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The β-Glucan Receptor, Dectin-1, Is Predominantly Expressed on the Surface of Cells of the Monocyte/Macrophage and Neutrophil Lineages[1]

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We recently identified dectin-1 (βGR) as a major β-glucan receptor on leukocytes and demonstrated that it played a significant role in the non-opsonic recognition of soluble and particulate β-glucans. Using a novel mAb (2A11) raised against βGR, we show here that the receptor is not dendritic cell-restricted as first reported, but is broadly expressed, with highest surface expression on populations of myeloid cells (monocyte/macrophage (Mϕ) and neutrophil lineages). Dendritic cells and a subpopulation of T cells also expressed the βGR, but at lower levels. Alveolar Mϕ, like inflammatory Mϕ, exhibited the highest surface expression of βGR, indicative of a role for this receptor in immune surveillance. In contrast, resident peritoneal Mϕ expressed much lower levels of βGR on the cell surface. Characterization of the nonopsonic recognition of zymosan by resident peritoneal Mϕ suggested the existence of an additional β-glucan-independent mechanism of zymosan binding that was not observed on elicited or bone marrow-derived Mϕ. Although this recognition could be inhibited by mannan, we were able to exclude involvement of the Mϕ mannose receptor and complement receptor 3 in this process. These observations imply the existence of an additional mannan-dependent receptor involved in the recognition of zymosan by resident peritoneal Mϕ. *The Journal of Immunology*, 2002, 169: 3876–3882.

Leukocyte β-glucan receptors were first described nearly 20 yr ago as opsonin-independent receptors for particulate activators of the alternative complement activation pathway (1, 2). These receptors are thought to mediate the potent biological effects of β-1,3-D-glucans, including anti-tumor and anti-infective properties (3–7). Receptors for these fungal-derived polymers have been reported on cells of the monocyte (M_o)[4]/macrophage (Mϕ)-lineage (including microglia), neutrophils, NK cells, and fibroblasts (8, 9). To date four β-glucan receptors have been identified as candidates mediating these activities, namely complement receptor 3 (CR3; CD11b/CD18) (10, 11), lactosylceramide (12), selected scavenger receptors (13), and dectin-1 (βGR) (14).

We identified dectin-1 (βGR) as a β-glucan receptor after screening a retroviral cDNA library derived from the Mϕ cell line RAW264.7 with the β-glucan-rich particle zymosan (14). βGR consists of a single C-type, lectin-like, carbohydrate recognition domain, a short stalk, and a cytoplasmic tail possessing an immunoreceptor tyrosine-based activation motif (15). The receptor recognized particles such as zymosan, *Saccharomyces cerevisiae*, and heat-killed *Candida albicans* in a β-glucan-dependent manner (14). The receptor could also bind to T cells, promoting cellular proliferation in the presence of suboptimal concentrations of anti-CD3 (15). T cell recognition was β-glucan independent, indicating the presence of a second binding site on this receptor (14). The human homologue of βGR has also been cloned (16–19) and was found to exhibit similar properties (18).

We recently assessed the role of βGR in the recognition of soluble and particulate (zymosan) β-glucans by macrophages (20). Using a novel anti-βGR mAb (2A11) that has the ability to block the β-glucan binding activity of the receptor, we found that βGR was a major receptor on Mϕ for the nonopsonic recognition of these carbohydrates, defining βGR as the missing leukocyte β-glucan receptor. Furthermore, we demonstrated that CR3 played no obvious role in this process (20), in contrast to previous reports (10, 21). βGR was initially considered dendritic cell (DC) restricted (15), a finding not consistent with the distribution ascribed to the leukocyte β-glucan receptor activity. However, we isolated βGR from a mouse Mϕ cell line and observed βGR transcript in murine and human Mϕ and peripheral blood neutrophils (14, 18, 20). Given the potential importance of the leukocyte β-glucan receptor in innate immunity, we sought to clarify the expression pattern of βGR and performed a comprehensive analysis of the distribution of this receptor in mice. We show, using mAb 2A11, that βGR is predominantly expressed on cells of the M_o/Mϕ and neutrophil lineages, but also on DC, as previously noted. Furthermore, a subset of splenic T cells that expressed the Gr-1 Ag also expressed βGR, albeit at low levels. Examination of βGR expression on various freshly isolated primary Mϕ showed that alveolar and peritoneal inflammatory Mϕ expressed high levels of the receptor, whereas resident peritoneal cells expressed relatively low levels. As βGR is responsible for the nonopsonic recognition of zymosan by other Mϕ populations (20), we studied zymosan

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[4] Abbreviations used in this paper: M_o, monocyte(s); Mϕ, macrophage; βGR, β-glucan receptor/dectin-1; DC, dendritic cell; BMDDC, bone marrow-derived DC; BMDMϕ, bone marrow-derived Mϕ; CR3, complement receptor 3; FSC, forward scatter; MR, mannose receptor; SSC, side scatter.

