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Ly49B Is Expressed on Multiple Subpopulations of Myeloid Cells

Frances Gays,* Jonathan G. Aust,* Delyth M. Reid,† Jane Falconer,* Noriko Toyama-Sorimachi,‡ Philip R. Taylor,§ and Colin G. Brooks2*†

Using a novel mAb specific for mouse Ly49B, we report here that Ly49B, the last remaining member of the C57 Ly49 family to be characterized, is expressed at low levels on ~1.5% of spleen cells, none of which are NK cells or T cells but which instead belong to several distinct subpopulations of myeloid cells defined by expression of CD11b and different levels of Gr1. Much larger proportions of bone marrow and peritoneal cells expressed Ly49B, all being CD11b+ and comprising multiple subpopulations defined by light scatter, F4/80, and Gr1 expression. Costaining for Ly49Q, also expressed on myeloid cells, revealed that Ly49B and Ly49Q were most strongly expressed on nonoverlapping subpopulations, Ly49Qhigh cells being mostly B220+CD4+ and/or CD8+, Ly49B+ cells lacking these markers. Myeloid populations that developed from bone marrow progenitors in vitro frequently coexpressed both Ly49B and Ly49Q, and Ly49B expression could be up-regulated by LPS, α-IFN, and γ-IFN, often independently of Ly49Q. PCR analysis revealed that cultured NK cells and T cells contained Ly49B transcripts, and Ly49B expression could be detected on NK cells cultured in IL-12 plus IL-18, and on an immature NK cell line. Immunohistochemical studies showed that Ly49B expression in tissues overlapped with but was distinct from that of all other myeloid molecules examined, being particularly prominent in the lamina propria and dome of Peyer’s patches, implicating an important role of Ly49B in gut immunobiology. In transfected cells, Ly49B was found to associate with SHP-1, SHP-2, and SHIP in a manner strongly regulated by intracellular phosphorylation events. The Journal of Immunology, 2006, 177: 5840–5851.

Since their discovery in 1983 (1), Ly49 receptors have been the subject of intense interest. It was eventually realized that they are encoded by a series of closely related genes (2) clustered at the telomeric end of the NK gene complex, a region of ~2 Mb on chromosome 6 that contains several families of genes expressed predominately in NK cells (3–5). The Ly49 polypeptides are type II transmembrane proteins belonging to the C-type lectin superfamily and comprise two subclasses, one of which possesses an ITIM in the cytoplasmic domain and delivers inhibitory signals following cross-linking by MHC class I ligands, while the other lacks an ITIM, has a charged residue in the transmembrane domain, and delivers activatory signals via the adaptor protein DAP12 following interaction with ligands that may include MHC class I molecules and virus-encoded class I homologs (5). Most of the Ly49 receptors that have been studied are expressed predominately on NK cells, although the inhibitory Ly49s can also be expressed in some circumstances on T cells (6). The only member of the Ly49 family in C57 mice that has so far not been studied is Ly49B (2). The gene encoding Ly49B is separated by >0.8 Mb from the cluster containing the remaining 10 functional Ly49 genes (4). It has an intron-exon structure similar to that of other Ly49 genes but appears to lack the upstream Pro1 element responsible for initiating the stochastic expression of Ly49 receptors in NK cells (7). Ly49B cDNAs have been obtained from several tissue sources and predict a polypeptide that lacks a transmembrane charge and bears an ITIM in the cytoplasmic domain but which is significantly divergent from other Ly49s. Interestingly, Ly49B is the only member of the mouse Ly49 family that has an identifiable ortholog in the rat (8, 9), suggesting it may have a distinctive and conserved function in murine species. We have now generated a series of mAbs that specifically recognize Ly49B and report here that Ly49B is not normally expressed on NK cells but instead is expressed on multiple subpopulations of myeloid cells that include granulocytes and macrophages.

Materials and Methods

Cells

PVG female rats and C57BL/6 and BALB/c female mice were obtained from Harlan-Olac, and all procedures were approved by the U.K. licensing authorities. Except where stated, 6- to 16-wk-old C57BL/6 mice were used as cell donors. Spleen cells were obtained by tearing of spleens with forceps, bone marrow (BM) cells by passing material eluted from femurs and tibias through narrow gauge needles, and peritoneal cells by flushing the peritoneum with PBS. In some experiments, animals were injected i.p. with 1 ml of a 10% solution of thioglycollate broth.

