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The Role of SIGNR1 and the β-Glucan Receptor (Dectin-1) in the Nonopsonic Recognition of Yeast by Specific Macrophages[1]

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We recently demonstrated that the β-glucan receptor Dectin-1 (βGR) was the major nonopsonic β-glucan receptor on macrophages (Mϕ) for the yeast-derived particle zymosan. However, on resident peritoneal Mϕ, we identified an additional mannan-inhibitable receptor for zymosan that was distinct from the Mϕ mannose receptor (MR). In this study, we have studied the mannose-binding potential of murine Mϕ and identified the dendritic cell-specific ICAM-3-grabbing nonintegrin homolog, SIGN-related 1 (SIGNR1), as a major MR on murine resident peritoneal Mϕ. Both SIGNR1 and βGR cooperated in the nonopsonic recognition of zymosan by these Mϕ. When SIGNR1 was introduced into NIH3T3 fibroblasts or RAW 264.7 Mϕ, it conferred marked zymosan-binding potential on these cells. However, in the nonprofessional phagocytes (NIH3T3), SIGNR1 was found to be poorly phagocytic, suggesting that other receptors such as βGR may play a more dominant role in particle internalization on professional phagocytes. Binding of zymosan to RAW 264.7 Mϕ expressing SIGNR1 resulted in TNF-α production. Treatment of RAW 264.7 Mϕ expressing SIGNR1, which express low levels of βGR, with β-glucans had little effect on binding or TNF-α production, indicating that there was no absolute requirement for βGR in this process. These studies have identified SIGNR1 as a major MR for fungal and other pathogens present on specific subsets of Mϕ. *The Journal of Immunology,* 2004, 172: 1157–1162.

We recently identified Dectin-1 as the leukocyte β-glucan receptor (βGR)[3] (1) and demonstrated that it was a major macrophage (Mϕ) receptor for the nonopsonic recognition of yeast (2). It is predominantly expressed on the surface of cells of the monocyte/Mϕ lineage, dendritic cells, and a subset of T cells (3). Interestingly, murine Mϕ exhibited some heterogeneity in the expression of βGR, with high surface expression particularly evident on alveolar Mϕ (3), which are constantly exposed to fungal-derived material. The human βGR homolog has also been identified (4–7) and recognizes β-glucans (6). More recently, we have been able to show that Dectin-1 is required in conjunction with Toll-like receptor 2 for the induction of TNF-α production in response to zymosan recognition (8). These studies have established βGR as a major pattern recognition receptor for the appropriate recognition and response to fungal infection.

Our examination of the nonopsonic recognition of zymosan by murine Mϕ led to the identification of an additional mannan-inhibitable receptor for the nonopsonic recognition of zymosan on the surface of resident peritoneal Mϕ (3). The heterogeneous expression of this receptor could represent the mannan-inhibitable receptor on peritoneal Mϕ described for *Saccharomyces cerevisiae*, zymosan, and *Leishmania donovani* promastigotes (9–11). Although at the time this activity was ascribed to the Mϕ mannose receptor (MR), we demonstrated with the aid of novel mAb that the expression of the Mϕ MR was inconsistent with this hypothesis (3). Candidates for this activity were the murine homologs of dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN) (12–14) and DC-SIGNR (14, 15), which now have well-established mannose-binding credentials (14). The mouse has five homologs of DC-SIGN(R) (16), and one of these homologs has been shown to be expressed by select Mϕ, and possess mannose-binding activities (17, 18). Another candidate, Endo180, is an additional member of the MR family, which has similar ligand specificity to the Mϕ MR (19, 20).

In this study, we demonstrate that the uncharacterized mannan-inhibitable receptor on peritoneal Mϕ is SIGN-related 1 (SIGNR1), the murine homolog of DC-SIGNR. SIGNR1 is involved in the nonopsonic recognition of the yeast-derived particle zymosan (exhibiting additive cooperation with βGR), facilitates the Mϕ TNF-α response, and contributes to the recognition of the human pathogen *Candida albicans* but is poorly phagocytic in isolation.

Materials and Methods

Preparation of primary cells and cell culture

All mice used in this study were C57BL/6J or BALB/c and between 8 and 12 wk at the time of study. Primary cells were isolated as previously described (3). Primary Mϕ were cultured in RPMI 1640 (Invitrogen, Paisley, U.K.) supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 2 mM L-glutamine. NIH3T3 (American Type Culture Collection, Manassas, VA) and RAW 264.7 (American Type Culture Collection) were maintained in DMEM (Invitrogen) supplemented as above.

Isolation of RNA and RT-PCR analysis

Peritoneal cells were enriched by overnight adherence to tissue culture plastic. This process is known not to alter the presence of the mannan-inhibitable

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[3] Abbreviations used in this paper: βGR, β-glucan receptor (Dectin-1); Mϕ, macrophage; MR, mannose receptor; DC-SIGN, dendritic cell-specific ICAM-3-grabbing nonintegrin; SIGNR1, SIGN-related 1; NIH3T3-SIGNR1, NIH3T3 fibroblast expressing SIGNR1; RAW-SIGNR1, RAW 264.7 Mϕ expressing SIGNR1; NIH3T3-βGR, NIH3T3 fibroblast expressing βGR; Man-PAA-Bio, synthetic biotinylated polymeric mannose probe on a polyacrylamide backbone; MFI, mean fluorescent intensity.