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recognition by the low βGR-expressing resident peritoneal cells. While βGR was responsible for β-glucan-dependent zymosan recognition by these cells, a second β-glucan-independent, mannan-inhibitable, nonopsonic recognition mechanism was also present.

Materials and Methods

RNA analysis

For RT-PCR analysis, total RNA from various cell lines (RAW264.7, J774, P388D1, and NIH-3T3) and primary cell types (BMDMϕ and BMDDC) was prepared using the guanidine isothiocyanate-based RNA isolation kit (Stratagene, La Jolla, CA). First-strand cDNA synthesis was performed using the Advantage RT-for-PCR kit with an oligo(dT) primer (Clontech, Palo Alto, CA), as described by the manufacturer. The βGR transcript was subsequently amplified using primers corresponding to the region of the cDNA encoding residues 66–244 of the primary protein sequence. Dihydrofolate reductase-specific primers (Stratagene) were used as a positive control. Commercially available membranes containing poly(A) mRNA isolated from various mouse tissues were purchased from Origene Technologies (Rockville, MD) and were probed as described by the manufacturer using a full-length βGR cDNA probe.

Tissue and cell preparation

All mice used in this study were C57BL/6J, unless otherwise stated, and were between 8 and 12 wk of age. Animals were kept and handled in accordance with institutional guidelines. Splenocytes were harvested by standard methods using a combination of digestion with Liberase Blendzyme II in RPMI (Roche, Indianapolis, IN) and mechanical dissociation. Femurs were collected, and fresh bone marrow was flushed from within using Liberase Blendzyme II and incubated for 10 min at 37°C to disaggregate cells. Enzymatic activity was quenched with RPMI/20% FCS, erythrocytes were lysed with Gey's solution, and cell debris was removed by centrifugation through 100% FCS at 300 × *g*.

Isolation of peripheral blood leukocytes

Mice were sacrificed, and peripheral blood was collected by cardiac puncture into 0.1 vol 100 mM EDTA. Cells were harvested by centrifugation and resuspended in 50 vol Gey's solution to lyse erythrocytes. Peripheral blood leukocytes were then recovered by centrifugation through FCS as described above.

Isolation of alveolar Mϕ

Bronchoalveolar lavage was performed by repeated washes with 1 ml PBS/5 mM EDTA. Resident alveolar Mϕ, the major leukocyte population in the lungs, were identified by size and autofluorescence using flow cytometry as previously described (22).

Induction of sterile peritonitis and recovery of peritoneal cells

To induce sterile peritonitis, mice were injected i.p. with 4% thioglycolate (BD Biosciences, Franklin Lakes, NJ) up to 4 days before peritoneal lavage. After humane killing of the animals, inflammatory cells were collected by peritoneal lavage with ice-cold 5 mM EDTA in PBS. Resident peritoneal cells were collected in the same way from untreated animals. Peritoneal Mϕ were identified by their expression of F4/80 and CR3 and were distinguished from eosinophils by forward scatter (FSC)/side scatter (SSC) profiles. To confirm the cellular composition of peritoneal exudates, differential counts were performed on cytospin preparations stained with Hema Gurr (VWR International, Poole, U.K.).

FACS analysis

FACS was performed according to conventional protocols at 4°C in the presence of 2 mM NaN_3. Cells were blocked with 5% heat-inactivated rabbit serum, 0.5% BSA, 5 mM EDTA, and 4 μg/ml 2.4G2 (anti-FcγRII and -III) before the addition of primary Abs. Biotinylated Abs were detected using streptavidin-allophycocyanin (BD PharMingen). Cells were fixed with 1% formaldehyde in PBS before analysis.

The following Abs were used in this study: B220-CyChrome (RA3-6B2; BD PharMingen), CD3-CyChrome (17A2; BD PharMingen), F4/80-PE (Serotec), CD11c-PE (HL3; BD PharMingen), Gr-1-PE (anti-Ly6C/G; BD PharMingen), CD49b-PE (DX5-Pan NK-cell; BD PharMingen), 5C6-FITC (anti-CR3/CD11b) (23), 2A11-biotin (rat IgG2b anti-βGR) (20), 5D3-biotin (rat IgG2a anti-Mϕ mannose receptor (MR)) (L. Martinez-Pomares, D. M. Reid, G. D. Brown, P. R. Taylor, R. Stillion, S. A. Linehan, S. Gordon, and S. Y. C. Wong, unpublished observations), and irrelevant rat IgG2b-biotin, IgG2a-biotin, and IgG2b-FITC control Abs.

