Immunity
Article

Interferon-β Production via Dectin-1-Syk-IRF5 Signaling in Dendritic Cells Is Crucial for Immunity to *C. albicans*

Carlos del Fresno,[1,7,8] Didier Soulat,[1,2,7] Susanne Roth,[3] Katrina Blazek,[4] Irina Udalova,[4] David Sancho,[5] Jürgen Ruland,[3,6] and Carlos Ardavín[1,*]

[1]Departamento de Inmunología y Oncología, Centro Nacional de Biotecnología/CSIC, C/ Darwin 3, 28049 Madrid, Spain
[2]Mikrobiologisches Institut – Klinische Mikrobiologie, Immunologie und Hygiene, Universitätsklinikum Erlangen and Friedrich-Alexander-Universität Erlangen-Nürnberg, Wasserturmstraβe 3-5, 91054 Erlangen, Germany
[3]Institut für Klinische Chemie und Pathobiochemie, Klinikum rechts der Isar, Technische Universität München, Ismaninger Straβe. 22, 81675, Munich, Germany
[4]Kennedy Institute of Rheumatology, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, London W6 8KH, UK
[5]Centro Nacional de Investigaciones Cardiovasculares Carlos III, C/ Melchor Fernández Almagro 3, 28029 Madrid, Spain
[6]Laboratory of Signaling in the Immune System, Helmholtz Zentrum München – German Research Center for Environmental Health, Ingolstädter Landstraβe 1, 85764, Neuherberg, Germany
[7]These authors contributed equally to this work
[8]Present address: Centro Nacional de Investigaciones Cardiovasculares Carlos III, C/ Melchor Fernández Almagro 3, 28029 Madrid, Spain
*Correspondence: ardavin@cnb.csic.es
http://dx.doi.org/10.1016/j.immuni.2013.05.010

SUMMARY

Type I interferon (IFN) is crucial during infection through its antiviral properties and by coordinating the immunocompetent cells involved in antiviral or antibacterial immunity. Type I IFN (IFN-α and IFN-β) is produced after virus or bacteria recognition by cytosolic receptors or membrane-bound TLR receptors following the activation of the transcription factors IRF3 or IRF7. IFN-β production after fungal infection was recently reported, although the underlying mechanism remains controversial. Here we describe that IFN-β production by dendritic cells (DCs) induced by *Candida albicans* is largely dependent on Dectin-1- and Dectin-2-mediated signaling. Dectin-1-induced IFN-β production required the tyrosine kinase Syk and the transcription factor IRF5. Type I IFN receptor-deficient mice had a lower survival after *C. albicans* infection, paralleled by defective renal neutrophil infiltration. IFN-β production by renal infiltrating leukocytes was severely reduced in *C. albicans*-infected mice with Syk-deficient DCs. These data indicate that Dectin-induced IFN-β production by renal DCs is crucial for defense against *C. albicans* infection.

INTRODUCTION

The type I interferon (IFN) cytokine family includes IFN-β and 14 members of the IFN-α family (Decker et al., 2005). Type I IFN was originally discovered by its antiviral properties, which rely primarily on its capacity to block gene translation, to inhibit viral replication, to trigger the production of nucleases degrading viral nucleic acids, and to induce the apoptosis of infected cells (Stetson and Medzhitov, 2006). However, type I IFN was subsequently demonstrated to play also a crucial role during infection by coordinating the function of different immunocompetent cells involved in the induction of defense against viruses and intracellular bacteria. In this regard, type I IFN can license dendritic cells (DCs) for cross-priming-dependent activation of $CD8^+$ T cells, trigger the effector function of cytotoxic $CD8^+$ T cells, activate natural killer (NK) cells through interleukin-15 (IL-15) production, and promote the production of leukocyte-attractant chemokines (González-Navajas et al., 2012). Type I IFN can be produced after recognition of viruses or intracellular bacteria through two categories of receptors: ubiquitously expressed cytosolic receptors such as RIG-I and MDA5 (that sense viral dsRNA), or NOD1 and NOD2 (that sense bacterial peptidoglycans), and membrane-bound Toll-like receptors (TLRs), such as TLR3, TLR4, TLR7, and TLR9 (Trinchieri, 2010). These type I IFN-inducing TLRs that are selectively expressed by specialized immunocompetent cells are located at the cell surface (TLR4, that senses Gram-negative bacteria LPS) or at endosomal compartments (TLR3, TLR7, and TLR9, that sense viral or bacterial nucleic acids). After viral or bacterial infection, the engagement of type I IFN-inducing receptors, except TLR7 and TLR9, leads to the activation of the transcription factor IFN response factor 3 (IRF3) that induces the production of IFN-β and IFN-α4 (Trinchieri, 2010). In contrast, engagement of endosomal TLR7 and TLR9 in DCs leads to the production of IFN-β and all types of IFN-α by a mechanism dependent on the transcription factor IRF7 (Decker et al., 2005; refer to Figure S7 available online for an integrated view of type I IFN-inducing receptors).

Recent studies have revealed that type I IFN can be produced by DCs in response to fungi, particularly to yeasts of the genus *Candida* (Biondo et al., 2011; Bourgeois et al., 2011), which can cause life-threatening infections in immunocompromised

patients (Pfaller and Diekema, 2007). As described for bacterial infections, type I IFN can be beneficial for the immune response to yeast, as described for *Candida albicans* (Biondo et al., 2011), or have a detrimental effect, as reported for *Candida glabrata* infection (Bourgeois et al., 2011). However, the mechanism by which type I IFN is produced during *Candida* infection remains controversial. Activation of DCs after interaction with *C. albicans* has been claimed to result from the engagement of TLR2 and TLR9, and particularly of Dectin-1 (Clec7a; Romani, 2011), a C-type lectin receptor recognizing the complex β-glucan cell wall of *C. albicans*, that is crucial for the induction of protective T helper 17 (Th17) cell responses (LeibundGut-Landmann et al., 2007). Dectin-1 engagement results in the recruitment of Syk, that leads to the activation of PLCγ2 (Xu et al., 2009) and to the assembly of the Card9 complex by a mechanism dependent on PKCδ (Strasser et al., 2012). Dectin-1-triggered Card9 signaling then drives IL-1β, IL-2, IL-6, IL-10, IL-12, IL-23, and tumor necrosis factor-α (TNF-α) production by an NF-κB and NFAT-dependent pathway (Sancho and Reis e Sousa, 2012; refer to Figure S7 for an integrated view of Dectin-1-mediated signaling).

In order to explore the involvement of Dectin-1 in the production of type I IFN during fungal infection, we have analyzed the signaling pathway controlling IFN-β expression by DCs in response to *C. albicans*. Our data have allowed us to describe a pathway leading to IFN-β production by DCs that depends on Dectin-1 and Dectin-2 activation. Dectin-1-induced IFN-β production was dependent on Syk- and Card9-driven signaling and on the transcription factor IRF5 and independent of IRF3 and IRF7. Experiments of in vivo infection with *C. albicans* performed in mice deficient in the IFNAR1 subunit of the type I IFN receptor IFN-α and IFN-β receptor-deficient (*Ifnar1*$^{-/-}$) and CD11c-Cre$^{+/-}$ *Syk*$^{fl/fl}$ mice, in which DCs are not responsive to Syk-dependent signaling, demonstrated that type I IFN exerted a protective role during *C. albicans* infection and that the production of type I IFN by renal leukocytic infiltrates was mainly controlled by DCs through Dectin-1 and Dectin-2 signaling. Taken together, our results support the hypothesis that the production of type I IFN by renal infiltrating DCs, mediated by Dectin-Syk-IRF5 signaling, plays a crucial role in defense against *C. albicans* infection.

RESULTS

Dectin-1 Engagement Induces IFN-β Production by Dendritic Cells

In order to address the involvement of Dectin-1 in the production of type I IFN by DCs, we first assessed by real-time PCR the capacity of bone-marrow-derived DCs (BMDCs) to express IFN-β-specific messenger RNA (mRNA) in response to Curdlan (a water-insoluble β-1,3 polysaccharide from *Alcaligenes faecalis* that acts as a Dectin-1 specific ligand), to Zymosan (a complex insoluble preparation from *Saccharomyces cerevisiae* that activates TLR2 and TLR6, TLR9, and Dectin-1), to heat-killed *C. albicans*, strain SC5314 (HKC), and to LPS from *Escherichia coli*, that was used as a control of type I IFN production in response to TLR4. Engagement of TLR4 was characterized by a fast and transient induction of IFN-β mRNA, which peaked 2 hr after LPS stimulation and returned to basal levels along the next 4 hr. In contrast, Curdlan and Zymosan induced a more sustained expression of IFN-β mRNA, which peaked at 3 hr, was maintained at maximal levels until 5 hr, and was still detectable 8 hr after stimulation (Figure 1A). The kinetics of IFN-β mRNA expression after stimulation with HKC revealed a slower response peaking at 5 hr. This delayed response compared to Curdlan or Zymosan could reflect a reduced exposure of DC-activating *Candida*-associated molecular patterns by HKC, and consequently a less efficient recognition of these ligands by the TLRs and C-type lectin receptors expressed by BMDCs.