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zymosan receptor on murine Mϕ (3). Isolation of total RNA and cDNA synthesis was performed as previously described (3). Primers for specific cDNA amplification were as follows: DC-SIGN, 5′-CATGAGTGATTCTAAG GAA-3′ and 5′-TCACTTGCTAGGGCAGGAAG-3′; SIGNR1, 5′-ACCAT GAGTGACTCCACAGAA-3′ and 5′-CTAGCCTTCAGTGCATGGGG-3′; and β_2-microglobulin, 5′-TGACCGGCTTGTATGCTATC-3′ and 5′-CAGT GTGAGCCAGGATATAG-3′.

Retroviral transduction of NIH3T3 and RAW 264.7

To obtain stable SIGNR1-expressing NIH3T3 fibroblasts (NIH3T3-SIGNR1) and RAW 264.7 Mϕ (RAW-SIGNR1), the SIGNR1 cDNA (a gift from Dr. T. Geijtenbeek (Vrije Universiteit Medical Centre, Amsterdam, The Netherlands)) was subcloned into the retroviral vector pFB(neo) (Stratagene, La Jolla, CA). NIH3T3-SIGNR1, RAW-SIGNR1, and βGR-expressing NIH3T3 fibroblasts (NIH3T3-βGR) transductants were generated as previously described (2, 6).

Carbohydrates and mannose-binding assays

Synthetic biotinylated polymeric mannose ligand (Man-PAA-Bio) was obtained from Lectinicity (Munich, Germany). Mannan (from *S. cerevisiae*), laminarin (from *Laminaria digitata*), and monosaccharides were purchased from Sigma-Aldrich (St. Louis, MO). Glucan phosphate was produced as previously described (21).

For the assessment of surface mannose-binding activity on murine Mϕ, freshly isolated cells were washed twice with ice-cold wash solution (0.5% (w/v) BSA, 2 mM NaN_3 in HBSS), and typically $0.5–1 \times 10^6$ cells per assay were blocked with 5% rabbit serum and 10 μg/ml 2.4G2 (anti-FcγRII and -III) in the wash solution for 30 min at 4°C. After blocking, the cells were incubated with 10 μg/ml Man-PAA-Bio on ice in the same wash buffer. FITC-labeled F4/80 was included for the identification of Mϕ. After 1 h on ice, cells were washed twice with the same wash solution, and Man-PAA-Bio was detected with streptavidin-allophycocyanin (BD PharMingen, San Diego, CA) in wash buffer. Cells were washed three times with wash solution before analysis, and Mϕ were identified by flow cytometry, gating on $F4/80^+$ cells.

Nonopsonic binding assays

For nonopsonic binding assays, a modification of our earlier assays (1–3) was developed for the analysis of freshly isolated cells. Freshly isolated peritoneal cells or subconfluent NIH3T3, which were lifted by scraping in 5 mM EDTA in PBS, were washed twice in ice-cold wash solution (see *Carbohydrates and mannose-binding assays*). A total of 2×10^5 peritoneal cells (1×10^5 NIH3T3) were placed in 96-well V-bottom bacterial plastic plates. Inhibitors (carbohydrates, Abs, or EDTA) were added (final concentrations stated where appropriate) and incubated on ice for 20 min before the addition of FITC-labeled zymosan (Molecular Probes, Eugene, OR), heat-killed *C. albicans*, or heat-killed *Mycobacterium tuberculosis* (a particle:cell ratio of 5:1 (25:1 for NIH3T3) was used). The cells and test particles were brought into contact by centrifugation at $350 \times g$ for 5 min and left for 1 h on ice before resuspension and fixation in 1% formaldehyde. Mϕ in mixed cultures were identified by flow cytometry by F4/80 expression or their characteristic forward scatter/side scatter and autofluorescent profiles. Particle binding was identified by the acquisition of FITC labeling (see *Flow cytometry*).

Results from binding assays were either expressed as a percentage of the cells present that had bound to the test particle or as a relative binding index, to gain a more accurate estimate of the total number of particles bound to these cells. The binding index is expressed as the percentage of cells interacting with particles multiplied by the mean fluorescent intensity (MFI) of those cells. A relative binding index was obtained by normalizing results to the mean binding of untreated Mϕ or the mean of the transfected cells with the highest binding, both of which were assigned a binding index of 100.

Phagocytosis assays

NIH3T3-SIGNR1 or NIH3T3-βGR were seeded at 2.5×10^5 cells/well in six-well dishes the day before assay. As a phagocytosis control, some cells were treated with 2 μM cytochalasin D (Sigma-Aldrich) 60 min before and throughout the assay. Plates were cooled on ice for 20 min, and then FITC-labeled zymosan (20 particles/cell) in ice-cold medium was added to each well, settled by gravity, and left for 60 min. After this time, unbound particles were removed by three washes with ice-cold medium, the medium was replaced, and the cells were returned to 37°C (5% CO_2) for 60 min to allow phagocytosis to occur. Plates were then returned to ice, where they remained while they were stained in situ for the presence of bound zymosan on the outside of the cells.

