Dectin-2 Is a Pattern Recognition Receptor for Fungi That Couples with the Fc Receptor γ Chain to Induce Innate Immune Responses*

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Antigen presenting cells recognize pathogens via pattern recognition receptors (PRR), which upon ligation transduce intracellular signals that can induce innate immune responses. Because some C-type lectin-like receptors (e.g. dectin-1 and DC-SIGN) were shown to act as PRR for particular microbes, we considered a similar role for dectin-2. Binding assays using soluble dectin-2 receptors showed the extracellular domain to bind preferentially to hyphal (rather than yeast/conidial) components of Candida albicans, M. audouinii, and Trichophyton rubrum. Selective binding for hyphae was also observed using RAW macrophages expressing dectin-2, the ligation of which by hyphae or cross-linking with dectin-2-specific antibody led to protein tyrosine phosphorylation. Because dectin-2 lacks an intracellular signaling motif, we searched for a signal adaptor that permits it to transduce intracellular signals. First, we found that the Fc receptor γ (FcRγ) chain can bind to dectin-2. Second, ligation of dectin-2 on RAW cells induced tyrosine phosphorylation of FcRγ, activation of NF-κB, internalization of a surrogate ligand, and up-regulated secretion of tumor necrosis factor α and interleukin-1 receptor antagonist. Finally, these dectin-2-induced events were blocked by PP2, an inhibitor of Src kinases that are mediators for FcRγ-dependent signaling. We conclude that dectin-2 is a PRR for fungi that employs signaling through FcRγ to induce innate immune responses.

To initiate immune responses against infection, antigen presenting cells (APC) must recognize and react to microbes. Recognition is achieved by interaction of particular surface receptors on APC with corresponding surface molecules on infectious agents (1). Complement and Fc receptors bind microbes coated with opsonin (1). By contrast, pattern recognition receptors (PRR) recognize and interact with pathogens directly (1, 2). PRR include the following: (a) scavenger receptors that bind low density lipoproteins or lipid A on some bacteria (3); (b) toll-like receptors (TLR) that bind zymosan, Staphylococcus aureus, lipopolysaccharide (LPS), bacterial flagellin, or CpG bacterial DNA (4–6); and (c) C-type lectin-like receptors (CLR) that bind carbohydrate moieties of many pathogens (1, 7). CLR include the following: (a) mannose receptors for mannose or its polymers (8); (b) mannose-binding lectins for encapsulated group B or C meningococci (9); (c) DC-SIGN and structurally related receptors (DC-SIGNR) for mannose on human immunodeficiency virus, Leishmania, and Mycobacteria (9–14); and (d) dectin-1 for β-glucan on yeasts (15, 16).

Binding of pathogens to particular PRR transduce intracellular signals and biologic consequences that may overlap, even synergize, with those of other PRR. For example, ligation of TLR2 alone on macrophages by zymosan (containing β-glucan) led to secretion of IL-12 and TNFα, and ligation of dectin-1 alone by zymosan resulted in production of reactive oxygen species (but not of IL-12 nor TNFα), whereas coligation of TLR-2 and dectin-1 by zymosan enhanced secretion of IL-12 and TNFα at levels higher than those induced by TLR-2 alone (17). On the other hand, ligation of DC-SIGN on dendritic cells (DC) inhibited TLR-induced IL-12 expression, while stimulating IL-10 expression (12).

Subtractive cDNA cloning of the XS52 line of epidermal Langerhans cell-like DC (18) minus J774 macrophages led us to discover dectin-1 (19) and dectin-2 (20). Both are type II- and structurally related transmembrane proteins with extracellular domains containing a carbohydrate recognition domain highly conserved among C-type lectins (19, 20). Dectin-1 is expressed widely by APC (21) and is a PRR for β-glucan in yeasts (15).
Dectin-2 is constitutively expressed at very high levels by mature DC and can be inducibly expressed on macrophages after activation (20, 22). Here we report that dectin-2 is a PRR for fungi that employ Fc receptor \( \gamma \) (FcR\( \gamma \)) chain signaling to induce internalization, activate NF-\( \kappa \)B, and up-regulate production of TNF\( \alpha \) and IL-1rA.

**EXPERIMENTAL PROCEDURES**

**Microbial Cell Cultures**—We obtained *Candida albicans* (ATCC 10231 and 14053), *Microsporum audouinii* (ATCC 10008), *Trichophyton rubrum* (ATCC 14001), and *Pseudomonas aeruginosa* (ATCC 10145) from the American Type Culture Collection; *Escherichia coli* DH5\( \alpha \) from Invitrogen; *Staphylococcus aureus* without protein A from Molecular Probes Inc. (Eugene, OR); *Saccharomyces cerevisiae* Y187 from Clontech; and group A Streptococci from the Section of Infectious Disease, Department of Pediatrics, the University of Texas Southwestern Medical Center (Dallas, TX). Each microbial strain was grown in media recommended by the ATCC. *C. albicans* yeast was transformed to pseudohyphae (herein referred to as hyphae) as follows. Freshly prepared yeast was resuspended in Hanks’ balanced salt solution (HBSS) containing 1.25 mM CaCl\(_2\), 1 mM MgCl\(_2\), 10 mM HEPES, pH 7.2, and 10% heat-inactivated FCS, seeded on 96-well plates or ELISA plates (2–4 \( \times \) 10\(^5\) cells/well), and then incubated at 37°C for 90 min.

**Construction of Expression Vectors**—To produce soluble dectin-1 and dectin-2 receptors, we inserted a nucleotide fragment encoding the extracellular domain of either molecule into an expression vector, pSTB-Fc (23), that allows secretion of the Fc chain to mammalian cells. Respective nucleotide fragments encoding for extracellular domains of dectin-1 and dectin-2 were obtained by PCR amplification of the full-length cDNA with primers containing BamHI (forward primer) and XbaI (reverse primer) restriction enzyme sites at the 5′-end for dectin-1 or containing HindIII and XbaI sites for dectin-2. PCR fragments remaining after digestion with restriction enzymes were ligated separately in-frame to the 5′-end of a nucleotide for the Fc in pSTB-Fc (pSTB-Dec1-Fc or pSTB-Dec2-Fc).

Lentiviral vectors encoding dectin-2 or dectin-1 tagged with the C-terminal V5 epitope were also constructed. Full-length dectin-2 or dectin-1 coding sequence was excised from an original cDNA clone (20) by PCR amplification using the forward primer (5′-CTCTGGTCAGCTGCTGTG-3′) and the reverse primer (5′-GTGATCTTG-3′). The resulting PCR fragment was inserted into amammalian expression vector, pSTB-Dec1-Fc or pSTB-Dec2-Fc.