In vitro non-opsonic zymosan binding assay

In vitro zymosan binding assays were performed as previously described (14, 18, 20). In brief, resident or 4-day thioglycolate-elicited peritoneal Mϕ were recovered, as described above, and plated at 5×10^5 and 2.5×10^5 cells/well, respectively, in 24-well plates in RPMI/10% FCS overnight. The following day the cells were cooled to 4°C and washed three times with prechilled medium. All experiments were performed at 4°C to prevent receptor internalization, to provide a direct measure of surface receptor involvement, and to prevent local release of opsonins, including complement (24, 25). Zymosan-FITC (Molecular Probes) was added to the Mϕ at a ratio of 25 particles/cell for 1 h on ice. For in vitro blocking assays, carbohydrates (laminarin, β-methylglucoside, and mannan; all from Sigma (St. Louis, MO) and used at 100 μg/ml) or Abs (2A11 (20); 5C6 (23), which has been shown to block the CR3-mediated lectin activity (26); or an irrelevant rat IgG2b control; all used at 100 μg/ml) were added to the chilled cells 20 and 60 min, respectively, before the addition of zymosan. After incubation, unbound zymosan was removed by extensive washing with medium, and cells were lysed with 3% Triton X-100. FITC in lysates was quantified using a Titer-Tek Fluoroskan II (Labsystems Group, Basingstoke, U.K.) as previously described (14, 18). For Ab modulation experiments poly-D-lysine-conditioned tissue culture plates were coated with Ab at 100 μg/ml as previously described (27). All experiments were repeated at least three times.

Statistical analysis

Statistics were calculated using GraphPad PRISM (version 2.0; GraphPad Software, Berkeley, CA). One-way ANOVA with Bonferroni multiple comparison test was applied throughout.

Results

Expression of βGR mRNA in macrophages and multiple mouse tissues

We studied the expression of βGR by RT-PCR in several Mϕ cell lines and in primary Mϕ and DCs. All Mϕ cell lines as well as bone marrow-derived Mϕ and DC showed evidence of βGR expression, whereas βGR transcript was not detectable in the mouse fibroblast cell line NIH-3T3 (Fig. 1*A*). Using the full-length coding sequence to screen a multiple tissue Northern blot, we found βGR expression in most murine tissues with the exception of brain, muscle, and skin (Fig. 1*B*). Notably there was only one discernible transcript detectable in these tissues.

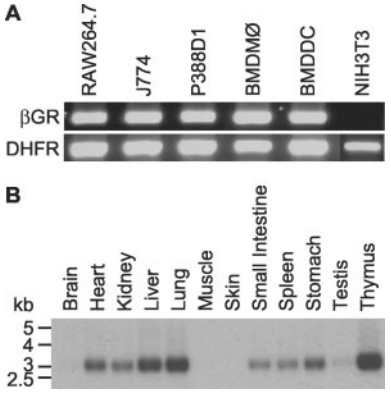

FIGURE 1. Expression of βGR by primary macrophages and in selected tissues. *A*, RT-PCR analysis showed the presence of βGR transcript in Mϕ cell lines (RAW264.7, J774, and P388D1) and primary Mϕ (BMDMϕ) and DC (BMDDC), but not in a mouse fibroblast cell line (NIH-3T3). The housekeeping gene dihydrofolate reductase (DHFR) was used as a PCR control. *B*, A multiple mouse tissue Northern blot probed with βGR cDNA showed widespread expression of βGR in the mouse. Control probing with β-actin confirmed equivalent loading between lanes (data not shown).

Distribution of βGR surface expression in the spleen

We examined the surface expression of βGR using 2A11 on freshly isolated splenocytes (Fig. 2). CD11c^{high} DC were found to express βGR (population 1) in a similar pattern to that reported previously (15). Notably, however, other CD11c$^{low/-}$ cells in the spleen, particularly those expressing CR3, exhibited high surface expression of βGR (population 2). To further delineate which cell types were expressing βGR, the cells were subdivided into six populations based on their expression of CR3 and Gr-1 (an mAb recognizing Ly-6G and Ly-6C) and their FSC/SSC profiles (Fig. 2*B*). Gr-1^{high}CR3^{high}SSC^{high} neutrophils (population 3) exhibited high surface expression of βGR, as did Gr-1^{low}CR3^{+}SSC^{low}Mϕ (population 4B), which also expressed F4/80 (data not shown). A second, unidentified, population of Gr-1^{low}CR3^{+} splenocytes with very high SSC (population 4A) did not show evidence of βGR surface expression. CR3^{+}Gr-1^{-} splenocytes, a mixed population containing DC (CD11c^{high}), NK cells, and other Mϕ (both CD11c^{int}), showed heterogeneity in expression of βGR (population 5). NK cells, which have been shown to recognize β-glucans (8) and are identified by high expression of the DX5 Ag (CD49b) (28), did not show significant labeling with the 2A11 Ab (data not shown). Gr-1^{low}CR3^{-} splenocytes (population 6), previously reported to be a T cell subset (29), expressed CD3 and low levels of surface βGR (Fig. 2*C*). Analysis of all splenic T cells (CD3^{+}) and B cells (B220^{+}) for βGR surface expression, however, indicated that only a distinct subset of T cells exhibited significant surface expression of βGR (Fig. 2*C*). βGR^{+}CD3^{+}T cells were predominantly Gr-1^{+} and CD8^{+}, but CD4^{+} cells were also observed (data not shown). Splenic autofluorescent F4/80^{+} Mϕ also expressed βGR, but at very low levels (data not shown). Plasmacytoid DC in the spleen, which were identified by their Gr-1^{+}B220^{+}CD11c^{int}CR3^{-} phenotype and analyzed in 129/SvEv and BALB/c mice because of the