One possible explanation for this sustained IFN-β mRNA expression is that IFN-β produced after engagement of Dectin-1 and/or TLRs by yeast compounds present in Curdlan and/or Zymosan triggers, in an autocrine manner, the signaling through the type I IFN receptor (IFNAR) IFN-α and IFN-β receptor (IFNAR1). This would lead to a feed-forward loop of IFN-β production that has been described for TLR7- or TLR-9-activated plasmacytoid DCs (Decker et al., 2005) but that was not operational in BMDCs activated by LPS (Figure S1). However, no differences in IFN-β mRNA expression were observed between *Ifnar1*$^{-/-}$ and wild-type (WT) BMDCs after stimulation with Curdlan or Zymosan (Figure S1). These results indicates that IFNAR signaling did not contribute to a feed-forward loop of IFN-β production in BMDCs under these activation conditions, although the autocrine IFNAR signaling pathway was operational because no expression of mRNA for the IFN-inducible antiviral gene *Mx2* was detected in *Ifnar1*$^{-/-}$ BMDCs (Figure S1). Therefore the sustained IFN-β mRNA expression induced by Curdlan and Zymosan was not due to IFNAR signaling, but most likely to a Dectin-1-specific transcriptional regulation of *Ifnb* gene expression, as discussed below.

As stated before, IRF3-mediated IFN-β production resulting from the recognition of viral or bacterial compounds by type I IFN-inducing cytosolic or TLR receptors has been claimed to be paralleled by IFN-α4 production (Trinchieri, 2010). To address whether this also occurred in response to *C. albicans*, we analyzed IFN-α mRNA expression in BMDCs stimulated with Curdlan or Zymosan by real-time PCR, by using pan-IFN-α primers. IFN-α mRNA expression was not detected in BMDCs stimulated with Curdlan or Zymosan (Figure S2). In contrast, IFN-α mRNA was induced in bone marrow-derived macrophages (BMMs) stimulated with either Curdlan or Zymosan (Figure S2A), indicating that Curdlan-induced signaling can lead to the induction of IFN-α mRNA, although this appears to be cell-type-specific. Interestingly, Curdlan or Zymosan stimulation induced a significantly higher IFN-β mRNA expression in BMDCs than in BMMs (Figure S2B).

To confirm that IFN-β production in response to Curdlan was due to Dectin-1 engagement, the expression of IFN-β mRNA and the production of IFN-β was analyzed by real-time PCR and ELISA, respectively, after stimulation of BMDCs from Dectin-1 deficient (*Clec7a*$^{-/-}$) mice (Figures 1B and 1C). Whereas no difference in the expression of mRNA for IFN-β was observed between WT and *Clec7a*$^{-/-}$ BMDCs stimulated with LPS (Figure 1B), IFN-β mRNA expression was dramatically reduced in *Clec7a*$^{-/-}$ BMDCs after Curdlan stimulation (Figure 1B), and, correspondingly, no IFN-β was detected at the protein level (Figure 1C). This result demonstrates that the production of IFN-β by Curdlan-stimulated BMDCs was dependent

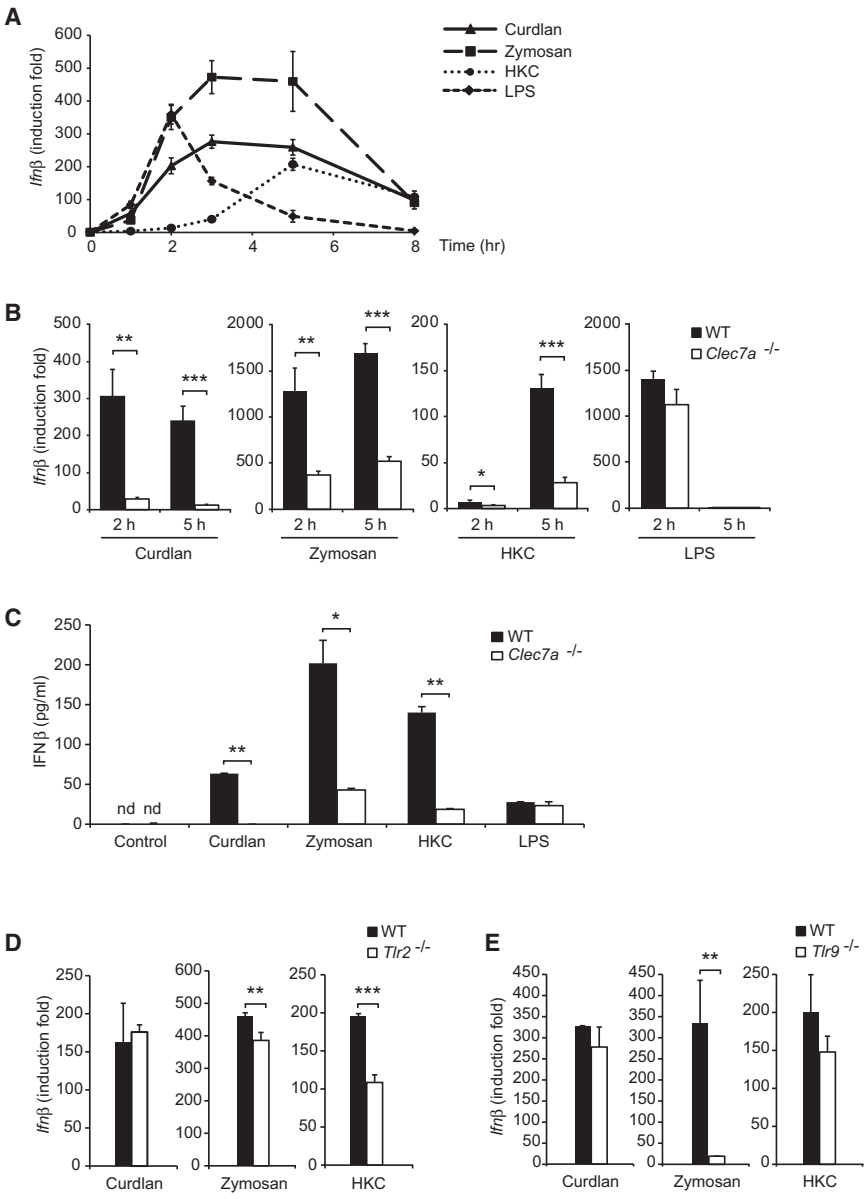

Figure 1. Dectin-1 Engagement Induces IFN-β Production by BMDCs

(A) Expression of mRNA for IFN-β by BMDCs stimulated for the indicated times with Curdlan, Zymosan, HKC, or LPS was assessed by real-time PCR, normalized to β-actin.

(B) BMDCs from WT or *Clec7a*$^{-/-}$ mice, unstimulated or stimulated for 2 or 5 hr with Curdlan, Zymosan, HKC, or LPS, were analyzed for the expression of mRNA for IFN-β by real-time PCR, normalized to β-actin.

(C) IFN-β production by BMDCs from WT or *Clec7a*$^{-/-}$ mice unstimulated (control) or stimulated for 16 hr with Curdlan, Zymosan, HKC, or LPS, was analyzed by ELISA.

(D and E) IFN-β mRNA expression by BMDCs from WT, *Tlr2*$^{-/-}$ (D), or *Tlr9*$^{-/-}$ (E) mice stimulated for 5 hr with Curdlan, Zymosan, or HKC was analyzed by real-time PCR, normalized to β-actin. Real-time PCR data are expressed as induction fold relative to unstimulated controls (mean ± SD of triplicates). ELISA data are expressed as mean ± SD of duplicate samples. The values for unstimulated controls were below the detection level of the ELISA kit used in this study (15.6 pg/ml), nd: not detectable. Significant differences, as determined by the unpaired t test, are indicated *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Data are representative of at least two independent experiments with similar results. See also Figures S1 and S2.

on Dectin-1 signaling. After stimulation with Zymosan or HKC, IFN-β mRNA expression was significantly lower in *Clec7a*$^{-/-}$ that in WT BMDCs (Figure 1B), and, accordingly, IFN-β production was reduced around 80% (Figure 1C). These data suggest that IFN-β production in response to *C. albicans* results, for the most part, from Dectin-1 signaling, and consequently to a lesser extent from TLR-mediated signaling. To further explore the relative contribution of TLR-mediated signaling to IFN-β production in response to *C. albicans*, we analyzed IFN-β mRNA expression by BMDCs from mice deficient in the receptors TLR2 or TLR9 that have been reported to contribute predominantly to TLR-mediated activation of DCs exposed to *C. albicans*, by interacting with *Candida* cell wall mannans and *Candida* DNA, respectively (Romani, 2011). As expected, neither TLR2 nor TLR9 deficiency affected the response of BMDCs to Curdlan stimulation (Figures 1D and 1E). The expression of IFN-β mRNA was reduced around 50% after stimulation of *Tlr2*$^{-/-}$ BMDCs with HKC compared to their WT counterparts, but was not significantly affected in HKC-stimulated BMDCs from *Tlr9*$^{-/-}$ mice. This result reflects that *Candida* DNA does not contribute significantly to HKC-mediated IFN-β production and revealed that HKC preparations contained a minimal amount of free *Candida* DNA. In contrast, IFN-β mRNA expression induced by Zymosan was strongly inhibited in *Tlr9*$^{-/-}$ BMDCs, indicating that yeast DNA from *S. cerevisiae* was readily exposed in Zymosan preparations. Globally, these results reveal that IFN-β production by BMDCs in response to *C. albicans* is mainly driven by Dectin-1 signaling and that IFN-β produced by BMDCs in response to Curdlan is TLR-independent and dependent on Dectin-1.

