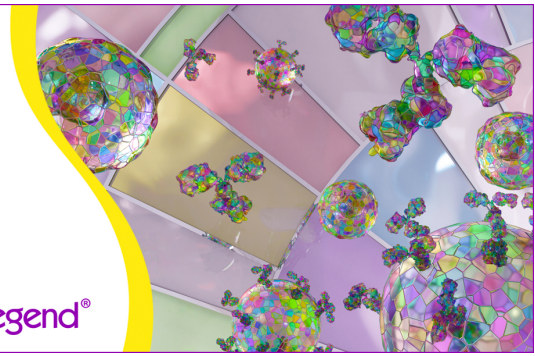


Prevent non-specific  
polymer dye interaction  
with True-Stain™  
Multi-Fluor Buffer

Learn more ▶

BioLegend®



## The Journal of Immunology

RESEARCH ARTICLE | OCTOBER 01 2002

### The $\beta$ -Glucan Receptor, Dectin-1, Is Predominantly Expressed on the Surface of Cells of the Monocyte/Macrophage and Neutrophil Lineages<sup>1</sup>

FREE

Philip R. Taylor; ... et. al

*J Immunol* (2002) 169 (7): 3876–3882.

<https://doi.org/10.4049/jimmunol.169.7.3876>

#### Related Content

The Role of SIGNR1 and the  $\beta$ -Glucan Receptor (Dectin-1) in the Nonopsonic Recognition of Yeast by Specific Macrophages

*J Immunol* (January,2004)

Costimulation of Dectin-1 and DC-SIGN Triggers the Arachidonic Acid Cascade in Human Monocyte-Derived Dendritic Cells

*J Immunol* (April,2008)

Characterizing the Mechanisms of Nonopsonic Uptake of Cryptococci by Macrophages

*J Immunol* (May,2018)

# The $\beta$ -Glucan Receptor, Dectin-1, Is Predominantly Expressed on the Surface of Cells of the Monocyte/Macrophage and Neutrophil Lineages<sup>1</sup>

Philip R. Taylor,<sup>2\*</sup> Gordon D. Brown,<sup>2,3\*</sup> Delyth M. Reid,<sup>†</sup> Janet A. Willment,\*  
Luisa Martinez-Pomares,\* Siamon Gordon,\* and Simon Y. C. Wong<sup>†</sup>

We recently identified dectin-1 ( $\beta$ GR) as a major  $\beta$ -glucan receptor on leukocytes and demonstrated that it played a significant role in the non-opsonic recognition of soluble and particulate  $\beta$ -glucans. Using a novel mAb (2A11) raised against  $\beta$ GR, 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 (M $\phi$ ) and neutrophil lineages). Dendritic cells and a subpopulation of T cells also expressed the  $\beta$ GR, but at lower levels. Alveolar M $\phi$ , like inflammatory M $\phi$ , exhibited the highest surface expression of  $\beta$ GR, indicative of a role for this receptor in immune surveillance. In contrast, resident peritoneal M $\phi$  expressed much lower levels of  $\beta$ GR on the cell surface. Characterization of the nonopsonic recognition of zymosan by resident peritoneal M $\phi$  suggested the existence of an additional  $\beta$ -glucan-independent mechanism of zymosan binding that was not observed on elicited or bone marrow-derived M $\phi$ . Although this recognition could be inhibited by mannan, we were able to exclude involvement of the M $\phi$  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 M $\phi$ . *The Journal of Immunology*, 2002, 169: 3876–3882.

Leukocyte  $\beta$ -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  $\beta$ -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 $_o$ )<sup>4</sup>/macrophage (M $\phi$ )-lineage (including microglia), neutrophils, NK cells, and fibroblasts (8, 9). To date four  $\beta$ -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 ( $\beta$ GR) (14).

We identified dectin-1 ( $\beta$ GR) as a  $\beta$ -glucan receptor after screening a retroviral cDNA library derived from the M $\phi$  cell line RAW264.7 with the  $\beta$ -glucan-rich particle zymosan (14).  $\beta$ GR 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 rec-

ognized particles such as zymosan, *Saccharomyces cerevisiae*, and heat-killed *Candida albicans* in a  $\beta$ -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  $\beta$ -glucan independent, indicating the presence of a second binding site on this receptor (14). The human homologue of  $\beta$ GR has also been cloned (16–19) and was found to exhibit similar properties (18).

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

\*Sir William Dunn School of Pathology, Oxford University, Oxford, United Kingdom; and <sup>†</sup>The Edward Jenner Institute for Vaccine Research, Compton, Berkshire, United Kingdom

Received for publication May 29, 2002. Accepted for publication August 5, 2002.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup> This work was funded by the Wellcome Trust, the Medical Research Council, the Arthritis Research Campaign, and the Yamanouchi Research Institute.

<sup>2</sup> P.R.T. and G.D.B. contributed equally to this work.