### Gene Delivery to Mammalian Cells

**—COS-1 cells** (5 \( \times \) 10\(^5\) cells/dish) were treated with an expression vector DNA (2 \( \mu \)g) and 6 \( \mu \)g of FuGENE 6 (Roche Applied Science) and then cultured for 2–3 days.

**RAW264.7 cells** (5 \( \times \) 10\(^5\)) were infected with lentivirus encoding dectin-2-V5 (or dectin-1-V5) at a multiplicity of infection (m.o.i.) of 20. The next day, the infected cells were treated with expression vector DNA (2 \( \mu \)g/ml Fc block (Pharmingen), infected RAW cells (5 \( \times \) 10\(^5\)) were incubated with mouse anti-V5 Ab (2 \( \mu \)g/ml; Serotec,}$
Recognition of Hyphae by Dectin-2

Raleigh, NC) and biotinylated goat anti-mouse IgG (5 µg/ml, Jackson ImmunoResearch, West Grove, PA) on ice for 60 min and treated with streptavidin-coated magnetic beads (Miltenyi, Auburn, CA). Bead-bound cells were collected and cultured in RPMI 1640 supplemented with 10% FCS. This enrichment was repeated 3–4 times, followed by analysis of the purity of the cell suspension by FACs. Greater than 90% of RAW cells expressed dectin-2-V5 (or dectin-1-V5) on their surfaces (Fig. 3B).

Purification of Fc Fusion Proteins—Three days after transfecting COS-1 cells with expression vectors for Fc fusion proteins, the culture supernatant was recovered, and Fc fusion proteins were purified by affinity chromatography as described previously (23). The protein concentrations of Fc fusion preparations were measured using the Bradford method and purity assessed by SDS-PAGE/Comassie Brilliant Blue staining (single band) and by Western blotting (reactivity for anti-dectin-2 Ab).

Binding Assays for Microbes—Aliquots of freshly cultured bacteria (0.1 OD600, S. cerevisiae and C. albicans yeasts (0.5–1×106 cells each), or hyphae (4×105 cells) were washed with Dulbecco’s PBS (DPBS) and incubated with staining buffer (0.1% BSA, 2 mM CaCl2, DPBS) containing 20 µg/ml Fc proteins on ice for 1 h. After extensive washing with buffer, cells were resuspended in 5 µg/ml of biotinylated goat anti-human IgG F(ab’)2 Ab (Jackson ImmunoResearch) on ice for 30 min, followed by incubation with 1:200-diluted FITC-avidin (Vector Laboratories Inc., Burlingame, CA). We also stained filamentous fungi (M. audouinii, and T. rubrum) as follows. Single colonies of fungi were grown on Sabouroud’s agar plates, harvested, and suspended in DPBS. After washing with DPBS and with water, small aliquots were spotted on slide glass, air-dried, and stained with Fc proteins as above. Binding of Fc proteins to microbes was examined using a Zeiss LSM510 laser scanning confocal microscope with 488 nm excitation and transmitted light detection (Carl Zeiss Microimaging, Thornwood, NY).

Quantitative Binding Assays—Fc protein (20 or 40 µg) was iodinated with 200 or 400 µCi of Na125I (ICN Biomedicals, Aurora, OH) at room temperature for 10 min in the presence of a rehydrated IODO-BEADS (Pierce). The reaction was stopped by removing the beads and diluting with 0.1% BSA/DPBS, followed by dialysis with CaCl2/DPBS until background levels of radioactivity were detected in the dialysis buffer. Radioactivity incorporated into Fc protein was measured by 125I cpm in the trichloroacetic acid-insoluble fraction. Specific activity was expressed as incorporated cpm/total input/µg (typically 1–2×106 cpm/µg).

Iodinated Fc proteins were used to quantitate binding of Fc proteins to C. albicans. Freshly cultured yeasts (5×105 cells) or hyphae (4×105 cells) were incubated with different doses of 125I-labeled Fc protein on ice for 1 h (two sets in triplicate). After extensive washing with CaCl2/DPBS, one set was left untreated, air-dried, and measured for radioactivity bound to C. albicans using a γ-counter. The other set was incubated with acidic buffer (0.15 M NaCl, 0.1 M glycine-HCl buffer, pH 2.3) or 10 mM EDTA (for Ca2+-dependent binding) on ice for 5 min, followed by washing. Residual radioactivity was regarded as background. Specific binding was expressed as the counts/min left after subtracting average background counts/min from untreated counts/min. The amount of Fc proteins bound to C. albicans was calculated as specific binding cpm/specific activity of 125I-Fc protein.

For experiments measuring specific binding of Dec2-Fc to hyphae, hyphae (2×105 cells) were pretreated with various concentrations of Fc protein on ice for 1 h (triplicate). After removing unbond Fc proteins by washing, 1 µg/ml of 125I-Dec2-Fc was added to pretreated and untreated hyphae and then incubated on ice for another 1 h. A set of tubes was incubated with 125I-Dec1-Fc or 125I-Dec2-Fc (1 µg/ml) in the presence of laminarin or mannann (both from Sigma) on ice for 1 h. After extensive washing, C. albicans-bound and background radioactivities were measured as before.

Binding of Transfectants to C. albicans—The following procedures were followed for binding of COS-1 transfectants to C. albicans hyphae. A day after transfecting COS-1 cells with expression vectors for full-length dectin-1-V5 or dectin-2-V5, or an empty vector, cells were re-seeded on 60-mm culture dishes (5×106 cells/dish) and metabolically labeled with [3H]thymidine (ICN Biochemicals, 1 Ci/dish) for 16 h. Cells were then harvested by pipetting in 0.02% EDTA/DPBS. After washing with 10% FCS/RPMI (cRPMI), specific activity of labeled cells (cpm/cell) was determined. Cells in increasing numbers were added to hyphae grown in 96-well plates (2×105 cells/well, in triplicate) and cultured in a CO2 incubator at 37 °C for 1 h. Amphotericin B (Sigma) was added to block fungal growth (final concentration of 2.5 µg/ml). Unbound COS-1 cells were removed by washing with cRPMI 10 times; cells bound to hyphae were lysed by incubation with 0.3% Triton X-100/DPBS (200 µl/well) at room temperature for 20 min.