IFN-β Production Triggered by Dectin-1 Is Dependent on Syk and Card9

As mentioned above, Dectin-1 engagement results in the recruitment of Syk and Card9, leading to cytokine production by an NF-κB and NFAT-dependent pathway (Sancho and Reis e Sousa, 2012). In order to assess whether Dectin-1-induced IFN-β production was dependent on Syk, IFN-β production was analyzed in BMDCs from CD11c-Cre$^{+/-}$ *Syk*$^{fl/fl}$ mice, in which *Syk* deletion is under the control of the promoter for CD11c, an integrin expressed by all mouse DC subtypes. As

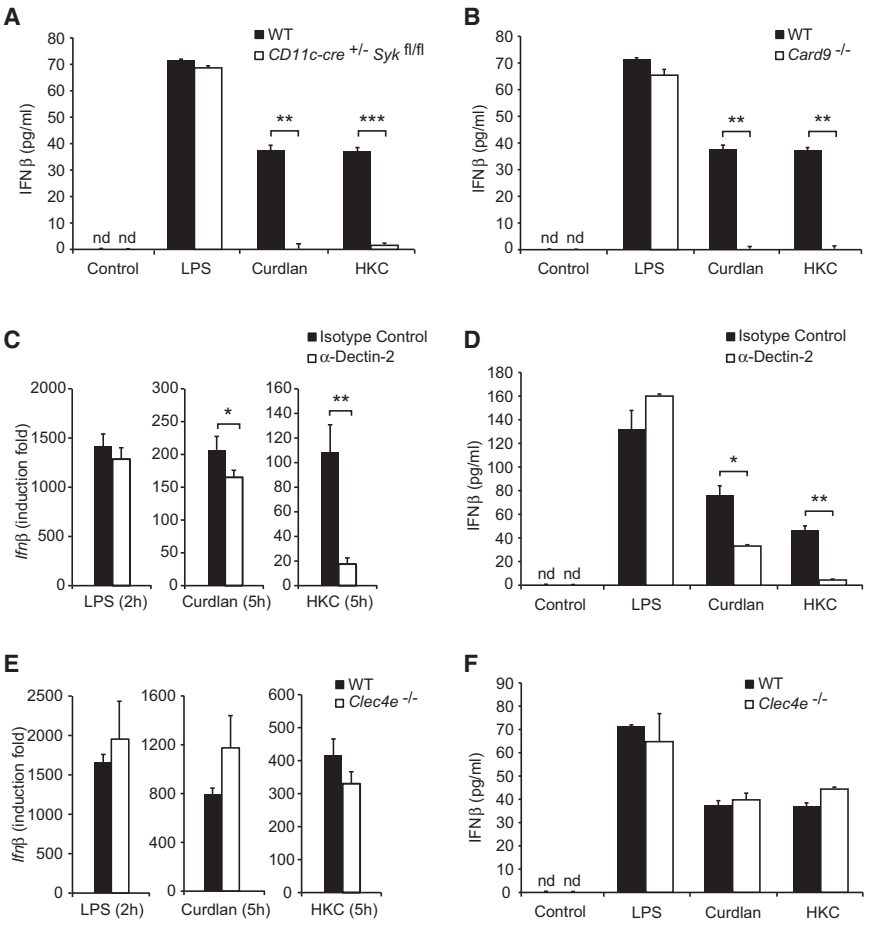

Figure 2. Contribution of Syk and Card9 Signaling to IFN-β Production by BMDCs in Response to *C. albicans*

(A and B) IFN-β production by BMDCs from WT, CD11c-Cre$^{+/-}$ *Syk*$^{fl/fl}$ (A), or *Card9*$^{-/-}$ (B) mice unstimulated (control) or stimulated for 16 hr with LPS, Curdlan, or HKC was analyzed by ELISA.

(C) IFN-β mRNA expression by BMDCs treated with anti-Dectin-2 or isotype control antibodies and stimulated for the indicated times with LPS, Curdlan, or HKC was analyzed by real-time PCR, normalized to β-actin.

(D) IFN-β production by BMDCs treated with anti-Dectin-2 or isotype control antibodies and stimulated or not (control) for 16 hr with LPS, Curdlan, or HKC was analyzed by ELISA.

(E) IFN-β mRNA expression by BMDCs from *Clec4e*$^{-/-}$ mice stimulated for the indicated times with LPS, Curdlan, or HKC was analyzed by real-time PCR, normalized to β-actin.

(F) IFN-β production by BMDCs from *Clec4e*$^{-/-}$ mice unstimulated (control) or stimulated for 16 hr with LPS, Curdlan, or HKC was analyzed by ELISA. Real-time PCR data are expressed as induction fold relative to unstimulated controls (mean ± SD of triplicates). ELISA data are expressed as mean ± SD of duplicate samples. Significant differences, as determined by the unpaired t test, are indicated *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Data are representative of at least two independent experiments with similar results. n.d., not detectable.

shown in Figure 2A, deletion of *Syk* had no effect on the synthesis of IFN-β by BMDCs stimulated by LPS as expected, but completely blocked its production by Curdlan-stimulated BMDCs, confirming the involvement of Syk in Dectin-1-mediated IFN-β production. Because Card9 is involved in NF-κB activation resulting from Dectin-1 engagement (Gross et al., 2006) and NF-κB is required for the transcriptional activation of the *Ifnb* gene (Panne et al., 2007), we hypothesized that Card9 could also control IFN-β production in response to Curdlan. To address this issue, we analyzed BMDCs from *Card9*$^{-/-}$ mice for their ability to produce IFN-β after stimulation with Curdlan or LPS. As expected, Card9 deficiency strongly inhibited the production of IFN-β in response to Curdlan, but not to LPS, confirming that Dectin-1-mediated IFN-β production is dependent on Card9 (Figure 2B). Interestingly, IFN-β production by HKC-stimulated BMDCs from either CD11c-Cre$^{+/-}$ *Syk*$^{fl/fl}$ or *Card9*$^{-/-}$ mice was barely detectable, indicating that production of IFN-β by BMDCs in response to *C. albicans* is essentially dependent on Syk and Card9 signaling.

The fact that the blockade of IFN-β production in response to HKC was even more pronounced in either CD11c-Cre$^{+/-}$ *Syk*$^{fl/fl}$ or *Card9*$^{-/-}$ BMDCs than in *Clec7a*$^{-/-}$ BMDCs, led us to explore the contribution of Dectin-2 and Mincle, the two other C-type lectin receptors recognizing *C. albicans* cell wall mannans and signaling through a Syk-Card9-mediated pathway (Sancho and Reis e Sousa, 2012), to IFN-β production in response to *C. albicans*. The production of IFN-β in response to Curdlan was reduced around 20% and 50% at the mRNA and protein level, respectively, after treatment with the anti-Dectin-2 blocking antibody D2.11E4 (Figures 2C and 2D). This result suggests that in BMDCs Curdlan was recognized by Dectin-2, although less efficiently than by Dectin-1, or alternatively that the Dectin-2 antibody D2.11E4 displayed some cross-reactivity with Dectin-1. Interestingly, HKC-induced production of IFN-β by anti-Dectin-2 treated BMDCs was reduced to a same extent than in *Clec7a*$^{-/-}$ BMDCs. In contrast, Mincle (Clec4e) deficiency had no effect on IFN-β production induced by either Curdlan or HKC (Figures 2E and 2F). These data suggest that Dectin-2, which requires to be associated with the ITAM-bearing adaptor FcRγ to recruit Syk (Sancho and Reis e Sousa, 2012), contributes significantly to IFN-β production in response to *C. albicans*, through a Dectin-2-FcRγ-Syk pathway. Therefore our data support that IFN-β production in response to *C. albicans* results essentially from the combined action of Dectin-1 and Dectin-2 signaling.