Cultures

The culture medium used was DMEM (52100-039; Invitrogen Life Technologies) made up in highly purified water and supplemented with 2× nonessential amino acids, 5 × 10−5 M 2-ME, and 10% FBS (F-7524; Sigma-Aldrich). Spleen and BM macrophages were obtained by culturing aliquots of respectively 50 or 5 million nucleated cells in 90-mm bacteriological grade petri dishes containing 10 ml of medium supplemented with 10% heat-inactivated horse serum and 10% supernatant from L929 cells

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2 Address correspondence and reprint requests to Dr. Colin G. Brooks, Institute of Cell and Molecular Biosciences, The Medical School, Newcastle NE2 4HH, U.K.

3 Abbreviations used in this paper: BM, bone marrow; cII, MHC class II; CRD, carbohydrate recognition domain; DC, dendritic cell; FL, Il3 ligand; HA, hemagglutinin; SHP, Src homology region 2 domain-containing phosphatase; SSC, side light scatter.
Immunoprecipitation and blotting

RNAK cells transfected with Ly49B bearing a C-terminal FLAG tag were collected with ice-cold PBS and suspended at 2 × 10^6 cells/75 µl in cold PBS containing 5% FBS. After addition of 25 µl of 4 mM Na$_2$VO$_3$ (per- vanadate; Sigma-Aldrich), the cells were incubated at 37°C for 5 min, followed by the addition of 1 ml of 1% Nonidet P-40 lysis buffer containing 100 mM NaF and 1 mM perrnvanadate. The cell lysates were incubated with agarose beads conjugated with anti-FLAG Ab M2 (Upstate Biotechnology), and the immunoprecipitates were resolved on 5–20% SDS-PAGE then transferred to polyvinylidene difluoride membrane. Proteins were detected by incubating the membranes with biotinylated Abs against FLAG (Upstate Biotechnology), followed by HRP-conjugated streptavidin, and visualized by SuperSignal West Dura Extended Duration Substrate (Pierce).

Reverse Ab-dependent cellular cytotoxicity assays

These were performed as described by others (18, 19). Briefly, RNAK cells transfected with wild-type Ly49B or Ly49B bearing a C-terminal HA tag were cultured in DMEM supplemented with 20% FBS and for the last day before assay also with 10^5 IU/ml IL-2. Titrated doses of these cells were then incubated in 96-well V-bottom plates for ~15 min at room temperature with/without control or anti-Ly49B mAbs. Cr-labeled target cells (5000) were then added, and the plates spun down and incubated at 37°C for 4–5 h before collecting and counting aliquots of supernatant. The target cells were the mouse macrophage lines P388D1 and J774, and the mouse B cell lymphoma A20, which have been maintained in our laboratory for many years by routine subculture in DMEM containing 5% FBS.

Results

Development and characterization of mAbs specific for Ly49B

Candidate anti-Ly49B mAbs were tested for specificity by staining cells transfected with each of the Ly49 molecules known to be expressed in C57 mice. Several mAbs specific for Ly49B were obtained. The mAb 1A1 strongly stained cells transfected with the C57 Ly49B molecule but showed no detectable staining of cells transfected with Ly49A, C, D, E, F, G, H, I, or Q derived from C57 mice (Fig. 1). It also failed to stain cells transfected with an HA-tagged BALB/c Ly49B molecule that was efficiently stained by anti-HA Ab and with several other Ly49B-specific mAbs that we generated.