For binding of RAW cells to C. albicans hyphae, the RAW parental cells or those expressing dectin-1-V5 or dectin-2-V5 were metabolically labeled with [3H]thymidine (1 µCi/culture) by overnight incubation. After measuring specific radioactivity (cpm/cell), labeled cells (3×105 cells/well) were incubated in ELISA wells just treated with 0.1% BSA/DPBS or where hyphae were grown (104 cells/well). After culturing at 37 °C for 30 min, wells were washed with 0.1% BSA/DPBS 10 times and lysed with 100 µl of 0.3% Triton X-100/PBS, and 3H counts were determined. The number of cells adherent to a well was computed by dividing 3H counts/min from a well by specific activity.

For binding of RAW cells to C. albicans yeasts (26), freshly grown yeasts were washed twice with PBS and resuspended in 0.1 mg/ml FITC (Sigma) at room temperature for 1 h. After extensive washing, FITC-labeled yeasts were resuspended in 10% FCS-HBSS. RAW cells (5×105) were incubated with FITC-labeled yeasts at indicated m.o.i. values for 30 min at room temperature. After removing unbound yeasts by extensive washing, cells were fixed with 1% paraformaldehyde for 1 h at 4 °C, washed, and then analyzed using FACScalibur (BD Biosciences). Histograms were made from fluorescent signals after
removal of free FITC-yeasts by gating out the small sized population using forward/side scatter analysis.

**Immunoprecipitation and Western Blotting**—To measure protein expression of dectin molecules in RAW cells, whole cell extracts were prepared from cells by lysis in RIPA buffer (0.05 μM Tris-HCl, pH 7.5, 0.15 μM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 20 mM EDTA) and subsequent centrifugation at 16,000 × g for 20 min at 4 °C. Small aliquots were applied to 4–20% SDS-PAGE, then transferred to a polyvinylidene difluoride membrane (Hybond P; Amersham Biosciences), followed by immunoblotting using mouse anti-V5 Ab (0.5 μg/ml), affinity-purified rabbit anti-dectin-1 oligopeptide (1 μg/ml) (19), or rat anti-dectin-2 mAb (0.5 μg/ml) (20) diluted with TTBS (20 mM Tris-HCl, pH 7.6, 137 mM NaCl, 0.1% Tween 20). After washing, the membrane was blotted further with horseradish peroxidase-conjugated secondary Ab and then developed using the ECL Plus system (Amersham Biosciences).

For protein tyrosine phosphorylation, RAW cells (2.5 × 10⁵) were starved by culturing for 1 h in serum-free DMEM, incubated at 37 °C for 1 h, and cocultured with yeast or hyphae (7.5 × 10⁶ each) in 24-well plates. At different time points after incubation at 37 °C, cells were chilled on ice and lysed by addition of 10 × lysis buffer (20 mM Tris-HCl, pH 7.6, 10% Triton X-100, 10 mM sodium orthovanadate, 10 mM EDTA) to terminate phosphorylation. The clear lysate was prepared by centrifugation at 14,000 rpm for 20 min and subjected to Western blot analysis using 1:1,000-diluted horseradish peroxidase anti-phosphotyrosine Ab (PY-plus, Zymed Laboratories Inc.).

To examine association of dectin-2 with the FcγR chain, whole cell extracts were prepared from Dec2V5-RAW or parental macrophages (1 × 10⁶ cells) using a lysis buffer (1% Brij 55, 50 mM Tris-HCl, pH 7.6, 1 mM Na₂VO₄, 50 mM NaF, proteinase inhibitor mixture (Sigma)) and incubated with mouse anti-V5 (2 μg) or mouse anti-human FcγR chain 7D3.5 mAb (Note: the mAb we originally developed has cross-reactivity to mouse FcγR) (3 μg) at 4 °C for 16 h, followed by precipitation with 10 μl of 50% slurry protein G-agarose (Roche Applied Science). After washing the agarose beads, the immunoprecipitates were dissociated from the beads by boiling and then subjected to Western blotting using anti-FcγR Ab or rat anti-dectin-2 mAb (each 2 μg/ml). The interaction was also examined in COS-1 cells (1 × 10⁶ cells) cotransfected with two expression vectors encoding for dectin-2-V5 and FcγR (pcDNA-m-chain), respectively.

To measure phosphorylation of the FcγR chain, Dec2V5-RAW or RAW parental cells (1 × 10⁶ cells in 100 μl of PBS) were incubated with anti-V5 Ab (5 μg/ml) at 4 °C for 40 min. After extensive washing, cells were treated with goat anti-mouse IgG (20 μg/ml) at 37 °C at various time periods and lysed using 100 μl of 2 × lysis buffer (1% Triton X-100, 50 mM Tris-HCl, pH 7.6, 1 mM Na₂VO₄, 50 mM NaF, proteinase inhibitor mix (Sigma)). In some experiments, RAW cells were pretreated with PP2 or PP3 kinase inhibitor at 37 °C for 2 h. Protein extracts were prepared, immunoprecipitated with anti-FcγR chain Ab, and then blotted with anti-phosphotyrosine Ab 4G10 (1 μg/ml) (Upstate Cell Signaling Solutions, Lake Placid, NY) or anti-FcγR chain Ab. RAW cells (2.5 × 10⁶) were also treated with C. albicans hyphae or yeasts (3 ×
Recognition of Hyphae by Dectin-2

10^5 cells/well) grown in 2-well chamber slides (Lab-Tek Products, Naperville, IL) were labeled with 100 μg/ml TRITC (Sigma) in 0.1 M sodium bicarbonate, pH 8.3, at room temperature for 30 min. Free TRITC was removed completely by incubating with 125I-Dec2-Fc or 125I-Dec2-Fc (100 ng/well), and Ca^2+ dependent binding was examined. Yeasts bind poorly to Dec2-Fc. Hyphae or yeasts in increasing numbers were incubated with a constant amount of 125I-Dec2-Fc (100 ng/well), and Ca^2+ dependent binding was determined as a percentage of control. C. albicans hyphae (2 x 10^5) were pretreated with concentrations (μg/ml) of cold Dec2-Fc or Fc before assaying binding to hyphae by using 1 μg/ml 125I-Dec2-Fc. Fc, Dec2-Fc binding is blocked by mannan. Hyphae (3 x 10^5) or yeasts (1 x 10^5) were incubated with 125I-Dec1-Fc or 125I-Dec2-Fc (1 μg/ml), respectively, in the absence (None) or presence or laminarin (Lam) or mannan (Man). Relative binding (%) to control (no saccharide added) is shown. All data are representative of at least three independent experiments.

