Hunter, C.A., and Cua, D.J. (2007). Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. Annu. Rev. Immunol. *25*, 221–242.

Kobayashi, H., Shibata, N., Mitobe, H., Ohkubo, Y., and Suzuki, S. (1989). Structural study of phosphomannan of yeast-form cells of *Candida albicans* J-1012 strain with special reference to application of mild acetolysis. Arch. Biochem. Biophys. *272*, 364–375.

Lee, S.J., Zheng, N.Y., Clavijo, M., and Nussenzweig, M.C. (2003). Normal host defense during systemic candidiasis in mannose receptor-deficient mice. Infect. Immun. *71*, 437–445.

LeibundGut-Landmann, S., Gross, O., Robinson, M.J., Osorio, F., Slack, E.C., Tsoni, S.V., Schweighoffer, E., Tybulewicz, V., Brown, G.D., Ruland, J., and Reis e Sousa, C. (2007). Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. Nat. Immunol. *8*, 630–638.

Mangan, P.R., Harrington, L.E., O'Quinn, D.B., Helms, W.S., Bullard, D.C., Elson, C.O., Hatton, R.D., Wahl, S.M., Schoeb, T.R., and Weaver, C.T. (2006). Transforming growth factor-beta induces development of the T(H)17 lineage. Nature *441*, 231–234.

McGeachy, M.J., Chen, Y., Tato, C.M., Laurence, A., Joyce-Shaikh, B., Blumenschein, W.M., McClanahan, T.K., O'Shea, J.J., and Cua, D.J. (2009). The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. Nat. Immunol. *10*, 314–324.

McGreal, E.P., Rosas, M., Brown, G.D., Zamze, S., Wong, S.Y., Gordon, S., Martinez-Pomares, L., and Taylor, P.R. (2006). The carbohydrate-recognition domain of Dectin-2 is a C-type lectin with specificity for high mannose. Glycobiology *16*, 422–430.

Milner, J.D., Brenchley, J.M., Laurence, A., Freeman, A.F., Hill, B.J., Elias, K.M., Kanno, Y., Spalding, C., Elloumi, H.Z., Paulson, M.L., et al. (2008). Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. Nature *452*, 773–776.

Nakae, S., Nambu, A., Sudo, K., and Iwakura, Y. (2003). Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. J. Immunol. *171*, 6173–6177.

Netea, M.G., Brown, G.D., Kullberg, B.J., and Gow, N.A. (2008). An integrated model of the recognition of *Candida albicans* by the innate immune system. Nat. Rev. Microbiol. *6*, 67–78.

Odds, F.C. (1988). Candia and Candidosis, Second Edition (London: Baillere-Tindall).

Poulain, D., and Jouault, T. (2004). *Candida albicans* cell wall glycans, host receptors and responses: elements for a decisive crosstalk. Curr. Opin. Microbiol. *7*, 342–349.

Robinson, M.J., Osorio, F., Rosas, M., Freitas, R.P., Schweighoffer, E., Gross, O., Verbeek, J.S., Ruland, J., Tybulewicz, V., Brown, G.D., et al. (2009). Dectin-2 is a Syk-coupled pattern recognition receptor crucial for Th17 responses to fungal infection. J. Exp. Med. *206*, 2037–2051.

Romani, L. (1999). Immunity to *Candida albicans*: Th1, Th2 cells and beyond. Curr. Opin. Microbiol. *2*, 363–367.

Romani, L. (2004). Immunity to fungal infections. Nat. Rev. Immunol. *4*, 1–23.

Saijo, S., Fujikado, N., Furuta, T., Chung, S.H., Kotaki, H., Seki, K., Sudo, K., Akira, S., Adachi, Y., Ohno, N., et al. (2007). Dectin-1 is required for host defense against *Pneumocystis carinii* but not against *Candida albicans*. Nat. Immunol. *8*, 39–46.

Sato, K., Yang, X.L., Yudate, T., Chung, J.S., Wu, J., Luby-Phelps, K., Kimberly, R.P., Underhill, D., Cruz, P.D., Jr., and Ariizumi, K. (2006). Dectin-2 is a pattern recognition receptor for fungi that couples with the Fc receptor gamma chain to induce innate immune responses. J. Biol. Chem. *281*, 38854–38866.