For detection of external zymosan, cells were first blocked for 30 min with ice-cold 5% (v/v) goat serum and 0.5% (w/v) BSA in HBSS (containing Ca^{2+} and Mg^{2+}). The block was replaced with a rabbit polyclonal anti-zymosan Ab (Molecular Probes) diluted 1/1000 in block for 1 h on ice. The anti-zymosan Abs are unable to bind to zymosan particles that have been internalized. The cells were washed three times with blocking solution, and an allophycocyanin-labeled goat anti-rabbit IgG (Molecular Probes) was added at a 1/200 dilution for a further 45 min. Cells were again washed three times with ice-cold blocking buffer and then detached by pipetting with HBSS before being fixed with 2% formaldehyde.

Flow cytometry

Flow cytometry was performed exactly as previously described (3). The labeled mAbs F4/80-PE/FITC/biotin (anti-F4/80), 5C6-FITC (anti-CD11b), and biotinylated mouse anti-rat IgM were obtained from Serotec (Oxford, U.K.). mAb ERTR9 (anti-SIGNR1) was obtained from DPC Biermann (Bad Nauheim, Germany), and control rat IgM was obtained from (BD PharMingen). mAb 2A11 (rat IgG2b, anti-βGR) (2) was produced in-house with the appropriate isotype control Abs. All FACS was performed using a BD Biosciences (Mountain View, CA) FACSCalibur and CellQuest, version 4, software.

For intracellular cytokine assays, resident peritoneal Mϕ or RAW 264.7 were plated in 24-well plates (5×10^5 cells/well) and, in the case of primary cells, washed 2 h later to remove nonadherent cells. The next day, the Mϕ were washed three times with ice-cold medium, and inhibitors were added for 30 min at 4°C (as detailed where appropriate). FITC-labeled zymosan was then added to the wells (20 particles per Mϕ) and incubated for 30 min at 37°C to allow binding and internalization to occur. Unbound zymosan was removed by repeated washing at 4°C; medium supplemented with GolgiPlug (BD PharMingen) was added; and the cells were warmed to 37°C for 90 min to allow TNF-α production. The cells were washed with ice-cold HBSS, lifted by scraping, and immediately fixed with 1% formaldehyde for 20 min at 4°C. Cells were permeabilized for staining by including 0.5% saponin (Sigma-Aldrich) in all blocking and washing buffers. Intracellular TNF-α was detected with allophycocyanin-conjugated anti-TNF-α (clone MP6-XT22; BD PharMingen) compared with control Ab (clone A110-1; BD PharMingen). Polymyxin B (Sigma-Aldrich) was included in all TNF-α assays (1000 U/ml) to control for endotoxin contamination of commercial mannan preparations.

Statistical analysis

Statistics were calculated using GraphPad Prism (version 2.0; GraphPad Software, San Diego, CA). Repeated measures one-way ANOVA with Tukey's multiple comparison test was applied where appropriate (*, $p < 0.05$; **, $p < 0.01$).

Results and Discussion

Evaluation of mannose-binding potential of murine Mϕ

During our previous studies, we identified a mannan-inhibitable component of zymosan recognition on resident peritoneal Mϕ (3). To investigate this further, we probed the surface of murine Mϕ (both resident and thioglycolate-elicited Mϕ) for mannose-binding potential using the synthetic probe Man-PAA-Bio. When these cells were assessed for mannose-binding potential, only the resident peritoneal Mϕ (Fig. 1, □) exhibited significant activity, and it is unlikely to be due to MR, because the expression of the Mϕ MR by these cells was poor, as previously reported (3). In addition, thioglycolate-elicited Mϕ, which express moderate levels of MR on the surface (3), showed virtually no activity in this assay (Fig. 1, ■). Binding of Man-PAA-Bio to resident peritoneal Mϕ was inhibited by mannan, but not the β-glucan laminarin, and was cation dependent (Fig. 1), which resembles the classical C-type lectins (22). Interestingly, the binding of the probe was inhibited by both mannose and galactose. Whereas classic Ca^{2+}-dependent lectins like the MR exhibit a significantly higher affinity for mannose than galactose (23, 24), this difference in affinity was found to be less marked for human DC-SIGN and its homolog DC-SIGNR

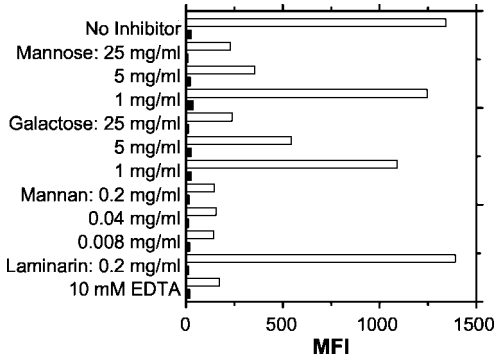

FIGURE 1. Assessment of the mannose-binding potential of murine Mϕ. Resident peritoneal Mϕ (□) exhibited strong and specifically inhibitable binding of Man-PAA-Bio; however, thioglycolate-elicited Mϕ (■) demonstrated negligible binding to this probe. Data represent MFI as a measure of Man-PAA-Bio binding and are representative of three independent experiments.

(14). Consequently, we investigated the expression of the murine DC-SIGN homologs by peritoneal Mϕ.