For inhibition of endocytosis (27), RAW cells (1 x 10^6) were pretreated with fresh complete DMEM containing 0.5% MeSO (control) or the indicated concentrations of PP2 or PP3 (Calbiochem) at 37 °C for 30 min. After removing medium, cells were incubated with 2 μg/ml FITC-anti-V5 Ab (Invitrogen) on ice for 1 h, followed by washing with 20 μg/ml goat anti-mouse IgG F(ab')_2 (Jackson ImmunoResearch) on ice for 30 min, washed, and labeled with mouse anti-Fcγ chain Ab (1 μg/ml) at room temperature for 1 h and stained with Alexa488-conjugated goat anti-mouse or 594-conjugated goat anti-rabbit IgG (each 1:1,000 dilution) (Molecular Probes). Fluorescence images were taken under confocal microscopy using 488 nm (for Fcγ) or 594 nm excitation (for dec2-2). In the case of COS-1 cells, cells (1 x 10^6) were seeded on a coverslip (12 mm diameter) in 24-well plates. Two days after transfection, cells were treated and analyzed in a similar manner.

Internalization and Its Inhibition by Tyrosine Kinase Inhibitor—Dec2V5—RAW cells were seeded on a 2-well chamber slide (LabTek) (1 x 10^6 cells/well) and cultured overnight. After washing cell layers once with PBS, RAW cells were pretreated with 2.5 μg/ml Fc block in 10% FCS/PBS on ice for 10 min and processed for surface labeling with 2 μg/ml FITC-anti-V5 or isotypic control Ab (Invitrogen). After eliminating unbound Ab, FITC-labeled dec2-2 was cross-linked with 10 μg/ml anti-mouse IgG F(ab')_2 (Jackson ImmunoResearch) on ice for 30 min, washed, and labeled with 200 nM LysoTracker Red (Molecular Probes) for 1 h at 37 °C. Cells were washed three times with 1% FCS/PBS and fixed with 10% formaldehyde. Optical sections were acquired using a Leica TCS SP1 laser scanning confocal microscope (Leica Micro-systems, Bannockburn, IL) as described previously.

Electromobility Shift Assay (EMSA)—RAW cells (3 x 10^7 cells/dish) were infected with C. albicans yeast or hyphae at a
m.o.i. of 3. After incubating at 37 °C for 1 h, cells were washed twice with ice-cold PBS and then lysed by incubating in ice-cold 0.6% Nonidet P-40-containing buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 1 mM dithiothreitol) for 5 min. Cell lysates were collected and centrifuged at 14,000 rpm for 20 s. The supernatant containing cytosolic proteins was aspirated, and pellets were resuspended in 1 ml of ice-cold buffer A without Nonidet P-40. Following centrifugation at 14,000 rpm for 4 °C for 20 s, the pellet was resuspended in 50 μl of ice-cold buffer C (20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 1 mM dithiothreitol) and incubated on ice for 1 h. Clear lysates (nuclear extracts) were prepared by centrifugation at 14,000 rpm for 30 min. Protein concentration was determined by the Bradford method (normally, 2–4 mg/ml), snap-frozen in liquid nitrogen, and stored at −85 °C until needed. Activation of NF-κB was examined by EMSA using an aliquot (4 μg) of prepared nuclear extract and a gel-shift assay kit (Promega, Madison, WI).

**Cytokine Expression Analysis**—Cytokine gene expression by RAW cells was examined using RNase protection assay performed according to the manufacturer’s recommended protocols (RiboQuant multiprobe ribonuclease protection assay system, Pharmingen). Briefly, 32P-labeled RNA probes were generated using the mCK-2b multiprobe template set and
mixed with 20 μg of total RNA isolated from RAW cells infected with/without yeasts or hyphae at a m.o.i. of 0.3. RAW cells (10⁷) were incubated with yeasts or hyphae (3.3 × 10⁶) in complete DMEM containing 2.5 μg/ml amphotericin B. After culturing for 3 h, total RNA was isolated from treated cells using RNA-STAT60 (Tel-Test B, Friendswood, TX) (28), hybridized with probe, and then digested with RNase. mRNA-protected probes were size-fractionated on 8 M urea, 5% polyacrylamide gel, and radioactivity was measured using a PhosphorImager analyzer, STORM820 (Amersham Biosciences).

To measure secretion of cytokines (29), parental or Dec2V5-RAW cells (set in triplicate) were cocultured using previous settings but for longer time periods (6 and 16 h). Protein amount of cytokine secreted in the culture media was measured using respective ELISA kits as follows: IL-1ra kit purchased from R&DSystems (Minneapolis, MN) and other cytokines from eBioscience (San Diego, CA).

RESULTS

Dectin-2 Binds Hyphal Components of C. albicans in a Calcium-dependent Manner—Because dectin-1 is a PRR for β-glucan on yeasts (30), we questioned whether dectin-2 also recognized microbial organisms. We created soluble receptors of dectin-2 and dectin-1, in which the respective extracellular domain was fused to the Fc portion of human IgG1 (Dec2-Fc; Dec1-Fc). We then performed binding assays to assess the ability of fluorescence-labeled Dec2-Fc, Dec1-Fc, or Fc alone (control) to recognize microbes. None of the probes bound to S. aureus, group A streptococci, P. aeruginosa, or E. coli (data not shown). As reported previously (26), Dec1-Fc bound to C. albicans yeasts especially at budding sites (data not shown). By contrast, Dec2-Fc bound to hyphal (but not yeast) components of C. albicans (Fig. 1A). We next questioned whether differences in the ability of dectin-1 and dectin-2 to recognize yeast versus hyphal forms extended to other fungi (Fig. 1B). Dec2-Fc bound to the filamentous (hyphal) but not conidial (yeast) form of the dermatophytes, M. audouinii and T. rubrum, whereas Dec1-Fc bound preferentially or predominantly to the conidial form (Fig. 1B).

To quantify binding activity, Candida yeast or hyphae were incubated with 125I-labeled Dec2-Fc in increasing doses (Fig. 2). After washing, Candida-bound 125I radioactivity (counts/min) was determined and nonspecific binding regarded as radioactivity left after treatment with acid buffer. Specific binding was expressed as counts/min after subtracting nonspecific binding from untreated counts/min. Using Candida-bound Dec2-Fc protein, calculated from specific activity of 125I-Fc proteins (cpm/μg), we observed binding of dectin-2 to hyphal components in a dose-dependent manner, whereas binding to yeast components was minimal, even at the highest dose tested (Fig. 2A).