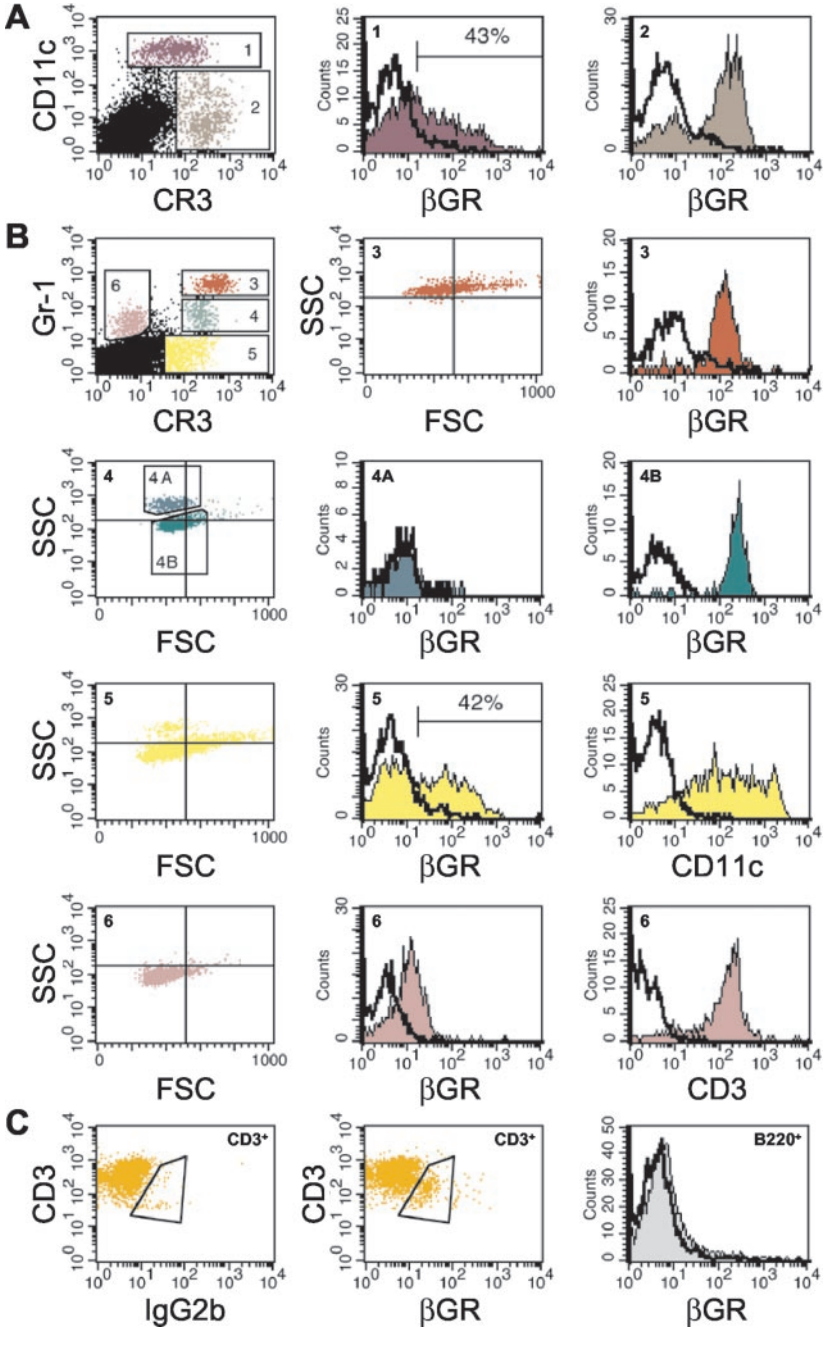

FIGURE 2. Distribution of βGR surface expression on freshly isolated splenocytes. *A*, CD11c^{high} DC (population 1; purple) were confirmed to express βGR, but highest surface expression was observed on most CR3^{+}CD11c$^{low/neg}$ splenocytes (population 2; brown). *B*, Gr-1^{high}CR3^{high}SSC^{high} neutrophils (population 3; red) exhibited high surface expression, as did the Gr-1^{low}CR3^{high}SSC^{low} Mϕ (population 4B; light green), which were also F4/80^{+} (data not shown). CR3^{+}Gr-1^{-} splenocytes (population 5; yellow), a mixed population that contains DC, Mϕ, and NK cells, showed heterogeneous βGR expression. Gr-1^{low}CR3^{-} splenocytes (population 6; pink) also exhibited βGR surface expression, albeit at a relatively low level, and these cells were confirmed to express CD3. *C*, Analysis of all T cells (gated on CD3^{+}; orange) showed that only a subset of T cells expressed βGR. B220^{+} B cells (gray) had no obvious βGR surface expression. Unshaded histograms, Control Ab staining; shaded histograms, correspond to the marker indicated.

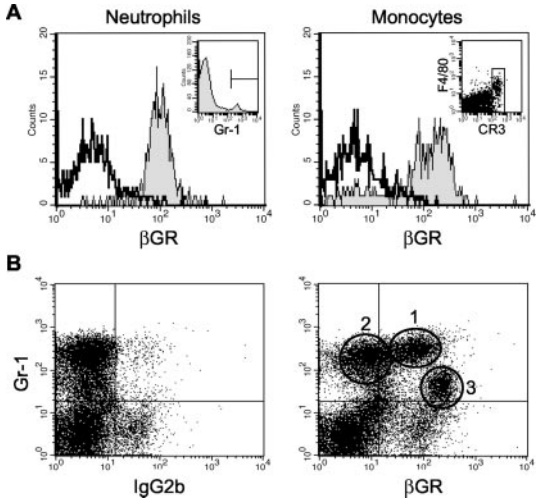

FIGURE 3. Expression of βGR by peripheral blood and bone marrow cells. *A*, Peripheral blood neutrophils (Gr-1^{high}) and M_o (F4/80$^+$CR3$^+$SSC^{low}), gated from total peripheral blood leukocytes as shown in the insets, both showed significant levels of surface staining with 2A11 (shaded histograms) compared with a rat IgG2b control (unshaded histograms). *B*, Bone marrow was analyzed for the expression of βGR, Gr-1, CR3, and F4/80. Dot plots show βGR expression in relation to Gr-1 staining. Gr-1^{high} neutrophils are subdivided into two populations by high and low βGR surface expression. βGR^{high} neutrophils (population 1) had higher SSC and CR3 surface expression than βGR^{low} neutrophils (population 2), indicating that βGR^{high} neutrophils may be more mature (data not shown). Gr-1^{low}βGR^{high} cells (population 3) in the bone marrow, most likely of the M_o/Mϕ lineage, exhibited the highest βGR surface expression.