<sup>3</sup> Address correspondence and reprint requests to Dr. Gordon D. Brown, Sir William Dunn School of Pathology, Oxford University, South Parks Road, Oxford, U.K. OX1 3RE. E-mail address: gbrown@molbiol.ox.ac.uk

<sup>4</sup> Abbreviations used in this paper: M $_o$ , monocyte(s); M $\phi$ , macrophage;  $\beta$ GR,  $\beta$ -glucan receptor/dectin-1; DC, dendritic cell; BMDDC, bone marrow-derived DC; BMDDM $\phi$ , bone marrow-derived M $\phi$ ; CR3, complement receptor 3; FSC, forward scatter; MR, mannose receptor; SSC, side scatter.

recognition by the low  $\beta$ GR-expressing resident peritoneal cells. While  $\beta$ GR was responsible for  $\beta$ -glucan-dependent zymosan recognition by these cells, a second  $\beta$ -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 (BMDM $\phi$  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  $\beta$ GR 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  $\beta$ GR 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  $\times$  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 M $\phi$

Bronchoalveolar lavage was performed by repeated washes with 1 ml PBS/5 mM EDTA. Resident alveolar M $\phi$ , 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 M $\phi$  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 Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. Cells were blocked with 5% heat-inactivated rabbit serum, 0.5% BSA, 5 mM EDTA, and 4  $\mu$ g/ml 2.4G2 (anti-Fc $\gamma$ RII 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- $\beta$ GR) (20), 5D3-biotin (rat IgG2a anti-M $\phi$  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 M $\phi$  were recovered, as described above, and plated at 5  $\times$  10<sup>5</sup> and 2.5  $\times$  10<sup>5</sup> 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 M $\phi$  at a ratio of 25 particles/cell for 1 h on ice. For in vitro blocking assays, carbohydrates (laminarin,  $\beta$ -methylglucoside, and mannan; all from Sigma (St. Louis, MO) and used at 100  $\mu$ g/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  $\mu$ g/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  $\mu$ g/ml as previously described (27). All experiments were repeated at least three times.

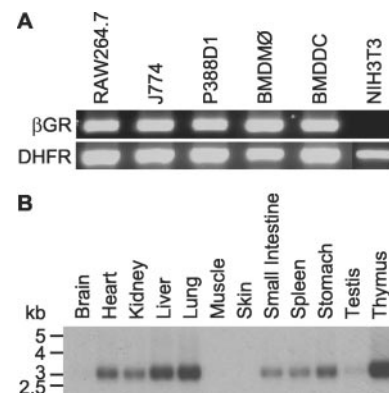
### Statistical analysis

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 $\beta$ GR mRNA in macrophages and multiple mouse tissues

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



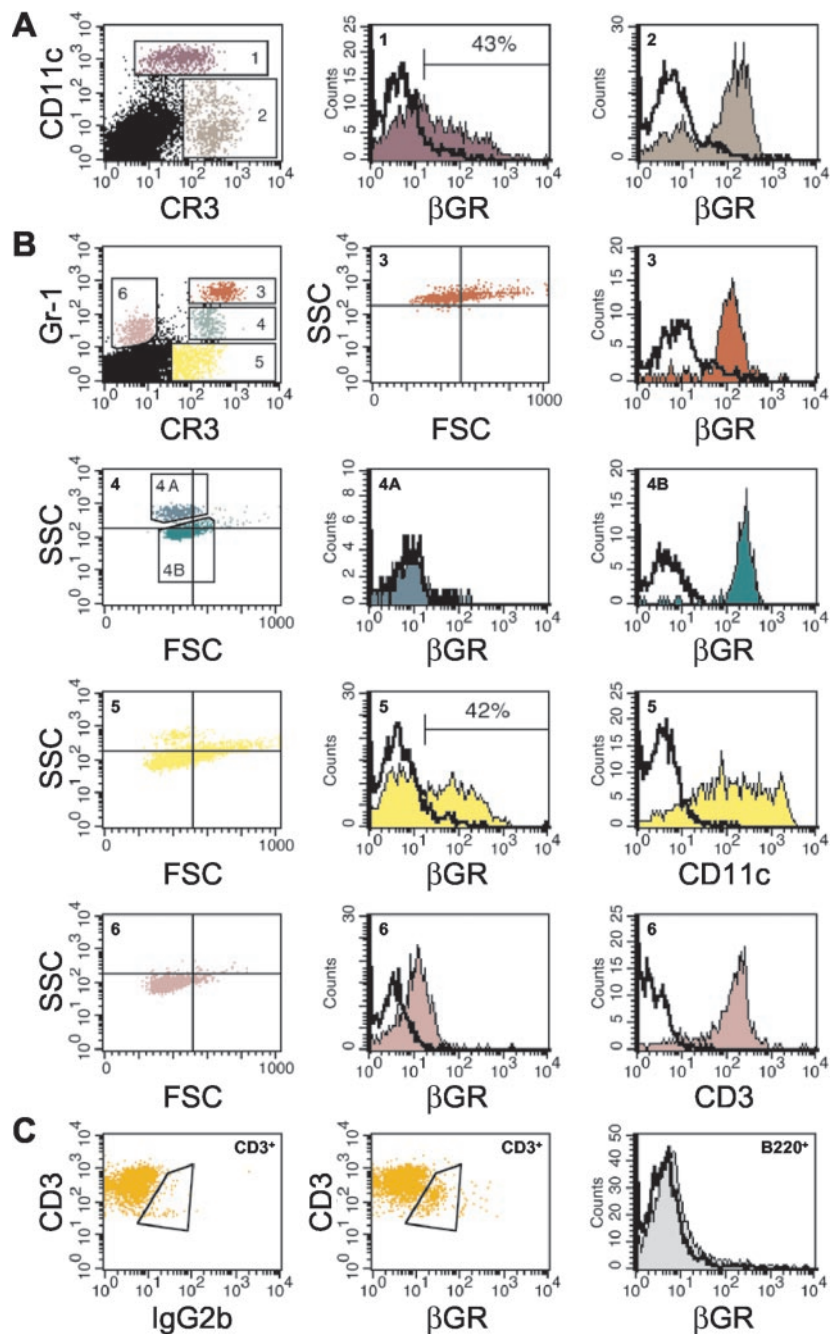
**FIGURE 1.** Expression of  $\beta$ GR by primary macrophages and in selected tissues. *A*, RT-PCR analysis showed the presence of  $\beta$ GR transcript in M $\phi$  cell lines (RAW264.7, J774, and P388D1) and primary M $\phi$  (BMDM $\phi$ ) and DC (BMDDC), but not in a mouse fibroblast cell line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