Because dectin-2 contains an EPN motif required for Ca²⁺-dependent carbohydrate binding by C-type lectins (31), we examined the effect of the calcium chelator, EDTA, on binding of Dec2-Fc to C. albicans hyphae (Fig. 2B). EDTA treatment (10 mM) abrogated such binding as strongly as did acid treatment. Moreover, incubation of a constant number of hyphae with increasing doses of 125I-Dec2-Fc in the presence of calcium revealed saturation of binding at a range of 30–100 μg/ml (Fig. 2C). These results suggest that putative ligands of dectin-2 are expressed abundantly on hyphae.

Because hyphae are larger than yeasts, we controlled for fungal size by culturing C. albicans yeast or hyphae (increasing numbers) with 125I-Dec2-Fc (constant dose) (Fig. 2D). At a dose range of less than 1 × 10⁶ cells, hyphae bound Dec2-Fc in a dose-dependent manner. By contrast, yeast bound to Dec2-Fc only minimally, if at all (Fig. 2D). To more rigorously evaluate specificity of Dec2-Fc binding to hyphae, we saturated putative ligands for dectin-2 on hyphae by pretreatment with cold Dec2-Fc or Fc control at increasing doses before measuring binding of 125I-labeled Dec2-Fc (Fig. 2E). Pretreatment with Dec2-Fc, but not Fc control, blocked binding in a dose-dependent manner, up to 80% at the highest dose tested (100 μg/ml) in which putative ligands of dectin-2 were presumed to be saturated with cold Dec2-Fc (Fig. 2C).

Because β-glucan is a ligand of dectin-1, we examined whether dectin-2 also recognizes β-glucan or its structurally related polysaccharide. Consistent with a previous report (26), laminarin almost completely blocked binding of Dec1-Fc to
RAW cells to culture wells may have masked binding to hyphae, we transected COS-1 cells with expression vectors for Dec2V5, Dec1V5, or control and determined their ability to bind hyphae (Fig. 3E). Prior to binding assays, we confirmed expression of Dec2V5 and Dec1V5 proteins by Western blotting and FACS. Surface expression levels were much lower levels than RAW transfectants (data not shown). Dec2V5-COS-1 cells bound hyphae markedly and in a dose-dependent manner, whereas Dec1V5-COS-1 cells displayed minimal binding (Fig. 3E). Finally, we performed confocal microscopy of FITC-anti-V5 Ab-treated Dec2V5-RAW cells incubated with rhodamine-labeled *C. albicans* pseudohyphae that contain yeast and hyphal components (Fig. 3F). Before incubating with *Candida*, dectin-2 was distributed evenly on the cell surface. After coculture, many RAW cells bound to hyphal components to the point of even engulfing these fungal parts (Fig. 3F). By contrast, we did not observe Dec2V5-RAW cells to bind yeast components. These results also indicate that dectin-2 preferentially recognizes hyphal rather than yeast components of *C. albicans*.

### Binding of Hyphae to Dectin-2 Leads to Protein Tyrosine Phosphorylation

Because dectin-2 lacks a tyrosine-based signal motif in its intracellular domain, we questioned whether ligand-bound dectin-2 receptor was capable of transducing intracellular signals. We subjected whole cell extracts of RAW cells cultured with *C. albicans* yeast or hyphae to Western blotting using anti-phosphotyrosine Ab to detect tyrosine-phosphorylated proteins (Fig. 4). Compared with correspondingly treated parental RAW cells, hyphae (but not yeast)-treated Dec2V5-RAW cells yielded increased amounts of tyrosine-phosphorylated proteins as early as 10 min after incubation (Fig. 4A). To evaluate specificity for dectin-2, we cross-linked dectin-2 with anti-V5 Ab plus secondary Ab (Fig. 4B); this treatment also induced tyrosine phosphorylation, albeit to a lesser degree than was achieved by hyphae. These results indicate that ligation of dectin-2 can transduce tyrosine-based signals in the absence of an intracellular signal motif.

### Dectin-2 Associates with the Fc Receptor γ Chain

DCAR is a C-type lectin shown recently to associate with the FcγR chain via an arginine in its transmembrane domain (32). Because dectin-2 shows 96% amino acid identity to the transmembrane of DCAR (25 of 26 amino acids, including the arginine connector (32)), we posited that dectin-2 also associates with FcRγ. We used anti-V5 Ab to immunoprecipitate Dec2V5 protein from extracts of Dec2V5-RAW (Fig. 5A) and then blotted it with anti-dectin-2 or anti-FcγR Ab. We found dectin-2 and FcγR proteins in precipitates from anti-V5 Ab (but not control Ab)-treated Dec2V5-RAW cells (Fig. 5A); dectin-2 was not detected in precipitates from RAW parental cells. We also used reverse

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**FIGURE 5. Dectin-2 associates with Fcγ chain.** A, Dec2V5 protein was immunoprecipitated (IP) from whole cell extracts of parental RAW or Dec2V5-RAW cells using anti-V5 or control IgG (Ctrl IgG). The precipitates were then immunoblotted (IB) with anti-FcγR or anti-dectin-2 (Dec2) mAb. Reverse precipitation was also performed. B, COS-1 cells were cotransfected with expression vectors for Dec2V5 or FcγR and then subjected similarly to immunoprecipitation analysis. C, localization of Dec2V5 and FcγR proteins. Dec2V5 protein on parental or Dec2V5-RAW cells or COS-1 cells cotransfected previously was surface-labeled with rabbit anti-V5 plus Alexa594-conjugated anti-rabbit Ab (shown in red fluorescence), fixed, permeabilized, and stained with mouse anti-FcγR plus Alexa488-anti-mouse Ab (green). Colocalization was examined using confocal microscopy.

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yeast but had almost no effect on binding of Dec2-Fc to hyphae (Fig. 2F). By contrast, mannan, a polysaccharide purified from *S. cerevisiae*, blocked binding of Dec2-Fc to hyphae in a dose-dependent manner while only minimally blocking binding of Dec1-Fc to yeast (Fig. 2F). These results indicate that dectin-2 and dectin-1 have disparate ligands.