Expression of SIGNR1 by murine Mϕ

We assessed the expression of both DC-SIGN and SIGNR1 by RT-PCR on enriched peritoneal Mϕ populations (Fig. 2*A*). Transcripts for DC-SIGN were present in cDNA preparations from both resident and elicited Mϕ. The expression of SIGNR1 was more restricted to resident peritoneal cells (Fig. 2*A*), and because SIGNR1 is known to bind mannose and to be expressed by some Mϕ and liver sinusoidal cells (17, 18), we evaluated its expression by FACS using mAb ERTR9 (17, 18). SIGNR1 was evident on the surface of freshly isolated resident peritoneal Mϕ, but not on thioglycolate-elicited peritoneal or resident alveolar Mϕ (Fig. 2*B*). To determine whether SIGNR1 was the major receptor responsible for the binding of Man-PAA-Bio to resident peritoneal Mϕ, the cells were pretreated with ERTR9, or a control rat IgM, before evaluation of binding activity (Fig. 2*C*). Pretreatment with ERTR9 prevented binding of the Man-PAA-Bio to resident peritoneal Mϕ to the level obtained with mannan, indicating that SIGNR1 was a major receptor mediating this activity (Fig. 2*C*). This shows that, in cold binding assays, Man-PAA-Bio is a fairly specific probe for the recognition of SIGNR1 activity, even when Mϕ MR is also present on the surface of the cell.

SIGNR1 and βGR cooperate in the nonopsonic recognition of zymosan by primary cells

We and others (3, 10) have reported that both β-glucan and mannan-inhibitable receptors are present on resident peritoneal Mϕ. Because we have shown in this study that SIGNR1 is a major MR on resident peritoneal Mϕ, we investigated whether SIGNR1 could cooperate with βGR in the recognition of zymosan. mAb against βGR (2A11) blocked binding of zymosan to a similar degree to that of the soluble β-glucan, laminarin (Fig. 2*D*). Notably, anti-SIGNR1 (ERTR9) blocked the recognition of zymosan in a comparable way to both mannan and EDTA (Fig. 2*D*). Furthermore, combination of β-glucan with mannan, or combination of anti-βGR with anti-SIGNR1, largely abrogated the nonopsonic recognition of zymosan by these cells (Fig. 2*D*). This demonstrates that βGR and SIGNR1 are primary nonopsonic pattern recognition receptors on resident peritoneal Mϕ that cooperate in an additive way in the recognition of zymosan.

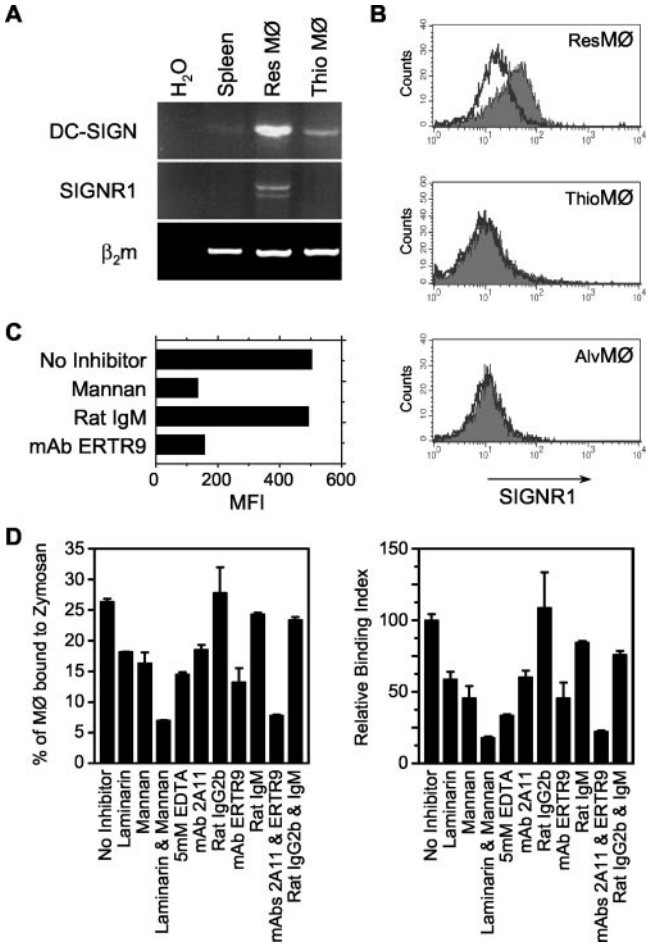

FIGURE 2. Expression of murine DC-SIGN homologs and role in Mϕ zymosan binding. *A*, mRNA for DC-SIGN was detected in both resident and thioglycolate-elicited Mϕ, and SIGNR1 was found only in resident peritoneal Mϕ. Also shown is the control PCR for β_2-microglobulin (β_2m). *B*, Resident peritoneal Mϕ (ResMϕ) exhibited reproducible surface staining with the SIGNR1-specific mAb ERTR9; however, SIGNR1 was not readily detectable on the surface of thioglycolate-elicited (ThioMϕ) or alveolar Mϕ (AlvMϕ). Each individual profile is representative of at least two experiments. *C*, Pretreatment of resident peritoneal Mϕ with mAb ERTR9 (anti-SIGNR1), but not a control rat IgM, blocked binding of Man-PAA-Bio in a similar way to mannan. The data are representative of two independent experiments. *D*, Blockade of either βGR (laminarin or mAb 2A11) or SIGNR1 (mannan, EDTA, or mAb ERTR9) partially inhibited the recognition of zymosan by resident peritoneal Mϕ. Combination of both polysaccharides or Abs largely abrogated zymosan recognition, showing that βGR and SIGNR1 cooperate in zymosan recognition. Results are shown as the percentage of Mϕ bound by FITC-zymosan and as a relative binding index. The data represent mean ± SEM of duplicate samples and are representative of three independent experiments.