IRF5, but Neither IRF3 Nor IRF7, Controls Dectin-1-Dependent IFN-β Production

To identify the molecular mechanism leading to IFN-β production after Dectin-1 engagement, we analyzed the activation of the main transcriptional activators required for the assembly of the IFN-β enhanceosome: NF-κB, c-Jun, and IRF3. For this purpose, the phosphorylation and nuclear translocation of the RelA subunit of NF-κB, c-Jun, and IRF3 was evaluated by immunoblot

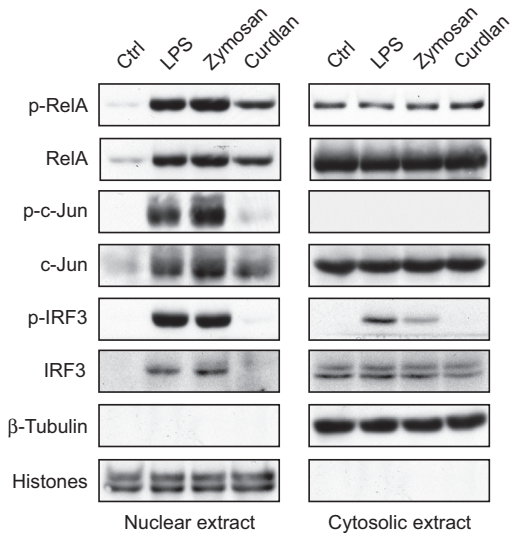

Figure 3. Activation of *Ifnb* Gene Transcriptional Activators after Dectin-1 Engagement in BMDCs

RelA, c-Jun, and IRF3 activation was determined on nuclear and cytosolic protein extracts from BMDCs stimulated with LPS, Zymosan, or Curdlan for 2 hr. Extracts were analyzed by immunoblot by using antibodies against phospho-RelA, phospho-c-Jun, or phospho-IRF3, RelA, c-Jun, and IRF3. Purity of the nuclear and cytosolic cell fractions was assessed by using antibodies against Histones (H1 and core proteins) and β-tubulin, respectively. Data are representative of two independent experiments with similar results.

on cell lysates from the nuclear or cytosolic fractions of Curdlan-, Zymosan-, or LPS-activated BMDCs. Whereas BMDC stimulation with either Curdlan, Zymosan, or LPS led to a correct activation of RelA (Figure 3), the phosphorylation and nuclear translocation of c-Jun was almost undetectable in BMDCs activated with Curdlan, indicating that IFN-β production in response to Curdlan occurred in the absence of a strong c-Jun activation. In contrast, both Zymosan and LPS induced a correct activation of c-Jun. The analysis of IRF3 activation, required for IFN-β production in response to the majority of type I IFN-inducing receptors, including cytosolic receptors as well as TLR3 and TLR4 (Trinchieri, 2010), revealed that IRF3 was not phosphorylated and consequently it was not translocated to the nucleus in response to Curdlan stimulation. In contrast, as expected, these processes occurred correctly in LPS- or Zymosan-activated BMDCs (Figure 3). This finding strongly supports the hypothesis that IRF3 was not required for IFN-β production in response to Curdlan. To confirm this hypothesis, we analyzed BMDCs from *Irf3*$^{-/-}$ mice for their capacity to produce IFN-β in response to Curdlan or LPS. IRF3 deficiency led to a dramatic reduction in IFN-β production by BMDCs stimulated with LPS, but had no effect on the synthesis of IFN-β in response to Curdlan (Figure 4A), confirming that IRF3 was not involved in Dectin-1-triggered IFN-β production. Given that IFN-β production induced after engagement of TLR7 or TLR9 on DCs has been demonstrated to be dependent on the transcription factor IRF7 (Decker et al., 2005), we used BMDCs from *Irf7*$^{-/-}$ mice to address whether IRF7 was involved in Dectin-1-mediated IFN-β production. Our data demonstrate that IRF7 was not involved in this process as IFN-β production by Curdlan-stimulated BMDCs was

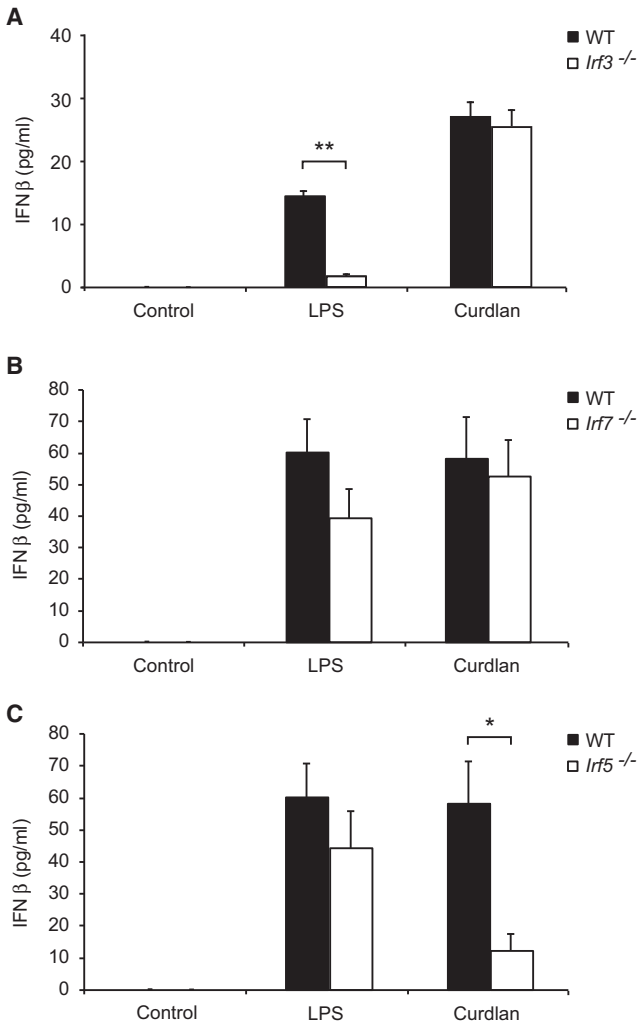

Figure 4. IRF5, but Neither IRF3 Nor IRF7, Controls Dectin-1-Dependent IFN-β Production

IFN-β production by BMDCs from wild-type (WT), *Irf3*$^{-/-}$ (A), *Irf7*$^{-/-}$ (B) or *Irf5*$^{-/-}$ (C) mice unstimulated (control) or stimulated for 16 hr with LPS or Curdlan was analyzed by ELISA. Data are expressed as mean ± SD of duplicate samples. Significant differences, as determined by the unpaired t test, are indicated *$p < 0.05$; **$p < 0.01$. Data are representative of three (A) or two (B and C) independent experiments with similar results. n.d., not detectable. See also Figure S3.

not affected by IRF7 deficiency (Figure 4B). In conclusion, these experiments confirmed that IFN-β production in response to Curdlan was dependent on neither IRF3 nor IRF7, that in contrast play a crucial role in the control of type I IFN production in response to virus and bacteria through cytosolic or TLR receptors. In search for a member of the IRF family of transcription factors that may control the transcriptional activation of the *Ifnb* gene resulting from Dectin-1 engagement, we explored the possible involvement of IRF5 in this process, based on two recent reports demonstrating a correlation between IRF5 and IFN-β production after bacterial infection (Gratz et al., 2011; Pandey et al., 2009). Interestingly, Dectin-1-mediated IFN-β production was found to be strongly dependent on IRF5, as assessed by analyzing BMDCs from *Irf5*$^{-/-}$ mice (Figure 4C). Regarding

these experiments performed with *Irf5*$^{-/-}$ mice, it is important to point out that a recent report (Purtha et al., 2012) has revealed that, in some cases, *Irf5*$^{-/-}$ mice harbor a spontaneous mutation in the *Dock2* gene that could interfere with the analysis of IRF5 deficiency. However, as shown in Figure S3, the genotyping of the *Irf5*$^{-/-}$ mice used in our study confirmed that they had a normal *Dock2* genotype.

In conclusion, our data firmly demonstrate the existence in BMDCs of a Dectin-1-mediated signaling pathway leading to the production of IFN-β that is dependent on the Syk kinase, the NF-κB activator Card9 and on the transcription factor IRF5. Importantly, our results also support that type I IFN production by BMDCs in response to *C. albicans* is mainly controlled by this Dectin-1-dependent pathway.

Type I IFN Production in Response to *C. albicans* Is Crucial for Defense against Candidiasis