Schulz, S.M., Köhler, G., Schütze, N., Knauer, J., Straubinger, R.K., Chackerian, A.A., Witte, E., Wolk, K., Sabat, R., Iwakura, Y., et al. (2008). Protective immunity to systemic infection with attenuated *Salmonella enterica* serovar enteritidis in the absence of IL-12 is associated with IL-23-dependent IL-22, but not IL-17. J. Immunol. *181*, 7891–7901.

Shibata, N., Kobayashi, H., Okawa, Y., and Suzuki, S. (2003). Existence of novel beta-1,2 linkage-containing side chain in the mannan of *Candida lusitaniae*, antigenically related to *Candida albicans* serotype A. Eur. J. Biochem. *270*, 2565–2575.

Shinohara, H., Nagi-Miura, N., Ishibashi, K., Adachi, Y., Ishida-Okawara, A., Oharaseki, T., Takahashi, K., Naoe, S., Suzuki, K., and Ohno, N. (2006). Beta-mannosyl linkages negatively regulate anaphylaxis and vasculitis in mice, induced by CAWS, fungal PAMPS composed of mannoprotein-beta-glucan complex secreted by *Candida albicans*. Biol. Pharm. Bull. *29*, 1854–1861.

Shiomi, S., Toriie, A., Imamura, S., Konishi, H., Mitsufuji, S., Iwakura, Y., Yamaoka, Y., Ota, H., Yamamoto, T., Imanishi, J., and Kita, M. (2008). IL-17 is involved in *Helicobacter pylori*-induced gastric inflammatory responses in a mouse model. Helicobacter *13*, 518–524.

Stark, M.A., Huo, Y., Burcin, T.L., Morris, M.A., Olson, T.S., and Ley, K. (2005). Phagocytosis of apoptotic neutrophils regulates granulopoiesis via IL-23 and IL-17. Immunity *22*, 285–294.

Tada, R., Nagi-Miura, N., Adachi, Y., and Ohno, N. (2008). The influence of culture conditions on vasculitis and anaphylactoid shock induced by fungal pathogen *Candida albicans* cell wall extract in mice. Microb. Pathog. *44*, 379–388.

Taylor, P.R., Tsoni, S.V., Willment, J.A., Dennehy, K.M., Rosas, M., Findon, H., Haynes, K., Steele, C., Botto, M., Gordon, S., and Brown, G.D. (2007). Dectin-1 is required for beta-glucan recognition and control of fungal infection. Nat. Immunol. *8*, 31–38.

Toy, D., Kugler, D., Wolfson, M., Vanden Bos, T., Gurgel, J., Derry, J., Tocker, J., and Peschon, J. (2006). Cutting edge: Interleukin 17 signals through a heteromeric receptor complex. J. Immunol. *177*, 36–39.

Umemura, M., Yahagi, A., Hamada, S., Begum, M.D., Watanabe, H., Kawakami, K., Suda, T., Sudo, K., Nakae, S., Iwakura, Y., and Matsuzaki, G. (2007). IL-17-mediated regulation of innate and acquired immune response against pulmonary *Mycobacterium bovis* bacille Calmette-Guerin infection. J. Immunol. *178*, 3786–3796.

van de Veerdonk, F.L., Marijnissen, R.J., Kullberg, B.J., Koenen, H.J., Cheng, S.C., Joosten, I., van den Berg, W.B., Williams, D.L., van der Meer, J.W., Joosten, L.A., and Netea, M.G. (2009). The macrophage mannose receptor induces IL-17 in response to *Candida albicans*. Cell Host Microbe *5*, 329–340.

Ye, P., Rodriguez, F.H., Kanaly, S., Stocking, K.L., Schurr, J., Schwarzenberger, P., Oliver, P., Huang, W., Zhang, P., Zhang, J., et al. (2001). Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. J. Exp. Med. *194*, 519–527.

Zelante, T., De Luca, A., Bonifazi, P., Montagnoli, C., Bozza, S., Moretti, S., Belladonna, M.L., Vacca, C., Conte, C., Mosci, P., et al. (2007). IL-23 and the Th17 pathway promote inflammation and impair antifungal immune resistance. Eur. J. Immunol. *37*, 2695–2706.

Zheng, Y., Valdez, P.A., Danilenko, D.M., Hu, Y., Sa, S.M., Gong, Q., Abbas, A.R., Modrusan, Z., Ghilardi, N., de Sauvage, F.J., and Ouyang, W. (2008). Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat. Med. *14*, 282–289.