Expression of Ly49B on lymphocytes

Splenic (and also BM, data not shown) B and T cells, both fresh and following activation in vitro, showed no detectable surface expression of Ly49B, the histograms of cells stained with IA1, isotype control mAb, or medium alone (this last not shown) being almost exactly superimposable (Fig. 2, A and B). Surprisingly, adult splenic and BM NK cells also showed no detectable surface expression of Ly49B (Fig. 2A), even after culture for 7 days in IL-2 (Fig. 2C) or IL-15 (data not shown), the control and Ly49B histograms again being almost exactly superimposable. The inclusion of IL-4, IL-12, IL-18, IL-21, α-IFN, γ-IFN, or LPS in the IL-2-containing cultures of spleen cells failed to induce detectable Ly49B expression (data not shown). However, the inclusion of both IL-12 and sialoadhesin (CD169), macroscin (CD68), F4/80, and Dectin-1 (all obtained from Serotec) and appropriate isotype controls (BD Biosciences) at 10 µg/ml, followed by mouse absorbed biotinylated anti-rat Ig (Vector Laboratories). For CD11c, a biotinylated mAb (clone HL3) from BD Biosciences was used. mAb labeling was developed with the avidin-biotin complex-HRP kit and NovaRed substrate, followed by hematoxylin according to the manufacturer’s instructions (Vector Laboratories). Double immunolabeling was performed with anti-Ly49B mAb plus AlexaFluor 488 anti-rat Ig together with biotin anti-CD11c and AlexaFluor 568 strepta- vidin (Molecular Probes) or the CR-Fe reagent, a lectin specific for sulfated glycoproteins predominantly expressed by metallophilic macrophages in the spleen and subcapsular sinus macrophages in lymph nodes (17) (do- noting by Dr. L. Martinez-Pomares, University of Oxford, Oxford, U.K.) + AlexaFluor 568 anti-human Ig. Specimens were examined on a Leica TCS NT confocal system.

Monoclonal Abs, staining, and flow cytometry

mAbs against Ly49B were generated by repeatedly immunizing PVG rats with RNAK cells expressing a C57 Ly49B molecule bearing a hemagglutinin (HA) tag at its C terminus. Following a final i.v. injection, spleen cells were prepared 3 days later and fused with NS0 cells according to standard pro- tocols. Several hybridomas that secreted mAbs that stained Ly49BHA-transfected but not untransfected RNAK cells were identified and recloned thrice as described previously (10), and long-term lines of cloned immature NK cells were maintained in medium containing 3.5 ng/ml IL-2. The LNK line (11) was provided by Dr. S. Anderson (National Cancer Institute, Freder- ick, MD), Ly49-transfected YB2/0 and RNAK cells were generated as described previously (12).

To activate B cells, 2 micrograms of 1–2 million spleen cells for 5–7 days in 24-well plates in 2 ml of medium containing 2 µg/ml LPS (L3129; Sigma-Aldrich) plus 3.5 ng/ml IL-2 to activate B cells, 2 µg/ml Con A (C7275; Sigma-Aldrich) plus 3.5 ng/ml IL-2 to activate T cells, or 350 ng/ml human IL-2 with or without 100 µg/ml mouse IL-12 (provided by Dr. M. Gately, Genetics Institute, Boston, MA) were cultured with IL-4 (R&D Systems) to activate NK cells and T cells. Immature NK cells were generated from day 14 fetal thymocytes as previously described (10), and long-term lines of cloned immature NK cells were maintained in medium containing 3.5 ng/ml IL-2. The LNK line (11) was provided by Dr. S. Anderson (National Cancer Institute, Freder- ick, MD). Ly49-transfected YB2/0 and RNAK cells were generated as de- scribed previously (12).

Polymerase chain reaction

RT-PCR was performed as previously described (12) using primers that amplified the complete coding sequence of Ly49B (primer set 1, fwd = atatcagacaagctagcctagtaggtatccagtgtagctagtgagctagttg, rev = atatcagacaagctagcctagtagttgacccctgagctagttgatcattttttt). The Ly49B transcript (primer set 2, fwd = cccctgagccatgtttttttt, rev = cccctgaggttttgggattttttt), the complete coding sequence of Ly49Q (fwd = atatcagacaagctagcctagtagttgacccctgagctagttgatcattttttt, rev = atatactacctttttt), or part of β-actin (fwd = atatactacctttttt, rev = atatactacctttttt) was amplified by PCR. The Ly49B full-length primers are described previously (12).

Morphological and histological analysis

Cytosin preparations of sorted cell populations were air-dried, fixed, and stained using Diff-Quik modified Giemsa reagent (Baxter) and mounted in DPX. For histological studies, frozen sections (5 µm) were fixed for 5 min at 4°C in ethanol, then incubated with anti-Ly49B mAb or with mAbs to