In order to explore the role of IFN-β produced in response to *C. albicans*, we first analyzed the survival of WT versus *Ifnar1*$^{-/-}$ mice after intravenous injection of LD_{50} dose of 1×10^5 *C. albicans*. During the 2 first weeks after infection, 100% (12/12) versus 16% (2/12) mice died in *Ifnar1*$^{-/-}$ and WT strains, respectively. Correspondingly, the survival at day 30 after infection was significantly higher in WT than in *Ifnar1*$^{-/-}$ mice (75% versus 0%, p value < 0.0001; Figure 5A). Because the kidney is the main target for *C. albicans* infection, we analyzed the renal fungal burden at day 6 after infection, corresponding to the time point when mice started to die. As expected from our survival data, *Ifnar1*$^{-/-}$ mice had a significantly higher fungal burden than WT mice (p value < 0.05; Figure 5B). In line with our data supporting that type I IFN production by DCs in response to *C. albicans* is essentially dependent on Dectin-Syk-IRF5 signaling, the renal fungal load in *C. albicans*-infected *Irf5*$^{-/-}$ mice was significantly higher than in their WT counterparts (p value < 0.05; Figure S4). In an attempt to unravel the mechanism by which IFN-β exerted a protective effect after *C. albicans* infection, we analyzed the recruitment to the kidney of the different leukocytic cell types infiltrating the renal parenchyma, in WT and *Ifnar1*$^{-/-}$ mice, from day 0 to day 6 after infection. The number of renal infiltrating leukocytes, characterized as CD45$^+$ cells, was reduced in *Ifnar1*$^{-/-}$ mice to 50% and 15% of controls at days 2 and 6 after infection, respectively (Figure 5C). This was paralleled by an average 50% reduction in the number of infiltrating NK cells, B cells, and DCs in *Ifnar1*$^{-/-}$ mice at day 2 postinfection. At day 6, the lymphoid and DC subsets underwent a more pronounced reduction, to around 30% of controls for B cells and DCs and to around 15% of controls for T cells and NK cells (Figures 5D and 5E). Interestingly, the number of neutrophils present in renal leukocyte infiltrates of *Ifnar1*$^{-/-}$ mice was reduced to around 25% of controls at day 2 and underwent a dramatic 95% reduction at day 6. No reduction was detected in any leukocyte-infiltrating subset at day 4 postinfection in line with previous data revealing the existence of two waves of leukocyte infiltration in the kidney after *C. albicans* infection peaking at days 3 and 5 after infection (Lionakis et al., 2011). To determine whether the impaired neutrophil recruitment to the kidney observed in *Ifnar1*$^{-/-}$ mice was linked to a defective expression of neutrophil-attractant chemokines, we first analyzed the production of CXCL1 and CXCL2 by CD45$^+$ renal infiltrating leukocytes at the mRNA and protein level, by real-time PCR and Luminex, respectively. In line with the strong reduction in the number of neutrophils observed in the renal infiltrates of *Ifnar1*$^{-/-}$ mice, the production of CXCL1 and CXCL2 by renal infiltrating leukocytes was also significantly reduced in *Ifnar1*$^{-/-}$ mice (Figures 5G and 5H). Because nonhematopoietic cells are known to contribute also substantially to the production of these chemokines, we analyzed the expression of CXCL1 and CXCL2 mRNA by the CD45$^-$ nonhematopoietic kidney fraction in *C. albicans*-infected WT and *Ifnar1*$^{-/-}$ mice. Interestingly, IFNAR1 deficiency did not affect the expression of these chemokines by nonhematopoietic cells (Figure S5). These data suggest that type I IFN promotes neutrophil recruitment to the kidney by a process that involves CXCL1 and CXCL2 production by infiltrating leukocytes. We subsequently explored the mechanism by which type I IFN positively regulates the production of these chemokines in the kidney during *C. albicans* infection. For this purpose we analyzed the effect of IFNAR1 deficiency on the expression of mRNA for CXCL1 and CXCL2 by BMDCs and neutrophils in vitro after stimulation with Curdlan or HKC, because DCs and neutrophils are the main leukocytes subsets potentially responsible for the production of theses chemokines in the kidney after *C. albicans* infection. Our data revealed that whereas no differences in CXCL1 and CXCL2 mRNA expression were found between BMDCs from WT or *Ifnar1*$^{-/-}$ mice, IFNAR1 deficiency lead to a strong reduction in the expression of mRNA for these chemokines by neutrophils after incubation with either Curdlan or HKC (Figure S6). As expected, no expression of mRNA for CXCL1 or CXCL2 was detected in T or B cells. These data support the idea that *C. albicans*-induced production of CXCL1 and CXCL2 by neutrophils is strongly dependent on type I IFN and suggest that the decreased production of these chemokines by renal infiltrating leukocytes during *C. albicans* infection of *Ifnar1*$^{-/-}$ mice results from their defective production by neutrophils in the absence of IFNAR signaling.

IFN-β Production during *C. albicans* Infection Is Controlled by DCs through Dectin and Syk Signaling

Our data from in vivo infection experiments in *Ifnar1*$^{-/-}$ mice revealed that type I IFN production is crucial for defense against *C. albicans*. In order to determine the contribution of DCs to type I IFN production through the Dectin-Syk-IRF5 signaling pathway, *C. albicans* infection experiments were performed in CD11c-Cre$^{+/-}$ *Syk*$^{fl/fl}$ mice, in which DCs are not responsive to Syk-dependent signaling and consequently are impaired in their capacity to produce IFN-β through Dectin and Syk. For this purpose, we first analyzed the kinetics of IFN-β production by renal infiltrating leukocytes after *C. albicans* infection by real-time PCR. Our data revealed that IFN-β production was undetectable at day 2 after infection, peaked at day 3 and was reduced along the next 24 hr (Figure 6A); IFN-β was not detectable on splenic DCs from day 2 to day 4 after infection (Figure 6B). On the basis of these data, we next analyzed IFN-β mRNA expression by renal infiltrating leukocytes at day 3 postinfection in WT and CD11c-Cre$^{+/-}$ *Syk*$^{fl/fl}$ mice. Interestingly, IFN-β production by renal-infiltrating leukocytes was reduced by around 70% in CD11c-Cre$^{+/-}$ *Syk*$^{fl/fl}$ mice (Figure 6C), and this reduction was not paralleled by a decreased number of infiltrating DCs (Figure 6D). In conclusion, our data strongly support the idea that,

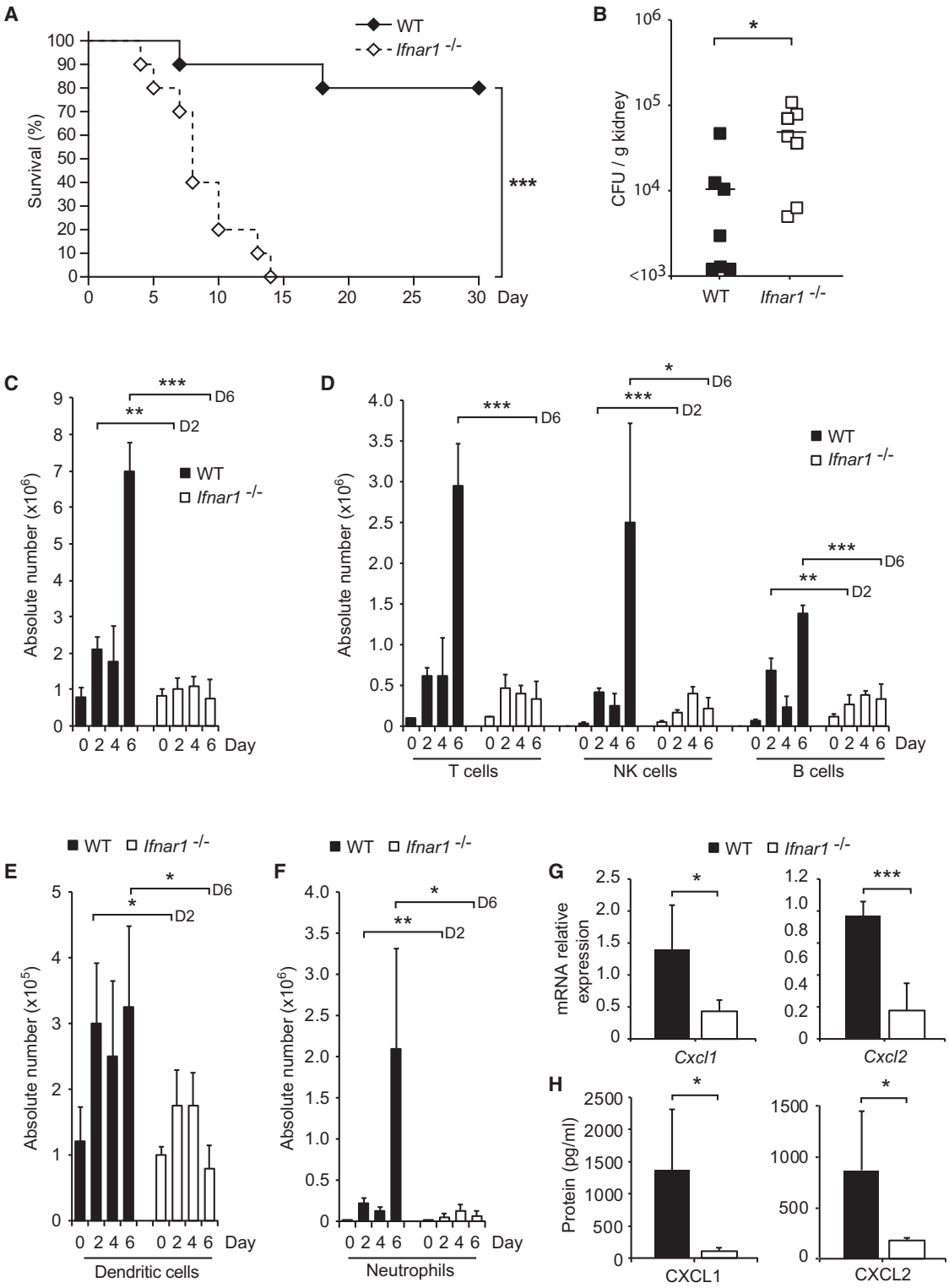

Figure 5. Type I IFN Produced in Response to *C. albicans* Is Crucial for Defense against Candidiasis

(A) Survival of WT (n = 12) and *Ifnar1*$^{-/-}$ (n = 12) mice infected intravenously with 1 × 10^5 *C. albicans*.

(B) Kidney fungal burden of WT (n = 7) and *Ifnar1*$^{-/-}$ (n = 7) mice at day 6 after infection with 1 × 10^5 *C. albicans*. Data are expressed as cfu/g kidney.

(C) Kinetics of infiltrating leukocytes (defined as CD45$^+$ cells) after infection with 1 × 10^5 *C. albicans* of WT or *Ifnar1*$^{-/-}$ mice. Data are expressed as mean ± SD of four mice per condition.

(D) Absolute number of T, NK, and B cells (defined as CD90$^+$, CD49b$^+$, and CD19$^+$ cells, respectively) in the renal leukocyte infiltrates of WT or *Ifnar1*$^{-/-}$ mice at the indicated times after infection with 1 × 10^5 *C. albicans*. Data are expressed as mean ± SD of four mice per condition.