Characterization of SIGNR1 transductants

To verify our findings in primary cells, we next examined this activity in SIGNR1-transduced NIH3T3 fibroblasts, which were confirmed to express SIGNR1 on their surface by FACS (Fig. 3*A*). Human DC-SIGN has been shown to interact with *M. tuberculosis* via mannose-capped lipoarabinomannan (25, 26), so to assess our transductants functionally, we measured the binding of FITC-labeled *M. tuberculosis*. We found that SIGNR1, like human DC-SIGN, exhibited mannan- and EDTA-inhibitable binding to *M. tuberculosis* (Fig. 3*B*). We observed that SIGNR1 was capable of mediating zymosan binding to the transduced cells, and that this binding was blocked with mannan but not laminarin (Fig. 3*C*).

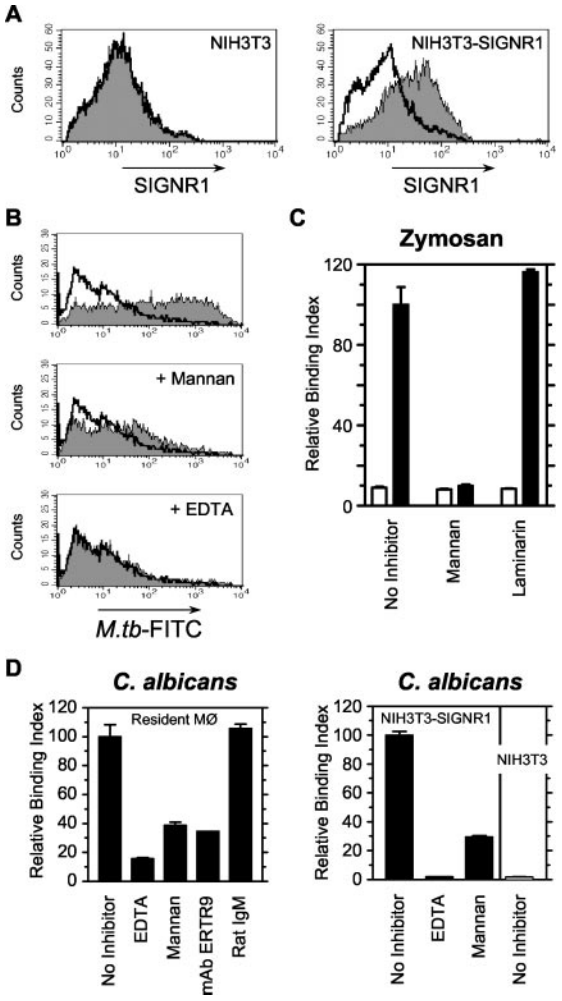

FIGURE 3. SIGNR1 is a receptor for zymosan and *C. albicans*. *A*, FACS analysis of NIH3T3-SIGNR1 demonstrates surface expression of SIGNR1 (shaded histogram) compared with isotype control staining (bold line). *B*, NIH3T3-SIGNR1 (shaded histograms) exhibit enhanced binding of *M. tuberculosis* (*M.tb*) in a mannan- and EDTA-inhibitable manner (NIH3T3 binding denoted by bold line). *C*, NIH3T3-SIGNR1 (■) bind significantly more zymosan than wild-type NIH3T3 (□), and this binding is inhibited by mannan but not β-glucan. Data represent mean ± SEM and are representative of three individual experiments. *D*, The binding of *C. albicans* to freshly isolated resident peritoneal Mϕ (*left panel*) was inhibited by mannan and the SIGNR1-specific mAb ERTR9, but not a control Ab. Binding was also found to be largely cation dependent. NIH3T3-SIGNR1 (*right panel*; ■) bind *C. albicans* in a mannan- and EDTA-inhibitable manner. NIH3T3 cells (*right panel*; □) exhibited only minimal binding. Data are representative of at least two independent experiments.

SIGNR1 contributes to the nonopsonic recognition of C. albicans

C. albicans infection of MR-deficient mice has recently been shown to be relatively normal (27). Furthermore, MR-deficient Mϕ exhibited no defect in the phagocytic uptake of *C. albicans*. We speculated that resident peritoneal Mϕ could use SIGNR1 for the nonopsonic recognition of *C. albicans* in the same way as zymosan (Fig. 3*D*, *left panel*). The binding of heat-killed *C. albicans* by resident peritoneal Mϕ was largely inhibited not only by mannan and EDTA but also by the SIGNR1-specific mAb ERTR9. Confirmation that SIGNR1 could interact directly with *C. albicans* was achieved using NIH3T3-SIGNR1 (Fig. 3*D*, *right panel*). This is consistent with the findings of Cambi et al. (28) who have recently reported that human DC-SIGN also binds *C. albicans*.