relative scarcity of these cells in C57BL/6 (30), also exhibited low, but detectable, levels of βGR expression (data not shown).

Expression of βGR on peripheral blood leukocytes

We and others have observed expression of βGR/dectin-1 on both human and mouse PBL by Northern blot (17, 18). We confirmed these observations by FACS by identifying a significant population of PBL that expressed βGR (data not shown). Consistent with the data obtained from the spleen, peripheral blood neutrophils (identified as Gr-1^{high}SSC^{high}) and peripheral blood M_o (identified as CR3$^+$F4/80$^+$SSC^{low}) exhibited high surface expression of βGR (Fig. 3*A*).

Surface expression of the βGR on myeloid cells in the bone marrow

Since we have previously observed the βGR transcript in human bone marrow (18), we examined murine bone marrow for the expression of βGR (Fig. 3*B*). Gr-1^{high}CR3$^+$ neutrophils were subdivided into two populations. Approximately one-third of the bone marrow Gr-1^{high} neutrophils had high βGR surface expression; the remaining two-thirds showed intermediate or marginal expression (populations 1 and 2, respectively; Fig. 3*B*). The βGR^{high} neutrophils had higher SSC and higher CR3 surface expression than the βGR^{low} neutrophils (data not shown), suggesting that the βGR^{high} neutrophils may be in a more advanced state of maturation. This is consistent with the high βGR surface expression detected on circulating peripheral blood neutrophils (Fig. 3*A*). The Gr-1^{low} subgroup of bone marrow cells that has been reported to include cells of the M_o/Mϕ lineage, myeloid precursors, and hemopoietic stem cells (31) contained cells with the highest βGR surface expression (population 3). The expression of CR3 and F4/80 indicated that these high βGR-expressing cells most likely belonged to the M_o/Mϕ lineage (data not shown). Additional unidentified Gr-1^{low} and Gr-1$^-$ bone marrow cells also showed evidence of βGR expression, but these were not characterized further (Fig. 3*B*).