(legend continued on next page)

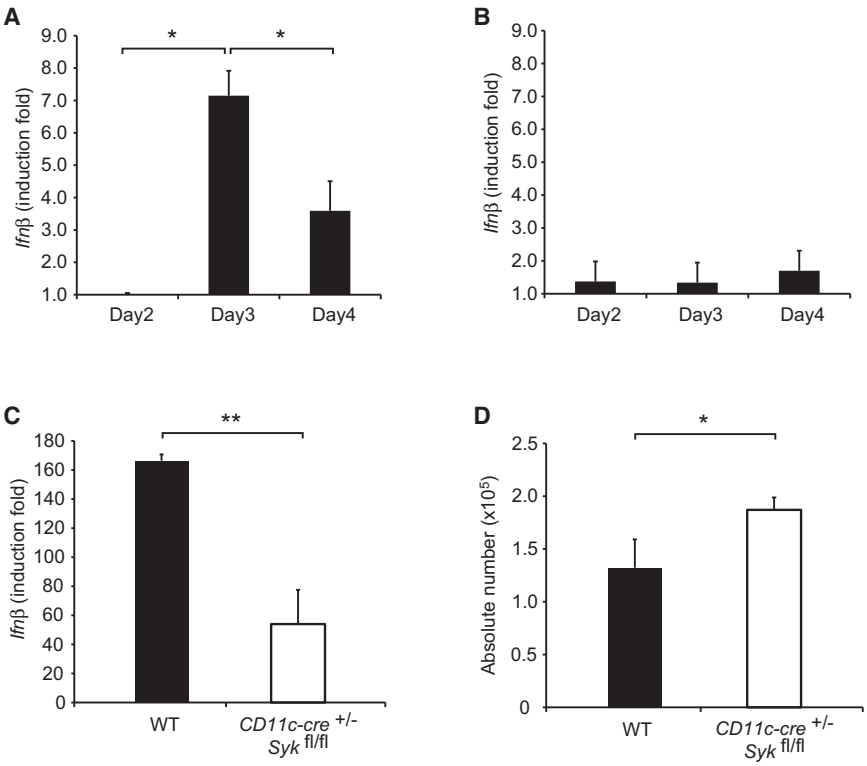

Figure 6. IFN-β Production in *Candida*-Infected CD11c-Cre$^{+/-}$ *Syk*$^{fl/fl}$ Mice

(A and B) Expression of IFN-β mRNA by purified leukocyte preparations from renal infiltrates (A) or purified splenic DCs (B) obtained at the indicated times after infection with 1 × 10^5 *C. albicans* assessed by real-time PCR, normalized to β-actin. Data are expressed as induction fold relative to the expression at day 2 after infection (mean ± SD of triplicates).

(C) Expression of IFN-β mRNA by purified renal infiltrating leukocyte preparations from WT or CD11c-Cre$^{+/-}$ *Syk*$^{fl/fl}$ mice at day 3 after infection assessed by real-time PCR, normalized to β-actin. Data are expressed as induction fold relative to the expression at day 2 post-infection for WT mice (mean ± SD of triplicates).

(D) Absolute number of DCs in the renal leukocyte infiltrates of WT or CD11c-Cre$^{+/-}$ *Syk*$^{fl/fl}$ mice at day 3 after infection. DCs were defined as CD11c$^+$ CD11b$^+$ cells after gating out cells expressing CD90 (T cells), B220 (B cells), CD49b (NK cells), and Ly-6G (neutrophils). Data are expressed as mean ± SD of three mice per condition. Significant differences as determined by the unpaired t test are indicated *p < 0.05; **p < 0.01. Data are representative of two independent experiments with similar results.

after *C. albicans* infection, IFN-β production by renal infiltrating leukocytes is controlled primarily by DCs and, importantly, that this production of IFN-β by renal infiltrating DCs is essentially driven by Dectin signaling via Syk.

DISCUSSION

Type I IFN is produced after engagement of cytosolic receptors or membrane-bound TLR receptors by viral or bacterial compounds through the activation of the transcription factors IRF3 and IRF7 (Trinchieri, 2010), as shown in Figure S7, summarizing the key signaling pathways emerging from the main type I IFN-inducing receptors. Fungi, particularly yeasts of the genus *Candida*, have also been recently reported to induce IFN-β production (Biondo et al., 2011; Bourgeois et al., 2011), but the mechanism responsible for the transcriptional activation of *Ifnb* gene in DCs, during *C. albicans* infection, is a matter of controversy. In this report we describe a signaling pathway leading to the production of IFN-β after stimulation of BMDCs with Curdlan, a Dectin-1 specific ligand that mimics the interaction of this C-type lectin receptor with β-glucans from *C. albicans* cell wall. IFN-β production triggered by Dectin-1 engagement is dependent on Syk, Card9, and the transcription factor IRF5, and independent of IRF3 and IRF7, as illustrated in Figure S7. To our knowledge, C-type lectin receptors had not been previously demonstrated to trigger the production of type I IFN. In addition, experiments using antibodies against Dectin-2, another Syk and Card9-dependent C-type lectin receptor recognizing *C. albicans* cell wall mannans, revealed that Dectin-2 signaling also contributes significantly to IFN-β production in response to *C. albicans*, a process that therefore results from a dual contribution of Dectin-1 and Dectin-2 signaling, in agreement with previous data demonstrating the cooperation of these receptors in inducing IL-2 and IL-10 production by BMDCs (Robinson et al., 2009). Importantly, our data based on BMDC stimulation with Curdlan and HKC demonstrate that IFN-β production in response to *C. albicans* is mostly controlled by Dectin-1 and Dectin-2 signaling with a minor contribution of TLR2 signaling. Therefore *Candida*-induced IFN-β production essentially results from the recognition of *C. albicans* cell-wall β-glucans by Dectin-1 and cell-wall mannans by Dectin-2 and TLR2. The differential relevance of TLR9 signaling to IFN-β production in response to Zymosan versus HKC reflects that whereas yeast DNA is readily exposed in Zymosan preparations and thus has

(E and F) Absolute number of DCs (E) and neutrophils (F), defined by flow cytometry on the basis of CD11c and Ly-6G expression, respectively, in the renal leukocyte infiltrates of WT or *Ifnar1*$^{-/-}$ mice, at the indicated times after infection with 1 × 10^5 *C. albicans*. Data are expressed as mean ± SD of four mice per condition.

(G) Expression of mRNA for CXCL1 and CXCL2 by purified leukocyte preparations from renal infiltrates obtained from WT or *Ifnar1*$^{-/-}$ mice at day 4 after infection with 1 × 10^5 *C. albicans* was assessed by real-time PCR, normalized to β-actin. Data are expressed as relative expression of each chemokine in *Ifnar1*$^{-/-}$ versus WT mice, considering as 1 the average expression for WT mice (mean ± SD of four mice per condition).

(H) Production of CXCL1 and CXCL2 by purified leukocyte preparations from renal infiltrates obtained from WT or *Ifnar1*$^{-/-}$ mice at day 4 after infection with 1 × 10^5 *C. albicans*, analyzed by Luminex. Significant differences as determined by the unpaired t test are indicated *p < 0.05; **p < 0.01; ***p < 0.001. Data are representative of at least two independent experiments with similar results. See also Figures S4 to S6.

a major contribution to Zymosan-induced IFN-β production, *Candida* DNA-mediated TLR9 signaling does not contribute substantially to IFN-β production in response to *C. albicans.*

Our data revealed that Dectin-1-mediated IFN-β production is driven by a Syk-Card9-dependent pathway, that has been demonstrated to activate NF-κB and MAP kinases (Sancho and Reis e Sousa, 2012), supporting the hypothesis that Dectin-1 signaling can lead to the activation of two key transcriptional factors required for the assembly of the IFN-β enhanceosome, NF-κB, and c-Jun (Panne et al., 2007). IRF3 and IRF7 were also demonstrated to contribute to the IFN-β enhanceosome controlling type I IFN production resulting from the activation of cytosolic or TLR receptors (Trinchieri, 2010). However, unexpectedly, Dectin-1-induced transcription of the *Ifnb* gene required neither IRF3 nor IRF7 but the transcription factor IRF5. A link between IRF5 and type I IFN production during viral infections was proposed in studies showing that infection by viruses, such as Newcastle disease virus (NDV), vesicular stomatitis virus (VSV), and herpes simplex virus type 1 (HSV-1), led to the transcription of *Ifna* and *Ifnb* genes, by an IRF5-dependent mechanism (Barnes et al., 2002). Type I IFN production induced in response to the TLR7 ligand R-848 was also found to depend on both IRF7 and IRF5 (Schoenemeyer et al., 2005). More recently, *Mycobacterium tuberculosis*-induced expression of IFN-β mRNA in macrophages has been reported to occur after recognition of a bacterial muramyl dipeptide by the cytosolic receptor NOD2 and was largely dependent on IRF5 (Pandey et al., 2009). In addition, BMDCs were demonstrated to produce IFN-β after recognition of RNA from *Streptococcus pyogenes* by a mechanism dependent on MyD88 and IRF5 (Gratz et al., 2011).