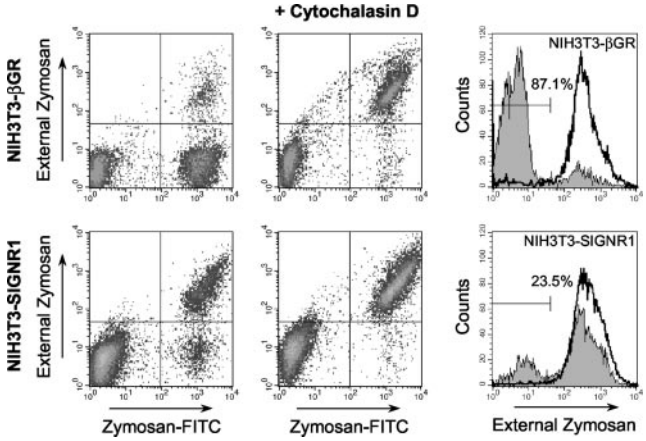

FIGURE 4. The role of SIGNR1 in the phagocytosis of zymosan. After incubation with zymosan, most of the bound particles are internalized by the NIH3T3-βGR (*upper left panel*), but not by the NIH3T3-SIGNR1 cells (*lower left panel*), and both were inhibited by cytochalasin D (*middle panels*). The histograms on the *right* are gated on the $FITC^+$ cells in the adjacent panels. In these examples, 87% of the bound zymosan was internalized by NIH3T3-βGR (*upper right panel*; shaded histogram), and this was clearly blocked by cytochalasin D (bold line), whereas NIH3T3-SIGNR1 internalized only 23% of the bound zymosan during the course of the assay (*lower right panel*; shaded histogram). Data shown are taken from one of three representative experiments.

Analysis of the phagocytic capacity of SIGNR1

It was recently suggested that human DC-SIGN is a phagocytic receptor capable of mediating the internalization of large particles such as *C. albicans* (28); however, these studies were conducted using normally phagocytic cells that are likely to express multiple receptors for this yeast. We assessed the ability of murine SIGNR1 to internalize zymosan in the normally nonphagocytic NIH3T3 background. Despite long incubation times at 37°C, NIH3T3-SIGNR1 were found to be only very poorly phagocytic for prebound zymosan particles (Fig. 4), particularly when compared with NIH3T3-βGR (Fig. 4 and J. Herre, A. S. J. Marshall, E. Caron, V. L. Tybulewicz, C. Reis-e-Sousa, S. Gordon, and G. D. Brown, manuscript in preparation), indicating that uptake of SIGNR1-ligated particulate material may be facilitated on professional phagocytes by other receptors such as βGR.

βGR, SIGNR1, and TNF-α production by Mϕ in response to zymosan

We recently demonstrated that, in elicited Mϕ, βGR cooperates with Toll-like receptor 2 in inducing a proinflammatory response to yeast and zymosan, including the production of TNF-α, which could be inhibited with soluble β-glucan (8). In resident peritoneal Mϕ, however, intracellular TNF-α accumulation could be partially inhibited with β-glucans (Fig. 5*A*). This was similar to our previous study of TNF-α secreted by resident peritoneal Mϕ in response to zymosan, indicating that an alternative βGR-independent mechanism for TNF-α production existed (29). Furthermore, mannan and the SIGNR1-specific mAb ERTR9 had no significant effect on this activity, supporting the idea that alternative mechanisms were responsible for TNF-α production (Fig. 5*A* and data not shown), suggesting that SIGNR1 was involved in binding but not in the induction of the TNF-α response.

To specifically study the role of SIGNR1 in the Mϕ response to zymosan, we expressed this receptor in RAW 264.7 Mϕ, which do not normally express SIGNR1 (data not shown), and compared

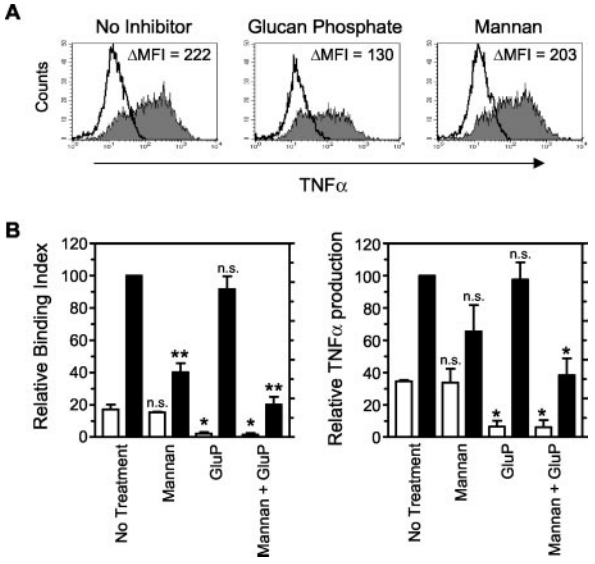

FIGURE 5. The role of βGR and SIGNR1 in the TNF-α response of Mϕ to zymosan. *A*, In the presence of glucan phosphate, cultured resident peritoneal Mϕ exhibited a marked impairment in the TNF-α response to zymosan particles. Mannan had no significant effect on the TNF-α response of these cells. Histograms are gated on zymosan-FITC-associated Mϕ and are representative of three individual experiments. Shaded histograms indicate TNF-α-specific staining, and bold lines represent isotype control Ab. Data are represented as ΔMFI (MFI with TNF-α specific mAb − MFI with control mAb). *B*, The graph on the *left* shows a comparison of zymosan-FITC relative binding index of RAW-FB (□) and RAW-SIGNR1 (■). The graph on the *right* shows the relative zymosan specific total TNF-α production (expressed as percentage of cells associated with zymosan multiplied by MFI of TNF-α of those cells normalized to 100). Data shown represent the mean ± SEM of two independent experiments. Statistical differences refer to the untreated control of the same cell type.