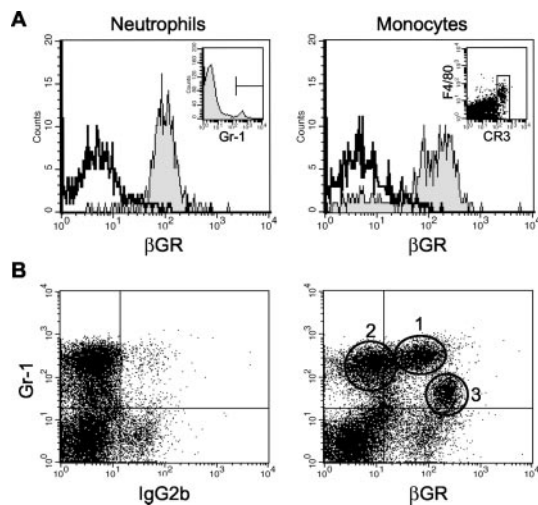
### Distribution of $\beta$ GR surface expression in the spleen

We examined the surface expression of  $\beta$ GR using 2A11 on freshly isolated splenocytes (Fig. 2). CD11c<sup>high</sup> DC were found to express  $\beta$ GR (population 1) in a similar pattern to that reported previously (15). Notably, however, other CD11c<sup>low/-</sup> cells in the spleen, particularly those expressing CR3, exhibited high surface expression of  $\beta$ GR (population 2). To further delineate which cell types were expressing  $\beta$ GR, 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. 2B). Gr-1<sup>high</sup>CR3<sup>high</sup>SSC<sup>high</sup> neutrophils (population 3) exhibited high surface expression of  $\beta$ GR, as did Gr-1<sup>low</sup>CR3<sup>high</sup>SSC<sup>low</sup> M $\phi$  (population 4B), which also expressed F4/80 (data not shown). A second, unidentified, population of Gr-1<sup>low</sup>CR3<sup>+</sup> splenocytes with very high SSC (population 4A) did not show evidence of  $\beta$ GR surface expression. CR3<sup>+</sup>Gr-1<sup>-</sup> splenocytes, a mixed population

containing DC (CD11c<sup>high</sup>), NK cells, and other M $\phi$  (both CD11c<sup>int</sup>), showed heterogeneity in expression of  $\beta$ GR (population 5). NK cells, which have been shown to recognize  $\beta$ -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<sup>low</sup>CR3<sup>-</sup> splenocytes (population 6), previously reported to be a T cell subset (29), expressed CD3 and low levels of surface  $\beta$ GR (Fig. 2C). Analysis of all splenic T cells (CD3<sup>+</sup>) and B cells (B220<sup>+</sup>) for  $\beta$ GR surface expression, however, indicated that only a distinct subset of T cells exhibited significant surface expression of  $\beta$ GR (Fig. 2C).  $\beta$ GR<sup>+</sup>CD3<sup>+</sup>T cells were predominantly Gr-1<sup>+</sup> and CD8<sup>+</sup>, but CD4<sup>+</sup> cells were also observed (data not shown). Splenic autofluorescent F4/80<sup>+</sup> M $\phi$  also expressed  $\beta$ GR, but at very low levels (data not shown). Plasmacytoid DC in the spleen, which were identified by their Gr-1<sup>+</sup>B220<sup>+</sup>CD11c<sup>int</sup>CR3<sup>-</sup> phenotype and analyzed in 129/SvEv and BALB/c mice because of the