The production of IFN-β by DCs in response to fungi, and particularly to yeasts of the genus *Candida*, has been described in two recent reports that proposed different signaling pathways to explain the transcriptional activation of the *Ifnb* gene induced by yeasts. Bourgeois et al. proposed that IFN-β production in response to *Candida glabrata* was triggered after engagement of TLR-7 by fungal RNA and was not affected by Dectin-1 deficiency; unexpectedly, this process appeared to be independent of IRF3 and IRF7 (Bourgeois et al., 2011). Alternatively, Biondo et al. reported that *C. albicans* 90028 strain induced IFN-β production by BMDCs after recognition of fungal DNA and proposed that this occurred by a mechanism partially dependent on TLR-7 and TLR-9 and on IRF-1, IRF3, and IRF7 (Biondo et al., 2011). In addition, these authors reported that Dectin-1 was not involved in the production of IFN-β in response to *C. albicans* yeast particles. Therefore, these two studies concur in proposing that IFN-β production in response to *Candida* was triggered by the activation of TLR receptors by fungal nucleic acids with no contribution of Dectin-1 signaling. The divergence between these reports and our data, proposing a major role for Dectin-1 in IFN-β production in response to *C. albicans* SC5314 strain (being the contribution of TLR signaling secondary), could be due to differences in the cell-wall composition between the yeast species or strains used in these studies that may dictate their ability to activate Dectin-1 signaling. In this regard, *Candida glabrata* is a nondimorphic yeast that is phylogenetically closer to *Saccharomyces cerevisiae* than to *C. albicans* (Kurtzman and Robnett, 1997), and thus *C. albicans* and *C. glabrata* may differ substantially in their ability to activate Dectin-1 signaling. Interestingly, host defense against two *C. albicans* strains was found to be differentially dependent on Dectin-1; whereas this receptor was required for the induction of antifungal immunity against the SC5314 strain (Taylor et al., 2007), protective immunity after infection with the IFO1385 strain was independent of Dectin-1 (Saijo et al., 2007).

The physiological relevance of the production of type I IFN in response to *C. albicans* was confirmed by our in vivo studies revealing that *Ifnar1*$^{-/-}$ mice had a lower survival after infection with *C. albicans* that was paralleled by a higher fungal burden in the kidney, the main target after systemic *C. albicans* infection. Our data, demonstrating that type I IFNs contribute to a more efficient immune response against *C. albicans*, confirm two previous reports on *C. albicans* (Biondo et al., 2011) and *Cryptococcus neoformans* (Biondo et al., 2008) infection that support the hypothesis that type I IFN had a beneficial effect for antifungal immunity. However, type I IFN had a deleterious effect during infection by the fungi *Histoplasma capsulatum* (Inglis et al., 2010) or *C. glabrata* (Bourgeois et al., 2011). In addition, *Ifnar1*$^{-/-}$ mice were recently reported to have an improved survival in response to *C. albicans*, although strikingly this increased survival was not paralleled by a lower fungal burden (Majer et al., 2012). Type I IFN has also been demonstrated to have opposing beneficial or detrimental effects on antiviral and antibacterial immunity depending on the infectious process (Trinchieri, 2010). During viral infections, type I IFNs contribute to host defense through their antiviral and immunomodulatory properties, but they can also mediate tissue damage and inflammatory or autoimmune reactions. Type I IFN can also contribute to protective antibacterial immunity essentially by blocking intracellular bacterial replication and through the induction of nitric oxide and proinflammatory cytokines. However, type I IFN production is often detrimental for defense against bacteria through several mechanisms. In this regard, type I IFN can increase the susceptibility of T cells and macrophages to apoptosis, interfere with microbicidal mechanisms, favor bacterial replication, limit T cell responses, and alter the recruitment and activation of myeloid cells to the site of infection (Stetson and Medzhitov, 2006).

Our results revealed that IFNAR1 deficiency led to lower survival after *C. albicans* infection that was paralleled by a defective renal neutrophil recruitment and a lower production of the neutrophil-chemoattractant chemokines CXCL1 and CXCL2. These findings support that type I IFN plays a crucial role in defense against *C. albicans* by promoting the mobilization of neutrophils to the kidney. Neutrophils are required for an efficient protection against systemic candidiasis due to their high capacity to kill *C. albicans* (Mansour and Levitz, 2002), as demonstrated by experiments of neutrophil depletion in *C. albicans*-infected mice, in which survival was severely compromised (Dejima et al., 2011). CXCL1 and CXCL2 have a critical function in the recruitment of neutrophils (Kobayashi, 2006), and interestingly they appear to be highly expressed in the kidney of *C. albicans*-infected mice, as revealed by gene expression microarray analysis (MacCallum, 2009). In this regard, our data suggest that type I IFN controls renal neutrophil infiltration by controlling the production of CXCL1 and CXCL2 by neutrophils, in contrast to previous evidence on the negative regulation of these chemokines by type I IFN (Trinchieri, 2010).

More importantly, the experiments revealing that the production of IFN-β by renal infiltrating leukocytes was severely

reduced in *C. albicans*-infected CD11c-Cre$^{+/-}$ *Syk*$^{fl/fl}$ mice, in which DCs are not responsive to Syk-dependent signaling, have been decisive to establish the link between the compromised survival of *Ifnar1*$^{-/-}$ mice and the Dectin and Syk-dependent pathway of IFN-β production by DCs. These data support the hypothesis that most of type I IFN required for protection against *C. albicans* was produced by renal-infiltrating inflammatory DCs through Dectin-Syk signaling and that there was only a minor contribution from DCs through Syk-independent signaling, and/or from the rest of non-DC infiltrating leukocytes, to type I IFN production during *C. albicans* infection.

In conclusion, the data presented in this report demonstrate that the production of type I IFN by DCs induced by *C. albicans* is essentially controlled by a Dectin-1-mediated signaling pathway, dependent on Syk, Card9 and IRF5. The existence of this pathway extends the physiological relevance of Dectin-1-mediated signaling, particularly in the context of the induction of antifungal immunity because, in addition to driving the synthesis of cytokines that are essential for the induction of Th17 cell responses, it triggers the production of IFN-β that, as revealed by our data, is crucial for defense against *C. albicans*.

EXPERIMENTAL PROCEDURES

Mice

C57BL/6 (10–12 weeks old) female mice were purchased from Harlan (Bicester, UK). *Ifnar1*$^{-/-}$ mice were kindly provided by Dr. U. Kalinke (Center for Experimental and Clinical Infection Research, Hannover, Germany). CD11c-Cre$^{+/-}$ *Syk*$^{fl/fl}$ mice and *Clec4e*$^{-/-}$ mice were provided by Dr. D. Sancho (Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain). C57BL/6, *Ifnar1*$^{-/-}$, and CD11c-Cre$^{+/-}$ *Syk*$^{fl/fl}$ mice were housed at the animal facility of Centro Nacional de Biotecnología-CSIC, Madrid, Spain. *Card9*$^{-/-}$ mice were generated by Dr. J. Ruland (Technische Universität München, München, Germany). Bone marrow from *Clec7a*$^{-/-}$ mice was generously provided by Dr. G. Brown (University of Aberdeen, Aberdeen, UK), from *Tlr2*$^{-/-}$ mice by Dr. M. Fresno (Centro de Biología Molecular Severo Ochoa-CSIC-UAM, Madrid, Spain), from *Tlr9*$^{-/-}$ mice by Dr. J. Pardo (Universidad de Zaragoza, Spain), from *Irf3*$^{-/-}$ and *Irf7*$^{-/-}$ mice by Dr. T. Decker (Max F. Perutz Laboratories, Vienna, Austria) and from *Irf5*$^{-/-}$ mice by Dr. T. Mak (University Health Network, Toronto, Ontario). All the experiments were approved by the Animal Care and Use Committee of the Centro Nacional de Biotecnología-CSIC, Madrid.

C. albicans

C. albicans (strain SC5314; kindly provided by Prof. C. Gil, Complutense University, Madrid) was grown on YPD plates (Sigma, St Louis, MO) at 30°C. HKC were obtained by incubation of *C. albicans*, strain SC5314, for 2 hr at 65°C.

Stimulation of BMDCs and BMMs

BMDCs or BMMs obtained as described in Supplemental Information, were rested for 6 hr in complete cell culture medium and subsequently stimulated at the indicated times with 100 ng.ml^{-1} LPS from *Escherichia coli* (Sigma), 10 μg.ml^{-1} Curdlan (Wako chemicals, Neuss, Germany), 10 μg.ml^{-1} Zymosan (Invivogen, San Diego, CA), or 5 × 10^5 HKC. To address the contribution of Dectin-2 to IFN-β production, we incubated BMDCs for 2 hr with 10 μg.ml^{-1} blocking anti-Dectin-2 antibodies (clone D2.11E4) kindly provided by Dr. P. Taylor (Cardiff Institute of Infection and Immunity, Cardiff, UK) or isotype-matched control antibodies, and subsequently stimulated as indicated above. The phenotypic analysis of BMDCs and BMMs was performed by flow cytometry. IFN-β mRNA expression by BMDCs and BMMs was analyzed by real-time PCR. RelA, c-Jun, and IRF3 activation on BMDCs was assessed by immunoblot. Flow cytometry, real-time PCR, and immunoblot methods are described in Supplemental Information.