their responses to zymosan with those of βGR-expressing and control RAW 264.7 cells (Fig. 5*B* and data not shown). As previously reported (8), RAW-FB control cells exhibited minimal zymosan binding and TNF-α production, which could be blocked with β-glucans, consistent with the low βGR expression on these cells. Cells overexpressing βGR showed a greatly enhanced β-glucan-dependent interaction with zymosan and an increased TNF-α response, as expected (8) (data not shown). RAW-SIGNR1 also exhibited an increase in their ability to recognize zymosan (Fig. 5*B*). The addition of mannan significantly inhibited the binding of zymosan to RAW-SIGNR1 cells but had only marginal, if any, effect on TNF-α production. Addition of β-glucan had no obvious effect, but combination of β-glucan with mannan reduced zymosan binding further, and this resulted in a significant reduction in the amount of TNF-α produced. Thus, these data are consistent with SIGNR1 contributing to the nonopsonic recognition of zymosan as observed with primary cells, but not playing a major role in TNF-α production, by these cells. Furthermore, these data, and the ability of zymosan to induce enhanced TNF-α production in RAW-FB cells when targeted to other surface receptors, such as complement receptor 3 (8), suggests that βGR is not essential for the TNF-α response to zymosan when an alternative binding/uptake receptor is present, but that it does contribute to the magnitude of this response.

In summary, we show in this study that expression of SIGNR1 by resident peritoneal Mϕ results in additive cooperation with βGR in the nonopsonic recognition of zymosan (Fig. 2*D*). We also demonstrate that SIGNR1 is poorly phagocytic, implicating additional receptors on professional phagocytes, such as βGR, in the internalization process. Finally, we have shown that uptake of zymosan can occur by either βGR or SIGNR1, and this results in TNF-α production by Mϕ, and although signals generated from the βGR are able to induce this response, there is a degree of receptor redundancy in this activity (8). The identification of SIGNR1 as a major MR on resident peritoneal Mϕ resolves the mechanism of a long-standing query over the nature of pattern recognition by these cells (9–11).

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References

1. Brown, G. D., and S. Gordon. 2001. Immune recognition: a new receptor for β-glucans. *Nature 413:36*.
2. Brown, G. D., P. R. Taylor, D. M. Reid, J. A. Willment, D. L. Williams, L. Martinez-Pomares, S. Y. Wong, and S. Gordon. 2002. Dectin-1 is a major β-glucan receptor on macrophages. *J. Exp. Med. 196:407*.
3. Taylor, P. R., G. D. Brown, D. M. Reid, J. A. Willment, L. Martinez-Pomares, S. Gordon, and S. Y. Wong. 2002. The β-glucan receptor, dectin-1, is predominantly expressed on the surface of cells of the monocyte/macrophage and neutrophil lineages. *J. Immunol. 169:3876*.
4. Hermanz-Falcon, P., I. Arce, P. Roda-Navarro, and E. Fernandez-Ruiz. 2001. Cloning of human *DECTIN-1*, a novel C-type lectin-like receptor gene expressed on dendritic cells. *Immunogenetics 53:288*.
5. Yokota, K., A. Takashima, P. R. Bergstresser, and K. Ariizumi. 2001. Identification of a human homologue of the dendritic cell-associated C-type lectin-1, dectin-1. *Gene 272:51*.
6. Willment, J. A., S. Gordon, and G. D. Brown. 2001. Characterization of the human β-glucan receptor and its alternatively spliced isoforms. *J. Biol. Chem. 276:43818*.
7. Sobanov, Y., A. Bernreiter, S. Derdak, D. Mechtcheriakova, B. Schweighofer, M. Duchler, F. Kalthoff, and E. Hofer. 2001. A novel cluster of lectin-like receptor genes expressed in monocytic, dendritic and endothelial cells maps close to the NK receptor genes in the human NK gene complex. *Eur. J. Immunol. 31:3493*.
8. Brown, G. D., J. Herre, D. L. Williams, J. A. Willment, A. S. J. Marshall, and S. Gordon. 2003. Dectin-1 mediates the biological effects of β-glucans. *J. Exp. Med. 197:1119*.
9. Sung, S. S., R. S. Nelson, and S. C. Silverstein. 1983. Yeast mannans inhibit binding and phagocytosis of zymosan by mouse peritoneal macrophages. *J. Cell Biol. 96:160*.
10. Giaimis, J., Y. Lombard, P. Fonteneau, C. D. Muller, R. Levy, M. Makaya-Kumba, J. Lazdins, and P. Poindron. 1993. Both mannose and β-glucan receptors are involved in phagocytosis of unopsonized, heat-killed *Saccharomyces cerevisiae* by murine macrophages. *J. Leukocyte Biol. 54:564*.
11. Channon, J. Y., M. B. Roberts, and J. M. Blackwell. 1984. A study of the differential respiratory burst activity elicited by promastigotes and amastigotes of *Leishmania donovani* in murine resident peritoneal macrophages. *Immunology 53:345*.
12. Geijtenbeek, T. B., R. Torensma, S. J. van Vliet, G. C. van Duijnhoven, G. J. Adema, Y. van Kooyk, and C. G. Figdor. 2000. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell 100:575*.
13. Geijtenbeek, T. B., D. S. Kwon, R. Torensma, S. J. van Vliet, G. C. van Duijnhoven, J. Middel, I. L. Cornelissen, H. S. Nottet, V. N. KewalRamani, D. R. Littman, et al. 2000. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances *trans*-infection of T cells. *Cell 100:587*.
14. Mitchell, D. A., A. J. Fadden, and K. Drickamer. 2001. A novel mechanism of carbohydrate recognition by the C-type lectins DC-SIGN and DC-SIGNR: subunit organization and binding to multivalent ligands. *J. Biol. Chem. 276:28939*.
15. Soilleux, E. J., R. Barten, and J. Trowsdale. 2000. DC-SIGN; a related gene, DC-SIGNR; and CD23 form a cluster on 19p13. *J. Immunol. 165:2937*.
16. Park, C. G., K. Takahara, E. Umemoto, Y. Yashima, K. Matsubara, Y. Matsuda, B. E. Clausen, K. Inaba, and R. M. Steinman. 2001. Five mouse homologues of the human dendritic cell C-type lectin, DC-SIGN. *Int. Immunol. 13:1283*.
17. Geijtenbeek, T. B., P. C. Groot, M. A. Nolte, S. J. van Vliet, S. T. Gangaram-Panday, G. C. van Duijnhoven, G. Kraal, A. J. van Oosterhout, and Y. van Kooyk. 2002. Marginal zone macrophages express a murine homologue of DC-SIGN that captures blood-borne antigens in vivo. *Blood 100:2908*.
18. Kang, Y. S., S. Yamazaki, T. Iyoda, M. Pack, S. A. Bruening, J. Y. Kim, K. Takahara, K. Inaba, R. M. Steinman, and C. G. Park. 2003. SIGN-R1, a novel C-type lectin expressed by marginal zone macrophages in spleen, mediates uptake of the polysaccharide dextran. *Int. Immunol. 15:177*.
19. East, L., S. Rushton, M. E. Taylor, and C. M. Isacke. 2002. Characterization of sugar binding by the mannose receptor family member, Endo180. *J. Biol. Chem. 277:50469*.
20. Sheikh, H., H. Yarwood, A. Ashworth, and C. M. Isacke. 2000. Endo180, an endocytic recycling glycoprotein related to the macrophage mannose receptor is