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



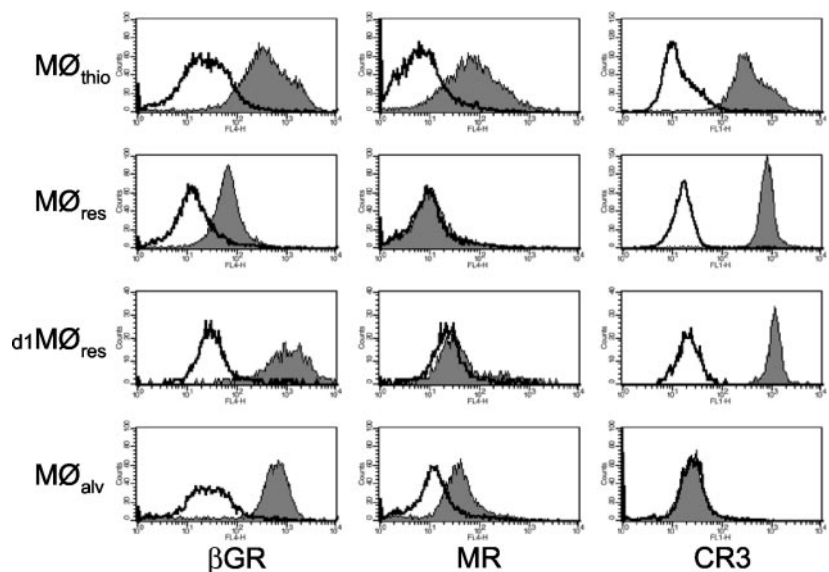
**FIGURE 3.** Expression of  $\beta$ GR by peripheral blood and bone marrow cells. *A*, Peripheral blood neutrophils ( $\text{Gr-1}^{\text{high}}$ ) and  $\text{M}_0$  ( $\text{F4/80}^+\text{CR3}^+\text{SSC}^{\text{low}}$ ), gated from total peripheral blood leukocytes as shown in the insets, both showed 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  $\beta$ GR, Gr-1, CR3, and F4/80. Dot plots show  $\beta$ GR expression in relation to Gr-1 staining.  $\text{Gr-1}^{\text{high}}$  neutrophils are subdivided into two populations by high and low  $\beta$ GR surface expression.  $\beta\text{GR}^{\text{high}}$  neutrophils (population 1) had higher SSC and CR3 surface expression than  $\beta\text{GR}^{\text{low}}$  neutrophils (population 2), indicating that  $\beta\text{GR}^{\text{high}}$  neutrophils may be more mature (data not shown).  $\text{Gr-1}^{\text{low}}\beta\text{GR}^{\text{high}}$  cells (population 3) in the bone marrow, most likely of the  $\text{M}_0/\text{M}\phi$  lineage, exhibited the highest  $\beta$ GR surface expression.

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

#### Expression of $\beta$ GR on peripheral blood leukocytes

We and others have observed expression of  $\beta$ GR/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  $\beta$ GR (data not shown). Consistent with the data obtained from the spleen, peripheral blood neutrophils (identified as  $\text{Gr-1}^{\text{high}}\text{SSC}^{\text{high}}$ ) and peripheral blood  $\text{M}_0$  (identified as  $\text{CR3}^+\text{F4/80}^+\text{SSC}^{\text{low}}$ ) exhibited high surface expression of  $\beta$ GR (Fig. 3A).

**FIGURE 4.** Primary  $\text{M}\phi$  express  $\beta$ GR. Freshly isolated 4-day thioglycolate-elicited peritoneal  $\text{M}\phi$  ( $\text{M}\phi_{\text{thio}}$ ), resident peritoneal  $\text{M}\phi$  ( $\text{M}\phi_{\text{res}}$ ) and alveolar  $\text{M}\phi$  ( $\text{M}\phi_{\text{alv}}$ ), and resident peritoneal  $\text{M}\phi$  that had been cultured for 1 day ( $\text{d1M}\phi_{\text{res}}$ ) were assessed for surface expression of  $\beta$ GR, MR, CR3, and F4/80 (not shown). Thioglycolate-elicited and resident alveolar  $\text{M}\phi$  expressed very high levels of  $\beta$ GR. Resident peritoneal  $\text{M}\phi$  expressed significantly less surface  $\beta$ GR than the other  $\text{M}\phi$  studied, but expression was markedly increased after 1 day in culture. Unshaded histograms, Control Ab staining; shaded histograms, receptor-specific staining. Peritoneal  $\text{M}\phi$  were distinguished from other cells by high expression of F4/80 and CR3, and alveolar  $\text{M}\phi$  were identified as previously described (22).