To confirm that full-length dectin-2 (as expressed on APC surfaces) can recognize hyphae, we transfected RAW264.7 macrophages with an expression vector that encodes dectin-2 or dectin-1 tagged with a C-terminal V5 epitope (Dec1V5 or Dec2V5, respectively). Note that parental RAW cells constitutively express dectin-2 and dectin-1 but at markedly lower levels compared with bone marrow-derived DC or the XS52 DC line (19, 20). Western blotting of Dec1V5 and Dec2V5 proteins was performed using anti-V5 antibody (Ab) to label dectin-2 (31 kDa) or dectin-1 (42 kDa) (Fig. 3A), and their identities were also confirmed by immunoreactivity to anti-dectin-2 and anti-dectin-1 Ab, respectively (data not shown). FACS analysis using anti-V5 Ab revealed cell surface expression of Dec1V5 or Dec2V5 at similar high levels (Fig. 3B). Western blotting of Dec1V5 and Dec2V5 proteins confirmed expression of Dec2V5 and Dec1V5 proteins by Western blotting and FACS. Surface expression levels were much lower levels than RAW transfectants (data not shown). Dec2V5-COS-1 cells bound hyphae markedly and in a dose-dependent manner, whereas Dec1V5-COS-1 cells displayed minimal binding (Fig. 3E). Finally, we performed confocal microscopy of FITC-anti-V5 Ab-treated Dec2V5-RAW cells incubated with rhodamine-labeled *C. albicans* pseudohyphae that contain yeast and hyphal components (Fig. 3F). Before incubating with *Candida*, dectin-2 was distributed evenly on the cell surface. After coculture, many RAW cells bound to hyphal components to the point of even engulfing these fungal parts (Fig. 3F). By contrast, we did not observe Dec2V5-RAW cells to bind yeast components. These results also indicate that dectin-2 preferentially recognizes hyphal rather than yeast components of *C. albicans*.

### Binding of Hyphae to Dectin-2 Leads to Protein Tyrosine Phosphorylation

Because dectin-2 lacks a tyrosine-based signal motif in its intracellular domain, we questioned whether ligand-bound dectin-2 receptor was capable of transducing intracellular signals. We subjected whole cell extracts of RAW cells cultured with *C. albicans* yeast or hyphae to Western blotting using anti-phosphotyrosine Ab to detect tyrosine-phosphorylated proteins (Fig. 4). Compared with correspondingly treated parental RAW cells, hyphae (but not yeast)-treated Dec2V5-RAW cells yielded increased amounts of tyrosine-phosphorylated proteins as early as 10 min after incubation (Fig. 4A). To evaluate specificity for dectin-2, we cross-linked dectin-2 with anti-V5 Ab plus secondary Ab (Fig. 4B); this treatment also induced tyrosine phosphorylation, albeit to a lesser degree than was achieved by hyphae. These results indicate that ligation of dectin-2 can transduce tyrosine-based signals in the absence of an intracellular signal motif.

### Dectin-2 Associates with the Fc Receptor γ Chain

DCAR is a C-type lectin shown recently to associate with the FcγR chain via an arginine in its transmembrane domain (32). Because dectin-2 shows 96% amino acid identity to the transmembrane of DCAR (25 of 26 amino acids, including the arginine connector (32)), we posited that dectin-2 also associates with FcγR. We used anti-V5 Ab to immunoprecipitate Dec2V5 protein from extracts of Dec2V5-RAW (Fig. 5A) and then blotted it with anti-dectin-2 or anti-FcγR Ab. We found dectin-2 and FcγR proteins in precipitates from anti-V5 Ab (but not control Ab)-treated Dec2V5-RAW cells (Fig. 5A); dectin-2 was not detected in precipitates from RAW parental cells. We also used reverse
**Recognition of Hyphae by Dectin-2**

**A**

| ICD | TM | ECD |
|-----|----|-----|
| WT  |     |     |
| R17V|     |     |
| A1/2ICD |   |     |
| 40LECD | CD40LECD |   |

**FIGURE 6. Intracellular region of dectin-2, proximal to the transmembrane, is required for the association.** A, amino acid structures of dectin-2 mutants are schematically depicted and aligned with the wild type (WT), consisting of an intracellular (ICD), a transmembrane (TM), and an extracellular domain (ECD). An inverted closed triangle represents the location of a point mutation (arginine to valine). B, COS-1 cells were transfected with an expression vector coding for WT or a mutant with (+) or without (−) vector for FcRγ chain. Two days post-transfection, whole cell extracts were prepared and subjected to immunoprecipitation and immunoblotting with the indicated Ab. Representative blotting data of two independent experiments are shown.

**B**

| Co-transfection with FcRγ | − | + | + | + | + | + | + |
|----------------------------|---|---|---|---|---|---|---|
| IP: FcRγ                    |   |   |   |   |   |   |   |
| IB: V5                      |   |   |   |   |   |   |   |
| IP: FcRγ                    |   |   |   |   |   |   |   |
| IB: FcRγ                    |   |   |   |   |   |   |   |

**FIGURE 7. Ligation of Dec2V5 on RAW cells transduces phosphorylation of FcRγ.** A, phosphorylation of FcRγ by cross-linking. At different time points after cross-linking of Dec2V5 on parental RAW or Dec2V5-RAW cells using anti-V5 Ab or control IgG (Ctrl IgG), whole cell extracts were prepared and FcRγ protein immunoprecipitated. Levels of FcRγ protein and of its phosphorylation were determined by immunoblotting with anti-FcRγ AB or anti-phosphotyrosine (p-Tyr). B, phosphorylation by C. albicans. RAW cells were cocultured without (No) or with C. albicans hyphae (Hy; 4 × 10⁵) or yeast (Y; 1 × 10⁶) and examined by Western blotting for phosphorylated and for total FcRγ protein. C, inhibition of phosphorylation by Src kinase inhibitor. RAW cells were pretreated with PP2 (an inhibitor for Src family kinases) or PP3 (a control derivative) (μM) prior to coculture with C. albicans. Hypoxia-induced tyrosine phosphorylation was determined as before. Two bands immunoreactive to anti-FcRγ Ab (indicated by arrows) represent the phosphorylated and unphosphorylated forms.

For the table, we only observe a partial dataset due to the image being partially visible. It appears to be experimental data showing the phosphorylation of FcRγ in RAW and Dec2V5-RAW cells under different conditions. The experiments include cross-linking, treatment with C. albicans, and inhibition with PP2 and PP3.