C. albicans In Vivo Infection

Mice were intravenously infected with 1 × 10^5 *C. albicans* and monitored daily for health and survival following the institutional guidance. Kidney fungal burden was determined at day 6 after infection by plating organ homogenates in serial dilutions on YPD plates; colony-forming units (cfu) were counted after growth for 48 hr at 30°C. Flow cytometry analysis of renal leukocyte infiltrates was performed on cell suspensions obtained from organ homogenates that were digested with 0.5 μg.ml^{-1} of collagenase A (Roche, Mannheim, Germany) for 10 min at 37°C and filtered through 40 μm cell strainers (BD PharMingen, San Diego, CA). The phenotypic analysis of renal leukocyte infiltration was performed by flow cytometry as described in Supplemental Information. For real-time PCR and Luminex analyses, performed as described in Supplemental Information, renal infiltrating leukocytes were purified from kidney cell suspensions by centrifugation on a gradient of Ficoll-Paque Plus (GE Healthcare, Uppsala, Sweden) and immunomagnetic positive selection after incubation with biotin-conjugated anti-CD45 and streptavidin-conjugated microbeads. Splenic DCs were purified by immunomagnetic positive selection after incubation with biotin-conjugated anti-CD11c and streptavidin-conjugated microbeads.

Cytokine Detection

IFN-β production was quantified by using the *Verikine* mouse IFN beta ELISA kit (PBL, Piscataway, NJ). IFN-β production values for unstimulated controls were below the detection level of this ELISA kit (15.6 pg/ml) for all the experiments analyzing IFN-β production. CXCL1 and CXCL2 production was analyzed by Luminex technology as described in Supplemental Information.

SUPPLEMENTAL INFORMATION

Supplemental Information includes seven figures, two tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2013.05.010.

ACKNOWLEDGMENTS

We thank C. Gil for *C. albicans* SC5314 strain and experimental advice, P. Taylor for Dectin-2 mAbs and scientific advice, G. Brown for *Clec7a*$^{-/-}$ bone marrow and scientific advice, G. Trinchieri for scientific advice, M. Fresno for *Tlr2*$^{-/-}$ bone marrow, J. Pardo for *Tlr9*$^{-/-}$ bone marrow, T. Decker for *Irf3*$^{-/-}$ or *Irf7*$^{-/-}$ bone marrow, T. Mak for *Irf5*$^{-/-}$ bone marrow, U. Kalinke for *Ifnar1*$^{-/-}$ mice, and M. Minguito and L. González for technical assistance. This work was supported by the Spanish Ministerio de Ciencia e Innovación (Grant SAF 2009-11592), Ministerio de Sanidad (Grant RD07/0064/0027), and Fundación Genoma España (MACIA project) to C.A.

Received: June 11, 2012
Accepted: March 22, 2013
Published: June 13, 2013

REFERENCES

Barnes, B.J., Kellum, M.J., Field, A.E., and Pitha, P.M. (2002). Multiple regulatory domains of IRF-5 control activation, cellular localization, and induction of chemokines that mediate recruitment of T lymphocytes. Mol. Cell. Biol. *22*, 5721–5740.

Biondo, C., Midiri, A., Gambuzza, M., Gerace, E., Falduto, M., Galbo, R., Bellantoni, A., Beninati, C., Teti, G., Leanderson, T., and Mancuso, G. (2008). IFN-alpha/beta signaling is required for polarization of cytokine responses toward a protective type 1 pattern during experimental cryptococcosis. J. Immunol. *181*, 566–573.

Biondo, C., Signorino, G., Costa, A., Midiri, A., Gerace, E., Galbo, R., Bellantoni, A., Malara, A., Beninati, C., Teti, G., and Mancuso, G. (2011). Recognition of yeast nucleic acids triggers a host-protective type I interferon response. Eur. J. Immunol. *41*, 1969–1979.

Bourgeois, C., Majer, O., Frohner, I.E., Lesiak-Markowicz, I., Hildering, K.S., Glaser, W., Stockinger, S., Decker, T., Akira, S., Müller, M., and Kuchler, K. (2011). Conventional dendritic cells mount a type I IFN response against

Candida spp. requiring novel phagosomal TLR7-mediated IFN-β signaling. J. Immunol. *186*, 3104–3112.

Decker, T., Müller, M., and Stockinger, S. (2005). The yin and yang of type I interferon activity in bacterial infection. Nat. Rev. Immunol. *5*, 675–687.

Dejima, T., Shibata, K., Yamada, H., Hara, H., Iwakura, Y., Naito, S., and Yoshikai, Y. (2011). Protective role of naturally occurring interleukin-17A-producing γδ T cells in the lung at the early stage of systemic candidiasis in mice. Infect. Immun. *79*, 4503–4510.

González-Navajas, J.M., Lee, J., David, M., and Raz, E. (2012). Immunomodulatory functions of type I interferons. Nat. Rev. Immunol. *12*, 125–135.

Gratz, N., Hartweger, H., Matt, U., Kratochvill, F., Janos, M., Sigel, S., Drobits, B., Li, X.D., Knapp, S., and Kovarik, P. (2011). Type I interferon production induced by Streptococcus pyogenes-derived nucleic acids is required for host protection. PLoS Pathog. *7*, e1001345.

Gross, O., Gewies, A., Finger, K., Schäfer, M., Sparwasser, T., Peschel, C., Förster, I., and Ruland, J. (2006). Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. Nature *442*, 651–656.

Inglis, D.O., Berkes, C.A., Hocking Murray, D.R., and Sil, A. (2010). Conidia but not yeast cells of the fungal pathogen Histoplasma capsulatum trigger a type I interferon innate immune response in murine macrophages. Infect. Immun. *78*, 3871–3882.

Kobayashi, Y. (2006). Neutrophil infiltration and chemokines. Crit. Rev. Immunol. *26*, 307–316.

Kurtzman, C.P., and Robnett, C.J. (1997). Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5′ end of the large-subunit (26S) ribosomal DNA gene. J. Clin. Microbiol. *35*, 1216–1223.

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.

Lionakis, M.S., Lim, J.K., Lee, C.C., and Murphy, P.M. (2011). Organ-specific innate immune responses in a mouse model of invasive candidiasis. J. Innate Immun. *3*, 180–199.

MacCallum, D.M. (2009). Massive induction of innate immune response to Candida albicans in the kidney in a murine intravenous challenge model. FEMS Yeast Res. *9*, 1111–1122.

Majer, O., Bourgeois, C., Zwolanek, F., Lassnig, C., Kerjaschki, D., Mack, M., Müller, M., and Kuchler, K. (2012). Type I interferons promote fatal immunopathology by regulating inflammatory monocytes and neutrophils during Candida infections. PLoS Pathog. *8*, e1002811.

Mansour, M.K., and Levitz, S.M. (2002). Interactions of fungi with phagocytes. Curr. Opin. Microbiol. *5*, 359–365.

Pandey, A.K., Yang, Y., Jiang, Z., Fortune, S.M., Coulombe, F., Behr, M.A., Fitzgerald, K.A., Sassetti, C.M., and Kelliher, M.A. (2009). NOD2, RIP2 and IRF5 play a critical role in the type I interferon response to Mycobacterium tuberculosis. PLoS Pathog. *5*, e1000500.

Panne, D., Maniatis, T., and Harrison, S.C. (2007). An atomic model of the interferon-beta enhanceosome. Cell *129*, 1111–1123.

Pfaller, M.A., and Diekema, D.J. (2007). Epidemiology of invasive candidiasis: a persistent public health problem. Clin. Microbiol. Rev. *20*, 133–163.

Purtha, W.E., Swiecki, M., Colonna, M., Diamond, M.S., and Bhattacharya, D. (2012). Spontaneous mutation of the Dock2 gene in Irf5-/- mice complicates interpretation of type I interferon production and antibody responses. Proc. Natl. Acad. Sci. USA *109*, E898–E904.

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. (2011). Immunity to fungal infections. Nat. Rev. Immunol. *11*, 275–288.

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.

Sancho, D., and Reis e Sousa, C. (2012). Signaling by myeloid C-type lectin receptors in immunity and homeostasis. Annu. Rev. Immunol. *30*, 491–529.

Schoenemeyer, A., Barnes, B.J., Mancl, M.E., Latz, E., Goutagny, N., Pitha, P.M., Fitzgerald, K.A., and Golenbock, D.T. (2005). The interferon regulatory factor, IRF5, is a central mediator of toll-like receptor 7 signaling. J. Biol. Chem. *280*, 17005–17012.

Stetson, D.B., and Medzhitov, R. (2006). Type I interferons in host defense. Immunity *25*, 373–381.

Strasser, D., Neumann, K., Bergmann, H., Marakalala, M.J., Guler, R., Rojowska, A., Hopfner, K.P., Brombacher, F., Urlaub, H., Baier, G., et al. (2012). Syk kinase-coupled C-type lectin receptors engage protein kinase C-σ to elicit Card9 adaptor-mediated innate immunity. Immunity *36*, 32–42.

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.

Trinchieri, G. (2010). Type I interferon: friend or foe? J. Exp. Med. *207*, 2053–2063.

Xu, S., Huo, J., Lee, K.G., Kurosaki, T., and Lam, K.P. (2009). Phospholipase Cgamma2 is critical for Dectin-1-mediated Ca2+ flux and cytokine production in dendritic cells. J. Biol. Chem. *284*, 7038–7046.