#### Surface expression of the $\beta$ GR on myeloid cells in the bone marrow

Since we have previously observed the  $\beta$ GR transcript in human bone marrow (18), we examined murine bone marrow for the expression of  $\beta$ GR (Fig. 3B).  $\text{Gr-1}^{\text{high}}\text{CR3}^+$  neutrophils were subdivided into two populations. Approximately one-third of the bone marrow  $\text{Gr-1}^{\text{high}}$  neutrophils had high  $\beta$ GR surface expression; the remaining two-thirds showed intermediate or marginal expression (populations 1 and 2, respectively; Fig. 3B). The  $\beta\text{GR}^{\text{high}}$  neutrophils had higher SSC and higher CR3 surface expression than the  $\beta\text{GR}^{\text{low}}$  neutrophils (data not shown), suggesting that the  $\beta\text{GR}^{\text{high}}$  neutrophils may be in a more advanced state of maturation. This is consistent with the high  $\beta$ GR surface expression detected on circulating peripheral blood neutrophils (Fig. 3A). The  $\text{Gr-1}^{\text{low}}$  sub-group of bone marrow cells that has been reported to include cells of the  $\text{M}_0/\text{M}\phi$  lineage, myeloid precursors, and hemopoietic stem cells (31) contained cells with the highest  $\beta$ GR surface expression (population 3). The expression of CR3 and F4/80 indicated that these high  $\beta$ GR-expressing cells most likely belonged to the  $\text{M}_0/\text{M}\phi$  lineage (data not shown). Additional unidentified  $\text{Gr-1}^{\text{low}}$  and  $\text{Gr-1}^-$  bone marrow cells also showed evidence of  $\beta$ GR expression, but these were not characterized further (Fig. 3B).

#### Expression of $\beta$ GR by isolated primary $\text{M}\phi$

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

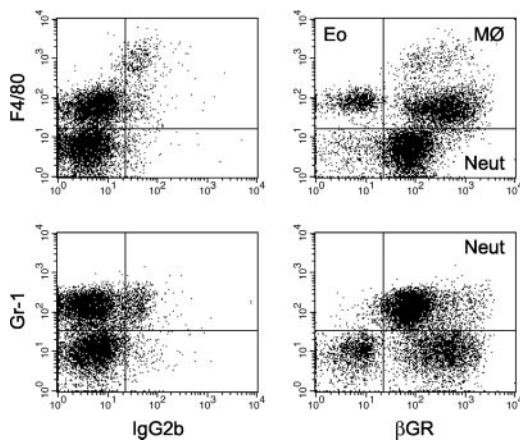
resident peritoneal cells (Fig. 4). Unlike  $\beta$ GR, however, the expression of MR on resident peritoneal cells was relatively unaffected by 1 day of culture (Fig. 4). As reported previously, we found that alveolar M $\phi$  expressed negligible CR3 (22) (Fig. 4) and low levels of F4/80 (data not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

#### Surface expression of $\beta$ GR during peritoneal inflammation

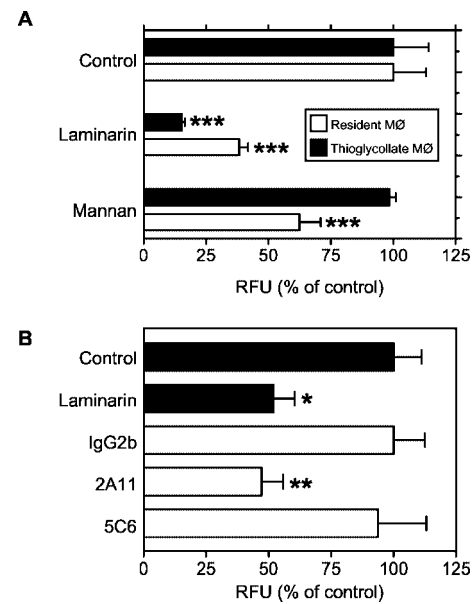
To study the expression of  $\beta$ GR in an inflammatory context, we examined peritoneal exudate cells 18 h after the i.p. administration of thioglycolate, a model of sterile peritonitis. F4/80<sup>+</sup>CR3<sup>high</sup>Gr-1<sup>-</sup> M $\phi$ , F4/80<sup>-</sup>CR3<sup>+</sup>Gr-1<sup>+</sup>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

#### Non-opsonic binding of zymosan to resident peritoneal M $\phi$

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



**FIGURE 5.** Expression of  $\beta$ GR 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 M $\phi$ /eosinophil marker F4/80 and the neutrophil marker Gr-1 for the expression of  $\beta$ GR. F4/80<sup>+</sup>Gr-1<sup>-</sup>M $\phi$  (M $\phi$ ) exhibited very high  $\beta$ GR surface expression. Gr-1<sup>+</sup>F4/80<sup>-</sup> neutrophils (Neut) also expressed  $\beta$ GR, but F4/80<sup>+</sup>Gr-1<sup>-</sup>SSC<sup>high</sup> eosinophils (Eo) did not show evidence of surface expression of this receptor.