(provided by Dr. J. H. Robinson, Newcastle University, Newcastle, U.K.) used as a source of M-CSF. BM dendritic cells (DC) were obtained by cultivating aliquots of 1–2 million cells in 25-cm² culture flasks with 5 ml of medium containing 10 ng/ml mouse GM-CSF (PeproTech) or aliquots of 5 million cells with 200 ng/ml mouse flt3 ligand (FL; PeproTech). In all cases, cultures were refed after 4–5 days, and both nonadherent and ad- herent cells were collected, the latter with the aid of a Ca²⁺ and Mg²⁺-free PBS containing 0.5 mM EDTA. The cells were then washed and aliquots of 0.5–1 million cells cultured for 1–2 days in low adherence 24-well plates (Corning) in 1 ml of medium containing LPS at 1 µg/ml, γ-IFN (Pepro- Tech) at 300 ng/ml, or rIFNαa/β (provided by Dr. M. Brunda, National Institutes of Health, Bethesda, MD) at 10⁴ U/ml. Cultures of macrophages and GM-CSF-derived DC were also supplemented with 5% L929 supernatant or 10 ng/ml GM-CSF, respectively. Nonadherent and adherent cells were harvested as above.

Cultured lymphocyte populations were generated by incubating aliquots of 1–2 million spleen cells for 5–7 days in 24-well plates in 2 ml of medium containing 2 µg/ml LPS (L3129; Sigma-Aldrich) plus 3.5 ng/ml IL-2 to activate B cells, 2 µg/ml Con A (C7275; Sigma-Aldrich) plus 3.5 ng/ml IL-2 to activate T cells, or 350 ng/ml human IL-2 with or without 100 µg/ml mouse IL-12 (provided by Dr. M. Gately, Genetics Institute, Boston, MA) to expand NK cells and T cells. Immature NK cells were generated from day 14 fetal thymocytes as previously described (10), and long-term lines of cloned immature NK cells were maintained in medium containing 3.5 ng/ml IL-2. The LNK line (11) was provided by Dr. S. Anderson (National Cancer Institute, Freder- ick, MD), Ly49-transfected YB2/0 and RNAK cells were generated as de- scribed previously (12).

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IL-18 caused NK cells but not “NKT” or T cells to express low but consistently detectable levels of Ly49B (Fig. 2D). Low but clear levels of Ly49B expression were also found on the immature NK cell line I2/22 derived from fetal thymic progenitors (Fig. 2E).

To investigate further the expression of Ly49B in lymphocytes, RT-PCR analysis was performed on RNA extracted from highly purified cells. In the first approach, spleen cells were stained with Abs to NK1.1, CD3, F4/80, and Gr1 and NK1.1+/CD3−/F4/80−/Gr1− cells purified by cell sorting. Following 5 days of culture in IL-2, NK1.1+/CD3−/F4/80−/Gr1− cells were purified again by a second round of cell sorting and returned to culture for an additional 7 days. RNA was prepared from these cells, >99.5% of which expressed NK1.1 and which also lacked any detectable CD3+, F4/80+, or Gr1+ cells. RT-PCR analysis was performed using two sets of Ly49B primers: primer set 1 whose primer binding sites overlapped the translation start and stop sites and which therefore amplified the entire coding sequence, and primer set 2 whose target sites lay in exons 1 and 3. Using RNA prepared from highly purified adult NK cells, the full-length primer set 1 failed to yield detectable product, whereas the 5′ primer set 2 consistently yielded a product of the expected size (Fig. 2F). This was indeed a specific Ly49B product as confirmed by sequencing. Throughout our studies, the 5′ primer set was consistently a more sensitive indicator of the presence of Ly49B transcripts presumably due to the greater innate efficiency of this primer pair and/or the greater abundance of Ly49B transcripts bearing 5′ exons caused by alternate splicing or degradation.

Next, we took advantage of the fact that day-14 fetal thymocytes are a rich source of NK progenitors (20). When cultured with IL-4 plus PMA, these progenitors proliferate rapidly with little differentiation, but when subsequently transferred to medium containing IL-15, most cells acquire NK1.1 within 1 day, and after 10 days all cells express NK1.1 and many express CD94/NKG2 receptors and Ly49E (20–22). At no stage of the differentiation of these essentially pure progenitor cells could we detect Ly49B transcripts using Ly49B primer set 1 (Fig. 2F). However, as in the case of adult NK cells, products of the expected size were reproducibly detected throughout the differentiation of these immature NK cells using primer set 2 (Fig. 2F). Because of the high level of proliferation that occurs in these cultures and because no feeder cells were present, it is extremely unlikely that these expanding NK cells contained significant numbers of non-NK cells. The possibility that the Ly49B transcripts originated from minute numbers of contaminating non-NK cells was formally ruled out by the finding that RNA prepared from cloned immature NK cell lines such as I2/22 and 1608b, and from the long-term BALB/c fetal liver NK cell line LNK, gave not only strong signals with primer set 2 but also, for
two of these three cells, clear signals with primer set 1. Surprisingly, these fetal NK cell lines also gave strong signals with primers that amplified the entire coding sequence of Ly49Q, and a weak but clear Ly49Q signal was reproducibly obtained with RNA from purified adult splenic NK cells (Fig. 2F). Sequencing of these various products again confirmed that they were derived from Ly49B and Ly49Q transcripts. The PCR data are consistent with the surface expression of Ly49B on I2/22 (Fig. 2E) but not 1608b (data not shown). Curiously, none of the three NK lines expressed detectable levels of Ly49Q at the cell surface (data not shown).