expressed on fibroblasts, endothelial cells and macrophages and functions as a lectin receptor. *J. Cell Sci. 113:1021*.

21. Muller, A., P. J. Rice, H. E. Ensley, P. S. Coogan, J. H. Kalbfleish, J. L. Kelley, E. J. Love, C. A. Portera, T. Ha, I. W. Browder, and D. L. Williams. 1996. Receptor binding and internalization of a water-soluble (1→3)-β-D-glucan biologic response modifier in two monocyte/macrophage cell lines. *J. Immunol. 156:3418*.
22. Weis, W. I., M. E. Taylor, and K. Drickamer. 1998. The C-type lectin superfamily in the immune system. *Immunol. Rev. 163:19*.
23. Ng, K. K., K. Drickamer, and W. I. Weis. 1996. Structural analysis of monosaccharide recognition by rat liver mannose-binding protein. *J. Biol. Chem. 271:663*.
24. Lee, R. T., Y. Ichikawa, M. Fay, K. Drickamer, M. C. Shao, and Y. C. Lee. 1991. Ligand-binding characteristics of rat serum-type mannose-binding protein (MBP-A): homology of binding site architecture with mammalian and chicken hepatic lectins. *J. Biol. Chem. 266:4810*.
25. Tailleux, L., O. Schwartz, J. L. Herrmann, E. Pivert, M. Jackson, A. Amara, L. Legres, D. Dreher, L. P. Nicod, J. C. Gluckman, et al. 2003. DC-SIGN is the major *Mycobacterium tuberculosis* receptor on human dendritic cells. *J. Exp. Med. 197:121*.
26. Geijtenbeek, T. B., S. J. Van Vliet, E. A. Koppel, M. Sanchez-Hernandez, C. M. Vandenbroucke-Grauls, B. Appelmelk, and Y. Van Kooyk. 2003. Mycobacteria target DC-SIGN to suppress dendritic cell function. *J. Exp. Med. 197:7*.
27. Lee, S. J., N. Y. Zheng, M. Clavijo, and M. C. Nussenzweig. 2003. Normal host defense during systemic candidiasis in mannose receptor-deficient mice. *Infect. Immun. 71:437*.
28. Cambi, A., K. Gijzen, J. M. de Vries, R. Torensma, B. Joosten, G. J. Adema, M. G. Netea, B. J. Kullberg, L. Romani, and C. G. Figdor. 2003. The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for *Candida albicans* on dendritic cells. *Eur. J. Immunol. 33:532*.
29. Willment, J. A., H. H. Lin, D. M. Reid, P. R. Taylor, D. L. Williams, S. Y. Wong, S. Gordon, and G. D. Brown. Dectin-1 expression and function are enhanced on alternatively activated and GM-CSF-treated macrophages and negatively regulated by IL-10, dexamethasone, and lipopolysaccharide. *J. Immunol. 171:4569*.