**FIGURE 6.**  $\beta$ -Glucan binding by resident and elicited M $\phi$ . *A*, Non-opsonic binding of zymosan particles to resident and thioglycolate-elicited M $\phi$  (□ and ■, respectively). Data are expressed as the mean  $\pm$  SD as a percentage of the binding to control untreated M $\phi$  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 M $\phi$ . *B*, Ab-mediated blocking of nonopsonic zymosan binding by resident peritoneal M $\phi$ . Nonopsonic binding assays were performed, as described in *Materials and Methods*, after preincubating the M $\phi$  with the mAbs, 2A11 (anti- $\beta$ GR) and 5C6 (a mAb against CR3 that has been shown to block the lectin activity of CR3 (26)), or the soluble  $\beta$ -glucans, laminarin and glucan phosphate (not shown). Only 2A11 inhibited nonopsonic zymosan binding to resident peritoneal M $\phi$ . Data are expressed as the mean  $\pm$  SD percentage of binding to control untreated M $\phi$ , measured as relative fluorescence units (RFU).

To find out which specific receptors were involved, we performed Ab blocking experiments on the resident peritoneal M $\phi$  (Fig. 6B). The anti- $\beta$ GR mAb, 2A11, blocked the nonopsonic binding of zymosan to resident peritoneal M $\phi$  to the same degree as the soluble  $\beta$ -glucans laminarin (Fig. 6B) and glucan phosphate (data not shown), consistent with  $\beta$ GR being a major  $\beta$ -glucan receptor on M $\phi$ . Anti-CR3 (5C6, which blocks the lectin activity of CR3) had no inhibitory effect (Fig. 6B) consistent with our previous results (20). As surface expression of M $\phi$  MR was low on resident peritoneal M $\phi$  and higher on thioglycolate-elicited M $\phi$ , 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 M $\phi$  (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- $\beta$ GR-coated tissue culture wells inhibited the nonopsonic binding of zymosan; anti-M $\phi$  MR and anti-CR3, did not (data not shown).

## Discussion

We recently demonstrated that  $\beta$ GR is a principal  $\beta$ -glucan receptor on primary M $\phi$  (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  $\beta$ -glucan receptor is believed to be more broadly expressed (8), we re-examined the distribution of dectin-1 using the novel anti- $\beta$ GR mAb 2A11. While we confirmed expression of dectin-1 by splenic DC, we discovered that a significant proportion of CR3<sup>+</sup> splenocytes exhibited higher surface expression of  $\beta$ GR. These cells were of the M $\phi$ /M $\phi$  lineage and



neutrophils. Furthermore, we also found that peripheral blood  $M_0$  and neutrophils expressed high surface levels of  $\beta$ GR. In the bone marrow, cells of the  $M_0/M\phi$  lineage were also the major surface expressers of  $\beta$ GR, but heterogeneous expression was evident on Gr-1<sup>high</sup> neutrophils. This heterogeneity appeared to be related to maturation, as  $\beta$ GR<sup>high</sup> cells had higher levels of CR3 and higher SSC. Overall, these results were consistent with the expected distribution of the  $\beta$ -glucan receptor (8).

Although NK cells are thought to recognize  $\beta$ -glucans (33), we detected no obvious surface expression on freshly isolated splenic NK cells. We cannot exclude, however, that expression of  $\beta$ GR on the surface of these cells may be regulated by activation. Surprisingly we also observed surface expression of dectin-1 on the Gr-1<sup>+</sup> 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  $\beta$ GR, 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  $\beta$ GR may play a fundamental role in the immunomodulatory effects of  $\beta$ -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  $M\phi$  and neutrophils exhibited high  $\beta$ GR surface expression. Although freshly isolated resident peritoneal  $M\phi$  exhibited low 2A11 binding, the levels of the receptor were up-regulated within 1 day of culture (Fig. 4), indicating that  $\beta$ GR surface expression on  $M\phi$  can be regulated. High levels of  $\beta$ GR were also detected on the CR3<sup>-</sup> resident alveolar  $M\phi$ , 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  $M\phi$  (both thioglycolate and Bio-Gel) and BMDM $\phi$  indicated that  $\beta$ GR was a major nonopsonic receptor for binding of the  $\beta$ -glucan-rich particle zymosan (20). The observation of lower  $\beta$ GR surface expression on resident peritoneal  $M\phi$  compared with the other  $M\phi$  we have studied prompted us to determine whether  $\beta$ GR was the major  $\beta$ -glucan receptor on this cell type. Although, we found that  $\beta$ -glucan-dependent binding of unopsonized zymosan to resident peritoneal  $M\phi$  was mediated by  $\beta$ GR, these cells also exhibited a second nonopsonic, mannan-inhibitable binding mechanism that was not found on all other  $M\phi$  examined (20). The surface expression pattern of the  $M\phi$  MR was not consistent with a role for the MR in this process. Our observations have hence uncovered the existence of a second  $\beta$ -glucan-independent, nonopsonic mechanism of binding zymosan used by resident peritoneal  $M\phi$  (but not by other  $M\phi$  tested) that was inhibitable by mannan. This additional receptor could represent the mannan-dependent yeast receptor previously observed on resident peritoneal  $M\phi$  (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  $M\phi$  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  $\beta$ GR is on cells of the  $M_0/M\phi$  lineage and neutrophils and to a lesser extent on splenic DC. We have also observed low surface expression of  $\beta$ GR on a specific subset of splenic T cells. This analysis of the surface expression of  $\beta$ GR has provided novel insights into the biology of this receptor and into the recognition of  $\beta$ -glucans by leukocytes.