Finally, pure CD4 and CD8 T cells were prepared by first culturing spleen cells, depleted respectively of CD8^+/H11001 and CD4^-/H11001 cells, for 3 days with Con A plus IL-2. Nonadherent cells were then sorted for CD4^-/H11001CD11b^-/H11002Gr1^-/H11002 or CD8^-/H11001F4/80^-/H11002Gr1^-/H11002 cells, respectively, and cultured for an additional 3 days in IL-2. The resulting nonadherent cells comprised >99% CD4^- or CD8^- cells with no detectable CD11b^-, F4/80^-, or Gr1^- cells. RNA prepared from the pure CD4 T cells gave a weak signal with primer set 2 but no detectable signal with primer set 1, whereas RNA prepared from CD8 T cells gave a clear signal with Ly49B primer set 2 and occasionally a detectable signal with Ly49B primer set 1 (Fig. 2F), although this latter was not always observed (Fig. 3). The nature of these PCR products was again directly confirmed by sequencing. RT-PCR analysis also indicated that CD4 and CD8 cells may express low levels of Ly49Q transcripts, and the thymic T cell lymphoma line SL8 appeared to express high levels of Ly49Q transcripts, although this last finding was not verified by sequencing. It should be noted that the input levels of RNA in terms of cell equivalents were four times higher for CD4 and CD8 T cells than for the other cells analyzed in Fig. 2F and that at lower input levels the PCR signals were more difficult to detect.

To gain a clearer impression of the relative expression of Ly49B transcripts in various cells, semiquantitative PCR analysis was performed with cDNA prepared from highly purified cell populations obtained by cell sorting as described above. As shown in Fig. 3, when NK cells were cultured in IL-12 plus IL-18, there was a substantive increase in Ly49B transcripts, which is consistent with their acquisition of detectable levels of surface Ly49B (Fig. 1). The level of transcripts expressed by NK cells following exposure to IL-12 plus IL-18 was comparable to that found in cultured splenic macrophages (see below), in both cases transcripts being detectable with the Ly49B full-length primers down to 150 cell equivalents of RNA.

![FIGURE 3](http://www.immunology.org)

**FIGURE 3.** Semiquantitative analysis of Ly49B transcripts in various cells. cDNA prepared from highly purified adult NK cells that had been cultured for 6 days in IL-2 either with IL-12 plus IL-18 (NK + IL-12/IL-18) or without (NK - IL-12/IL-18), highly purified CD8 T cells cultured with Con A plus IL-2, and splenic macrophages cultured in M-CSF for 7 days (Spleen mph) were titrated in PCR with various primers, with each PCR containing cDNA equivalent to 5,000, 1,500, 500, 150, or 50 cells, except for CD8 T cells where the corresponding figures were 25,000, 8,000, 2,500, 800, or 250 cells. The results shown are representative of those obtained in five experiments.