Expression of βGR by isolated primary Mϕ

Freshly isolated resident and thioglycolate-elicited peritoneal Mϕ were assayed for surface expression of βGR. We also looked for βGR expression on alveolar Mϕ, as we had found a high level of transcript in the lung (Fig. 1). Both freshly isolated alveolar Mϕ and thioglycolate-elicited Mϕ expressed high surface levels of βGR, whereas resident peritoneal Mϕ exhibited lower expression (Fig. 4). Interestingly, we observed an up-regulation of βGR on the surface of resident peritoneal Mϕ after 1 day of culture (Fig. 4). Surface expression of βGR on thioglycolate-elicited Mϕ was relatively unaffected by 1 day of culture (data not shown). Since the Mϕ MR is also a candidate receptor for the nonopsonic recognition of zymosan by resident peritoneal Mϕ (see below), we analyzed the surface expression of this receptor on the same cells. Similar to βGR, we found the highest surface expression of the MR on thioglycolate-elicited Mϕ, moderate expression on resident alveolar Mϕ, but only very limited expression on the surface of

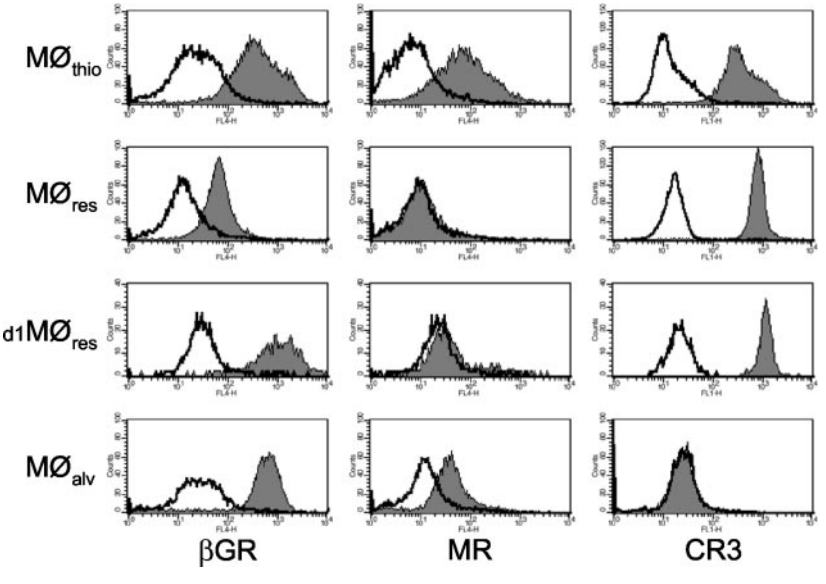

FIGURE 4. Primary Mϕ express βGR. Freshly isolated 4-day thioglycolate-elicited peritoneal Mϕ ($M\phi_{thio}$), resident peritoneal Mϕ ($M\phi_{res}$) and alveolar Mϕ ($M\phi_{alv}$), and resident peritoneal Mϕ that had been cultured for 1 day (d1 $M\phi_{res}$) were assessed for surface expression of βGR, MR, CR3, and F4/80 (not shown). Thioglycolate-elicited and resident alveolar Mϕ expressed very high levels of βGR. Resident peritoneal Mϕ expressed significantly less surface βGR than the other Mϕ studied, but expression was markedly increased after 1 day in culture. Unshaded histograms, Control Ab staining; shaded histograms, receptor-specific staining. Peritoneal Mϕ were distinguished from other cells by high expression of F4/80 and CR3, and alveolar Mϕ were identified as previously described (22).

resident peritoneal cells (Fig. 4). Unlike βGR, however, the expression of MR on resident peritoneal cells was relatively unaffected by 1 day of culture (Fig. 4). As reported previously, we found that alveolar Mϕ expressed negligible CR3 (22) (Fig. 4) and low levels of F4/80 (data not shown).

Surface expression of βGR during peritoneal inflammation

To study the expression of βGR in an inflammatory context, we examined peritoneal exudate cells 18 h after the i.p. administration of thioglycolate, a model of sterile peritonitis. F4/80$^+$CR3^{high}Gr-1$^-$ Mϕ, F4/80$^-$CR3$^+$Gr-1$^+$ neutrophils, and F4/80$^+$CR3$^+$Gr-1$^-$SSC^{high} eosinophils (32) were then tested for βGR surface expression (Fig. 5). Elicited peritoneal Mϕ exhibited the highest βGR expression, and significant amounts were also present on the inflammatory neutrophils. In contrast, recruited eosinophils showed no obvious surface expression of βGR (Fig. 5).