## Acknowledgments

We thank the staff of our animal facility for the care of the animals used in this study. We also thank Dr. Steve Cobbold for his advice and critical reading of the manuscript.

## References

- Kadish, J. L., C. C. Choi, and J. K. Czap. 1986. Phagocytosis of unopsonized zymosan particles by trypsin-sensitive and  $\beta$ -glucan-inhibitable receptors on bone marrow-derived murine macrophages. *Immunol. Res.* 5:129.
- Czap, J. K., D. T. Fearon, and K. F. Austen. 1978. Opsonin-independent phagocytosis of activators of the alternative complement pathway by human monocytes. *J. Immunol.* 120:1132.
- Williams, D. L., A. Mueller, and W. Browder. 1996. Glucan-based macrophage stimulators. *Clin. Immunother.* 5:392.
- Ross, G. D., V. Vetvicka, J. Yan, Y. Xia, and J. Vetvickova. 1999. Therapeutic intervention with complement and  $\beta$ -glucan in cancer. *Immunopharmacology* 42:61.
- Williams, D. L., J. A. Cook, E. O. Hoffmann, and N. R. Di Luzio. 1978. Protective effect of glucan in experimentally induced candidiasis. *J. Reticuloendothel. Soc.* 23:479.
- Kokoshis, P. L., D. L. Williams, J. A. Cook, and N. R. Di Luzio. 1978. Increased resistance to *Staphylococcus aureus* infection and enhancement in serum lysozyme activity by glucan. *Science* 199:1340.
- Itoh, W. 1997. Augmentation of protective immune responses against viral infection by oral administration of schizophyllan. *Mediat. Inflamm.* 6:267.
- Williams, D. L. 1997. Overview of (1,3)- $\beta$ -D-glucan immunobiology. *Mediat. Inflamm.* 6:247.
- Kougiaris, P., D. Wei, P. J. Rice, H. E. Ensley, J. Kalbfleisch, D. L. Williams, and I. W. Browder. 2001. Normal human fibroblasts express pattern recognition receptors for fungal (1-3)- $\beta$ -D-glucans. *Infect. Immun.* 69:3933.
- Ross, G. D., J. A. Cain, and P. J. Lachmann. 1985. Membrane complement receptor type three (CR3) has lectin-like properties analogous to bovine conglutinin as functions as a receptor for zymosan and rabbit erythrocytes as well as a receptor for iC3b. *J. Immunol.* 134:3307.
- Thornton, B. P., V. Vetvicka, M. Pitman, R. C. Goldman, and G. D. Ross. 1996. Analysis of the sugar specificity and molecular location of the  $\beta$ -glucan-binding lectin site of complement receptor type 3 (CD11b/CD18). *J. Immunol.* 156:1235.
- Zimmerman, J. W., J. Lindermuth, P. A. Fish, G. P. Palace, T. T. Stevenson, and D. E. DeMong. 1998. A novel carbohydrate-glycosphingolipid interaction between a  $\beta$ -(1-3)-glucan immunomodulator, PGG-glucan, and lactosylceramide of human leukocytes. *J. Biol. Chem.* 273:22014.
- Pearson, A., A. Lux, and M. Krieger. 1995. Expression cloning of dSR-CI, a class C macrophage-specific scavenger receptor from *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 92:4056.
- Brown, G. D., and S. Gordon. 2001. Immune recognition: a new receptor for  $\beta$ -glucans. *Nature* 413:36.
- Ariizumi, K., G. L. Shen, S. Shikano, S. Xu, R. Ritter III, T. Kumamoto, D. Edelbaum, A. Morita, P. R. Bergstresser, and A. Takashima. 2000. Identification of a novel, dendritic cell-associated molecule, dectin-1, by subtractive cDNA cloning. *J. Biol. Chem.* 275:20157.
- Hernandez-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.
- 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.
- Willment, J. A., S. Gordon, and G. D. Brown. 2001. Characterisation of the human  $\beta$ -glucan receptor and its alternatively spliced isoforms. *J. Biol. Chem.* 276:2020.
- 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.
- Brown, G. D., P. R. Taylor, D. M. Reid, J. A. Willment, D. L. Williams, L. Martinez-Pomares, S. Y. C. Wong, and S. Gordon. 2002. Dectin-1 is a major  $\beta$ -glucan receptor on macrophages. *J. Exp. Med.* 196:407.
- Le Cabec, V., C. Cols, and I. Maridonneau-Parini. 2000. Nonopsonic phagocytosis of zymosan and *Mycobacterium kansasii* by CR3 (CD11b/CD18) involves distinct molecular determinants and is or is not coupled with NADPH oxidase activation. *Infect. Immun.* 68:4736.
- Maus, U., S. Herold, H. Muth, R. Maus, L. Ermert, M. Ermert, N. Weissmann, S. Rosseau, W. Seeger, F. Grimmlinger, et al. 2001. Monocytes recruited into the alveolar air space of mice show a monocytic phenotype but upregulate CD14. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 280:L58.
- Rosen, H., and S. Gordon. 1987. Monoclonal antibody to the murine type 3 complement receptor inhibits adhesion of myelomonocytic cells in vitro and inflammatory cell recruitment in vivo. *J. Exp. Med.* 166:1685.
- Ezekowitz, R. A., R. B. Sim, M. Hill, and S. Gordon. 1984. Local opsonization by secreted macrophage complement components: role of receptors for complement in uptake of zymosan. *J. Exp. Med.* 159:244.
- Ezekowitz, R. A., R. B. Sim, G. G. MacPherson, and S. Gordon. 1985. Interaction of human monocytes, macrophages, and polymorphonuclear leukocytes with zymosan in vitro: role of type 3 complement receptors and macrophage-derived complement. *J. Clin. Invest.* 76:2368.