**Dectin-2 Transduces Tyrosine Phosphorylation of Fc Receptor γ Chain**—We next questioned whether ligation of dectin-2 leads to tyrosine phosphorylation of the FcRγ chain (Fig. 7). At different time points after cross-linking dectin-2 on RAW cells with anti-V5 Ab or control IgG, whole cell extracts were prepared from treated RAW cells; FcRγ protein was immunoprecipitated, and tyrosine phosphorylation of FcRγ was examined by immunoblotting with anti-phosphotyrosine Ab (to detect phosphorylation levels) or anti-FcRγ Ab (to measure precipitated FcRγ). A single band immunoreactive to anti-phosphotyrosine Ab was detected as early as 2 min, and it peaked at 5 min followed by a rapid decrement (Fig. 7A). The phosphorylated form, which migrated slower than the unphosphorylated form (34), was also detected in immunoblots with anti-FcRγ (Fig. 6A). Absence of phosphorylation in parental cells treated with anti-V5 Ab and in Dec2V5-RAW cells treated with control Ab confirmed specificity for dectin-2. We next determined whether ligation of dectin-2 by hyphae (versus yeast as control) immunoprecipitation to show that anti-FcRγ Ab coprecipitated Dec2V5 protein. In addition, we employed COS-1 cells cotransfected with Dec2V5 and FcRγ genes to confirm that dectin-2 associates with FcRγ (Fig. 5B). Finally, we used confocal microscopic analysis to locate dectin-2 and FcRγ proteins within Dec2V5-RAW and cotransfected COS-1 cells (Fig. 5C). These cells were surface-labeled with phycoerythrin-anti-V5 Ab (Fig. 5C, red fluorescence), fixed, and then stained with FITC-anti-FcRγ Ab (Fig. 5C, green). In RAW cells, the majority of endogenous FcRγ protein resided on the cell surface colocalizing with surface-labeled dectin-2 (Fig. 5C, yellow). In cotransfected COS-1 cells, similar colocalization was observed, although the majority of FcRγ resided intracellularly (Fig. 5C).

To determine whether the transmembrane arginine (Arg-17) in dectin-2 is required to associate with the FcRγ chain, we assayed the binding of an R17V mutant, in which the positively charged arginine was replaced by the neutrally charged valine (Fig. 6). FcRγ protein was immunoprecipitated from extracts of COS-1 cells cotransfected with the R17V mutant (tagged with the C-terminal V5) and FcRγ, and then immunoblotted with anti-V5 to detect dectin-2 (Fig. 6B). Wild-type dectin-2 and the R17V mutant each coprecipitated FcRγ efficiently, indicating that transmembrane arginine is not essential for the association. We next examined the importance of the intracellular and extracellular domains of dectin-2 by constructing three other mutants as follows: ΔICD mutant with the entire intracellular domain (amino acids 1–14) deleted; Δ1/2 ICD lacking the N-terminal half of the intracellular domain (amino acids 1–7); and 40LECD in which the extracellular domain is replaced by the CD40 ligand (CD40L) (33), a type II transmembrane receptor that does not associate with FcRγ. Immunoprecipitation revealed binding of Δ1/2 ICD or 40LECD with FcRγ as avidly as that of the wild type, whereas ΔICD mutant bound poorly, indicating that a short stretch of the intracellular domain of dectin-2 (amino acids 8–14) proximal to the transmembrane domain is required for associating with FcRγ.
RAW cells with PP2 (but not PP3) blocked FcR

Ligation of Dectin-2 Triggers Internalization Likely through Src Family Kinases—We next used confocal microscopy to study internalization and intracellular trafficking after cross-linking of dectin-2 on Dec2V5-RAW cells with FITC-anti-V5 Ab (as a surrogate ligand) plus a secondary Ab (Fig. 8A). As early as 15 min after cross-linking, ligand-loaded dectin-2 was internalized and formed endosomes, most of which were not fused to lysosomes (stained by LysoTracker). We then examined whether the Src family kinases are involved in the internalization (Fig. 8B). PP2 (50 μM) pre-treatment blocked internalization by 70%, whereas PP3 had little effect. Thus, internalization of ligated dectin-2 is achieved through activation of Src family kinases.

### FIGURE 8. Dectin-2 rapidly internalizes a surrogate ligand (anti-V5 Ab) through activation of Src kinases.

A, Dec2V5-RAW cells were surface-labeled with FITC-anti-V5 Ab (0 min) and then cross-linked with secondary Ab. At various time points after incubation, cells were fixed and stained with Red-LysoTracker (red fluorescence). Confocal images of doubly stained cells are shown. B, internalizing capacity of Dec2V5-RAW cells was quantified by FITC fluorescent intensity of internalized anti-V5 Ab in the absence (100%) or the presence of PP2 or PP3 at varying concentrations (μM). Data shown are representative of two (A) and three (B) experiments.

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NFκB—NFκB is a major transcription pathway utilized by many immunoregulatory receptors to mediate their downstream biologic effects (36). Because FcRγ can activate NFκB, we examined the effects of ligated dectin-2 on NFκB activation using the EMSA on nuclear extracts from transfected or parental RAW cells infected with C. albicans (hyphae versus yeasts) or LPS as a nonspecific control. Parental RAW cells failed to induce NFκB activation beyond steady-state levels (Fig. 9A). By contrast, Dec2V5-RAW cells activated NFκB in response to hyphal infection but not yeasts. Moreover, specificity for hyphae-ligated dectin-2 was confirmed by the finding of close to equal nuclear translocation of NFκB among the cytokine genes tested (TNFα was unintentionally not included), IL-1ra was most markedly up-regulated, 7-fold increase in Dec2V5-RAW cells treated with LPS (Fig. 9B). Hyphae-bound Dectin-2 Up-regulates IL-1ra and TNFα Expression—To determine whether ligated dectin-2 stimulates RAW cells to produce cytokines, we again cocultured parental and Dec2V5-RAW cells with C. albicans hyphae or yeast, and we examined cytokine gene expression by multiple RNase protection assay (Fig. 9, A and B). Among the cytokine genes tested (TNFα was unintentionally not included), IL-1ra was most markedly up-regulated, 7-fold increase in Dec2V5-RAW cells treated with hyphae versus 2-fold increase induced by yeast (Fig. 10, A and B). IL-6 and IL-18 gene expression was up-regulated minimally in hyphae-treated cells. We next measured production of five cytokines by RAW cells at 6 and 16 h after infection with C. albicans (Fig. 10C). Consistent with mRNA results, hyphae induced considerable secretion of IL-1ra protein, whereas yeast did so only minimally. Hyphae-induced TNFα production was even more greatly induced. A time course study revealed hyphae-induced augmentation as early as 2 h for TNFα and 6 h for IL-1ra (Fig. 10, D and E).

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### FIGURE 9. Hyphae stimulate NFκB activation in dectin-2-expressing RAW cells.