Non-opsonic binding of zymosan to resident peritoneal Mϕ

We observed that freshly isolated resident peritoneal Mϕ had a lower level of surface βGR expression than that on other Mϕ studied (Fig. 4). As we had previously shown that βGR was a major receptor for zymosan on thioglycolate-elicited and BMDMϕ (20), we wanted to determine whether this was also true for resident peritoneal Mϕ. We compared the contribution of βGR on both resident and elicited Mϕ and found that the binding of unopsonized zymosan to elicited Mϕ was significantly inhibited by β-glucans, as previously reported (Fig. 6) (20). βGR was still a major receptor for zymosan on resident Mϕ, but it contributed less to this process than in the thioglycolate-elicited cells. Furthermore, we found that mannan had an inhibitory effect on the binding of zymosan to the resident Mϕ, but not to the thioglycolate-elicited cells (Fig. 6*A*). The combination of β-glucans and mannan did not have an additive effect (data not shown). As with the elicited Mϕ, methylglucoside failed to inhibit the initial binding of zymosan to resident peritoneal Mϕ, suggesting no involvement of CR3 in this process (data not shown). These results implied that a secondary β-glucan-independent, mannan-inhibitable, nonopsonic binding mechanism was operational on resident Mϕ, but was not present on other Mϕ examined.

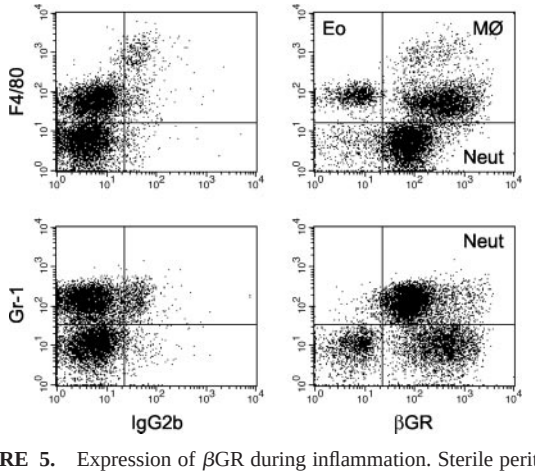

FIGURE 5. Expression of βGR during inflammation. Sterile peritonitis was induced by i.p. administration of thioglycolate broth 18 h before analysis. Peritoneal exudate cells were examined by FACS with the Mϕ/eosinophil marker F4/80 and the neutrophil marker Gr-1 for the expression of βGR. F4/80$^+$Gr-1$^-$Mϕ (Mϕ) exhibited very high βGR surface expression. Gr-1$^+$F4/80$^-$ neutrophils (Neut) also expressed βGR, but F4/80$^+$Gr-1$^-$SSC^{high} eosinophils (Eo) did not show evidence of surface expression of this receptor.

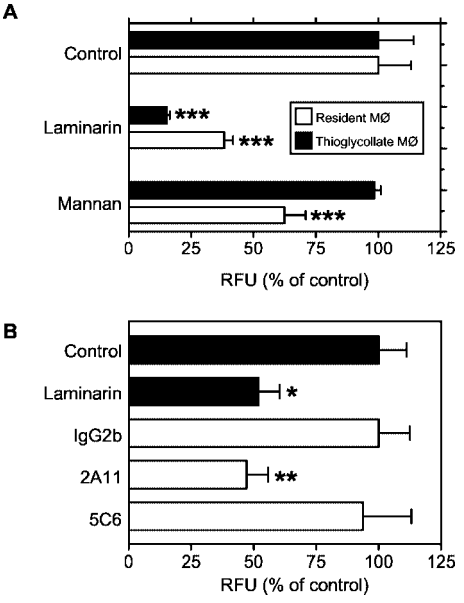

FIGURE 6. β-Glucan binding by resident and elicited Mϕ. *A*, Nonopsonic binding of zymosan particles to resident and thioglycolate-elicited Mϕ (□ and ■, respectively). Data are expressed as the mean ± SD as a percentage of the binding to control untreated Mϕ measured as relative fluorescence units (RFU). Laminarin significantly inhibited the nonopsonic recognition of zymosan by both cell types. Mannan, however, only inhibited recognition by resident peritoneal Mϕ. *B*, Ab-mediated blocking of nonopsnic zymosan binding by resident peritoneal Mϕ. Nonopsonic binding assays were performed, as described in *Materials and Methods*, after preincubating the Mϕ with the mAbs, 2A11 (anti-βGR) and 5C6 (a mAb against CR3 that has been shown to block the lectin activity of CR3 (26)), or the soluble β-glucans, laminarin and glucan phosphate (not shown). Only 2A11 inhibited nonopsonic zymosan binding to resident peritoneal Mϕ. Data are expressed as the mean ± SD percentage of binding to control untreated Mϕ, measured as relative fluorescence units (RFU).