26. Xia, Y., V. Vetvicka, J. Yan, M. Hanikyrova, T. Mayadas, and G. D. Ross. 1999. The  $\beta$ -glucan-binding lectin site of mouse CR3 (CD11b/CD18) and its function in generating a primed state of the receptor that mediates cytotoxic activation in response to iC3b-opsonized target cells. *J. Immunol.* 162:2281.
27. Michl, J., M. M. Pieczonka, J. C. Unkeless, and S. C. Silverstein. 1979. Effects of immobilized immune complexes on Fc- and complement-receptor function in resident and thioglycollate-elicited mouse peritoneal macrophages. *J. Exp. Med.* 150:607.
28. Arase, H., T. Saito, J. H. Phillips, and L. L. Lanier. 2001. Cutting edge: the mouse NK cell-associated antigen recognized by DX5 monoclonal antibody is CD49b ( $\alpha_2$  integrin, very late antigen-2). *J. Immunol.* 167:1141.
29. Lagasse, E., and I. L. Weissman. 1996. Flow cytometric identification of murine neutrophils and monocytes. *J. Immunol. Methods* 197:139.
30. Nakano, H., M. Yanagita, and M. D. Gunn. 2001. CD11c<sup>+</sup>B220<sup>+</sup>Gr-1<sup>+</sup> cells in mouse lymph nodes and spleen display characteristics of plasmacytoid dendritic cells. *J. Exp. Med.* 194:1171.
31. Biermann, H., B. Pietz, R. Dreier, K. W. Schmid, C. Sorg, and C. Sunderkotter. 1999. Murine leukocytes with ring-shaped nuclei include granulocytes, monocytes, and their precursors. *J. Leukocyte Biol.* 65:217.
32. McGarry, M. P., and C. C. Stewart. 1991. Murine eosinophil granulocytes bind the murine macrophage-monocyte specific monoclonal antibody F4/80. *J. Leukocyte Biol.* 50:471.
33. Ross, G. D., and V. Vetvicka. 1993. CR3 (CD11b, CD18): a phagocyte and NK cell membrane receptor with multiple ligand specificities and functions. *Clin. Exp. Immunol.* 92:181.
34. McMahon, C. W., and D. H. Raulet. 2001. Expression and function of NK cell receptors in CD8<sup>+</sup> T cells. *Curr. Opin. Immunol.* 13:465.
35. 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.
36. Giaimis, J., Y. Lombard, P. Fonteneau, C. D. Muller, R. Levy, M. Makaya-Kumba, J. Lazdins, and P. Poindron. 1993. Both mannose and  $\beta$ -glucan receptors are involved in phagocytosis of unopsonized, heat-killed *Saccharomyces cerevisiae* by murine macrophages. *J. Leukocyte Biol.* 54:564.
37. Goldman, R. 1988. Characteristics of the  $\beta$ -glucan receptor of murine macrophages. *Exp. Cell Res.* 174:481.
38. 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.
39. Fernandes, M. J., A. A. Finnegan, L. D. Siracusa, C. Brenner, N. N. Iscove, and B. Calabretta. 1999. Characterization of a novel receptor that maps near the natural killer gene complex: demonstration of carbohydrate binding and expression in hematopoietic cells. *Cancer Res.* 59:2709.