A, nuclear extracts (NE) were prepared from RAW cells treated without (None) or with yeast or hyphae (A) or with LPS (1 μg/ml) (B) and assayed for NFκB (A and B) activation by EMSA. Specific (NFκB) and non-specific (NS) bands are shown by arrows. Second experiment showed similar results.

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Because FcRγ is phosphorylated by the Src kinases, Lyn and Fyn (35), we examined whether PP2, an inhibitor of Src kinases, can block dectin-2-induced FcRγ phosphorylation; the biologically inert derivative, PP3, was used as control. Pretreatment of RAW cells with PP2 (but not PP3) blocked FcRγ phosphorylation by 60% (Fig. 7C). Altogether, our results indicate that dectin-2 can associate with FcRγ and that such association is likely to transduce Src-dependent phosphorylation.
Finally, to determine whether Src kinase played a role, we assayed the inhibitory effect of PP2 on TNFα and IL-1ra secretion (Fig. 10F). PP2 blocked hyphae-induced TNFα production completely, whereas it inhibited IL-1ra secretion by 80%. These results indicate that hyphae-ligated dectin-2 stimulates RAW cells to produce IL-1ra and TNFα likely through activation of Src kinases.

**DISCUSSION**

We showed that dectin-2 (soluble extracellular domain or full-length form on RAW cells) preferentially binds hyphal forms rather than yeast forms of *C. albicans*, *M. audouinii*, and *T. rubrum*. Dectin-2 ligated by hyphae or cross-linked by Ab induces phosphorylation of protein tyrosine, internalizes a surrogate ligand, activates NF-κB, and up-regulates expression of TNFα and IL-1ra. Transduction of these events after recognition of hyphae is achieved by coupling of dectin-2 with the signal adaptor, FcR, which bears an immunoreceptor tyrosine-based activation motif (ITAM). Because ITAM-dependent signaling in leukocytes appears critical to the differentiation, proliferation, regulation, and survival of several immune effector cells (27), we speculate that dectin-2 on APC contributes to the initiation and modulation of anti-fungal immunity.

Selective binding of dectin-2 to hyphae led us to screen carbohydrates unique to hyphae (not found in yeasts) as candidates for the dectin-2 ligand, including chitin (37, 38); (1–3)- and (1–2)-linked glucans (39, 40); high molecular weight mannoproteins like CaCYC3 (41, 42); other mannoproteins (43, 44); and other lipids (45). However, neither chitin, its carbohydrate unit (N-acetylglucosamine), nor any of the glucans blocked binding of dectin-2 to hyphae (data not shown). We also tested simple hexose carbohydrates for their ability to bind to dectin-2 and found none to do so significantly (data not shown). Rather, we discovered that high dose mannan blocks binding of dectin-2 to hyphae (data not shown). We also tested simple hexose carbohydrates for their ability to bind to dectin-2 and found none to do so significantly (data not shown). Rather, we discovered that high dose mannan blocks binding of dectin-2 to hyphae (data not shown).
be a minor oligomannoside of this polysaccharide preparation. *C. albicans* yeasts and hyphae both contain mannan in their cell walls, yet dectin-2 binds preferentially to the latter. Thus, it is possible that one of the minor oligomannosides is synthesized more abundantly by hyphae (*versus* yeast) or that its presence in hyphae (*versus* yeast) is more accessible for binding to dectin-2. For example, dectin-1 binds preferentially to yeasts at budding sites, where β-glucan is more accessible (26). Transformation of yeasts to pseudohyphae may alter the three-dimensional structure of the cell wall (44) that better displays the putative dectin-2 ligand.

Type II-configured CLR on APC can be sorted into the following two groups based on the presence/absence of signaling motifs in the intracellular domain: CLR having the motif include dectin-1 which carries a YXXL (an ITAM-like sequence) (19, 47), and DCIR which has an immunoreceptor tyrosine-based inhibitory motif (48). CLR without the motif include DCAR and dectin-2. Recently, it has been reported that DCAR associates with the FcR chain, enabling it to induce signals leading to Ca^{2+} influx (32). The same authors claimed that dectin-2 was unable to couple with the FcR in COS-1 cells cotransfected with FcRy and dectin-2 genes (32). Our results are at odds with this report; not only is dectin-2 capable of binding with the FcR chain (coprecipitation of endogenous or genetically engineered FcRy from RAW cells or from cotransfected COS-1 cells using anti-V5 Ab) (Fig. 5, A and B) but also dectin-2 and FcRy chain colocalize within these cells (Fig. 5C). Furthermore, ligation of dectin-2 by hyphae or V5-cross-linked Ab induces phosphorylation of the FcRy chain (Fig. 7). Note that both dectin-2 and DCAR possess transmembrane domains with almost identical amino acid sequence (one miss-match among 26 amino acids), including a positively charged arginine residue essential for interaction of many Ig superfamly members with the FcRy chain (49). In contrast to DCAR (32) and other Ig-like receptors (49), the association of dectin-2 with FcRy was achieved via the intracellular domain proximal to the transmembrane and not through transmembrane arginine. Relevant to this finding is platelet receptor GPVI, which was also shown to associate with FcRy through its intracellular domain (50).

To study the function of dectin-2 in innate immunity, we used dectin-2-overexpressing RAW cells as a model of inflammatory macrophages and DC expressing high levels of dectin-2 (22). Expression levels by the RAW cells are likely to be more abundant than levels physiologically expressed by those inflammatory cells. Thus, some of our data may not reflect precisely the real significance of dectin-2 on DC. Recognition of pathogens by DC is not achieved by a single receptor. Rather, DC employ concurrently multiple receptors. In this regard, we speculate that inflammatory macrophages and DC employ dectin-2 to recognize hyphae, with the dectin-2-induced downstream events we found contributing in part to overall changes induced by DC.

Interaction between particular microbes and PRR on APC leads to intracellular and secretory events that may govern whether effector responses generated against infection are protective or promiscuous. Ligation of dectin-1 by zymosan (containing β-glucan) led to phosphorylation of the ITAM-like motif of dectin-1, activation of Syk tyrosine kinase, and up-regulated secretion of IL-2 and IL-10 (47). By contrast, we showed that ligation of dectin-2 by hyphae led to phosphorylation of FcRy and up-regulated secretion of TNFα and IL-1ra. This disparity between cytokines produced by each pathway may account at least partially for differences in the biologic outcome of infection by dimorphic fungi, with yeast-dominant infections fostering protective immunity and hyphae-dominant infections engendering greater tissue invasion.

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Recognition of Hyphae by Dectin-2