To find out which specific receptors were involved, we performed Ab blocking experiments on the resident peritoneal Mϕ (Fig. 6*B*). The anti-βGR mAb, 2A11, blocked the nonopsonic binding of zymosan to resident peritoneal Mϕ to the same degree as the soluble β-glucans laminarin (Fig. 6*B*) and glucan phosphate (data not shown), consistent with βGR being a major β-glucan receptor on Mϕ. Anti-CR3 (5C6, which blocks the lectin activity of CR3) had no inhibitory effect (Fig. 6*B*) consistent with our previous results (20). As surface expression of Mϕ MR was low on resident peritoneal Mϕ and higher on thioglycolate-elicited Mϕ, which do not have a mannan-inhibitable component of zymosan binding, the MR appears not to be involved in the nonopsonic recognition of zymosan by the Mϕ (Fig. 4) (20). We also performed Ab modulation experiments using specific mAb to deplete surface receptors from the upper ligand binding surface. Only 2A11, anti-βGR-coated tissue culture wells inhibited the nonopsonic binding of zymosan; anti-Mϕ MR and anti-CR3, did not (data not shown).

Discussion

We recently demonstrated that βGR is a principal β-glucan receptor on primary Mϕ (20). As our data (14, 18, 20) did not agree with the previously reported DC-restricted expression of dectin-1 (15), and as the leukocyte β-glucan receptor is believed to be more broadly expressed (8), we re-examined the distribution of dectin-1 using the novel anti-βGR mAb 2A11. While we confirmed expression of dectin-1 by splenic DC, we discovered that a significant proportion of CR3$^+$ splenocytes exhibited higher surface expression of βGR. These cells were of the M$_o$/Mϕ lineage and

neutrophils. Furthermore, we also found that peripheral blood M_o and neutrophils expressed high surface levels of βGR. In the bone marrow, cells of the M_o/Mϕ lineage were also the major surface expressers of βGR, but heterogeneous expression was evident on Gr-1^{high} neutrophils. This heterogeneity appeared to be related to maturation, as βGR^{high} cells had higher levels of CR3 and higher SSC. Overall, these results were consistent with the expected distribution of the β-glucan receptor (8).

Although NK cells are thought to recognize β-glucans (33), we detected no obvious surface expression on freshly isolated splenic NK cells. We cannot exclude, however, that expression of βGR on the surface of these cells may be regulated by activation. Surprisingly we also observed surface expression of dectin-1 on the Gr-1^+ subset of splenic T cells, although it is not without precedent that NK-like C-type lectins can be expressed on T cells (reviewed in Ref. 34). It is plausible that the expression of βGR, as a T cell binding receptor, on a subset of T cells may be part of a novel mechanism for the regulation of the T cell response by specific subsets of T cells as well as by APC.

We have postulated that βGR may play a fundamental role in the immunomodulatory effects of β-glucans and the host response to fungal pathogens (14), and so looked for expression of this receptor on inflammatory cells. Consistent with this, in a model of peritoneal inflammation, elicited Mϕ and neutrophils exhibited high βGR surface expression. Although freshly isolated resident peritoneal Mϕ exhibited low 2A11 binding, the levels of the receptor were up-regulated within 1 day of culture (Fig. 4), indicating that βGR surface expression on Mϕ can be regulated. High levels of βGR were also detected on the CR3$^-$ resident alveolar Mϕ, highlighting the important role this receptor may play in immune surveillance and host defense at this portal of entry, where the availability of complement and Ig is restricted.

Our recent studies with elicited Mϕ (both thioglycolate and Bio-Gel) and BMDMϕ indicated that βGR was a major nonopsonic receptor for binding of the β-glucan-rich particle zymosan (20). The observation of lower βGR surface expression on resident peritoneal Mϕ compared with the other Mϕ we have studied prompted us to determine whether βGR was the major β-glucan receptor on this cell type. Although, we found that β-glucan-dependent binding of unopsonized zymosan to resident peritoneal Mϕ was mediated by βGR, these cells also exhibited a second nonopsonic, mannan-inhibitable binding mechanism that was not found on all other Mϕ examined (20). The surface expression pattern of the Mϕ MR was not consistent with a role for the MR in this process. Our observations have hence uncovered the existence of a second β-glucan-independent, nonopsonic mechanism of binding zymosan used by resident peritoneal Mϕ (but not by other Mϕ tested) that was inhibitable by mannan. This additional receptor could represent the mannan-dependent yeast receptor previously observed on resident peritoneal Mϕ (35, 36) and implicates this receptor as a pattern recognition receptor with a potentially important role in host defense. The presence of this receptor activity specifically on resident peritoneal Mϕ may also explain the controversy surrounding the contribution of a mannan-dependent receptor to yeast/zymosan recognition (35–37). Candidate receptors that may play a role in this process and have demonstrated mannose binding capabilities are DC-SIGN/DC-SIGNR (38) and NKCL (39).

In summary, we have shown that the major surface expression of βGR is on cells of the M_o/Mϕ lineage and neutrophils and to a lesser extent on splenic DC. We have also observed low surface expression of βGR on a specific subset of splenic T cells. This analysis of the surface expression of βGR has provided novel insights into the biology of this receptor and into the recognition of β-glucans by leukocytes.

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