![FIGURE 4](http://www.immunology.org)

**FIGURE 4.** Expression of Ly49B on fresh spleen and BM cells. A, Upper plots, The costaining of spleen and BM cells with anti-CD45 mAb and anti-Ly49B mAb or isotype control. Center plots, The forward scatter (FSC) and side scatter (SSC) of ungated (total) cells and Ly49B-gated cells. The R1-R2 regions delineate SSC^int and SSC^high BM populations. Lower plots, The expression of CD11b and Gr1 on ungated (total) cells and Ly49B-gated cells. B, Upper plots, The CD11b and Gr1 staining of whole spleen cells, BM cells having intermediate levels of side scatter (SSC^int), and BM cells having high levels of side scatter (SSC^high). Lower plots, The staining of the CD11b/Gr1 subpopulations defined by gates R1-R4 with anti-Ly49B (solid) or isotype control IgG1 (dotted) mAbs. The results shown are representative of more than three experiments of each type.
equivalents per reaction. By contrast, NK cells cultured in the absence of IL-12 plus IL-18 gave only weak signals with these primers at 5,000 cell equivalents per reaction, and CD8 T cells in these experiments gave no detectable signal even with 25,000 cell equivalents per reaction. As noted above, NK cells cultured without IL-12 plus IL-18 expressed detectable full-length Ly49Q transcripts, although the level of these was markedly lower than in splenic macrophages. Remarkably, whereas the level of Ly49Q transcripts increased when NK cells were exposed to IL-12 plus IL-18, the level of Ly49Q transcripts declined. The level of Ly49G transcripts was unaffected.

The nature of the full-length Ly49Q transcripts obtained from NK cells cultured with IL-12/IL-18 was confirmed by sequencing. Remarkably, this revealed the existence of transcripts in NK cells that contained a previously unknown additional exon of 99 nt located 6.3 kb downstream of exon 6 and 1.6 kb upstream of exon 7 (GenBank accession no. EF025059). Transcripts containing this sequence if translated would generate an Ly49Q isoform having an extra 33 aa in the C-type lectin domain. No exon homologous to this has been reported in any other Ly49 cDNA sequence. It will be important to determine whether such transcripts do indeed give rise to expressed proteins and whether these are selectively expressed in NK cells.

Expression of Ly49B on myeloid cells

Although freshly prepared B, T, and NK cells lacked detectable surface expression of Ly49B, ∼1.5% of CD45+ C57BL/6 spleen cells (above a background of 0.7% autofluorescent cells) showed low but clear staining with the 1A1 mAb (Fig. 4A). Most of these Ly49B+ spleen cells had relatively high levels of side light scatter (SSC) and comprised three distinct subpopulations, all of which expressed high levels of CD11b together with low, intermediate, or high levels of Gr1, representing ∼0.3, 0.6, and 0.8% of all spleen cells, respectively (a fourth population of cells that appear to be CD11blowGr1high constitute autofluorescent cells that are inadvertently captured in the Ly49B gate). Conversely, most if not all cells in each of these three CD11b/Gr1-defined spleen cell populations expressed Ly49B (Fig. 4B). Using the additional myeloid-specific cell markers F4/80, Dectin-1, and 7/4 (15, 23–29), it was found that the Gr1high cells expressed little or no F4/80 but low levels of Dectin-1 and high levels of the 7/4 Ag, whereas the Gr1int and Gr1low cells mostly expressed high levels of F4/80 and Dectin-1 and low levels of 7/4.

A much higher proportion of C57BL/6 BM cells, typically ∼10% of CD45+ cells, expressed low levels of Ly49B (Fig. 4A). Ly49B-expressing BM cells were concentrated amongst those with intermediate (SSCint) or high (SSCint) levels of light scatter and were present in multiple subpopulations of CD11b/Gr1-defined cells. Among SSCint cells, Ly49B was most prominently expressed on a small subpopulation of CD11b+Gr1low cells that represented 0.7% of BM cells (gate R2, upper middle panel of Fig. 4B) and which expressed F4/80, Dectin-1, and generally low levels of 7/4 (data not shown). Ly49B was also expressed at low levels on a second subpopulation of SSCintCD11b+ cells that expressed intermediate levels of Gr1 together with mostly high levels of F4/80, Dectin-1, and 7/4 (data not shown) and represented ∼2% of BM cells (gate R3, upper middle panel of Fig. 4B). However, it was notably absent from a third SSChighCD11b+ population that was Gr11high (gate R4, upper middle panel of Fig. 4B). These cells were F4/80low Dectin-1+ 7/4+ and uniformly expressed CD117 (data not shown), suggesting that they were immature myeloid cells. By contrast, among SSChigh BM cells Ly49B expression was confined to the major CD11b+Gr11high subpopulation that represents 15–20% of all BM cells (gate R3, upper right panel of Fig. 4B). These cells expressed low levels of Dectin-1 and F4/80 and intermediate levels of 7/4 (data not shown); however, only ∼50% of the cells in this population expressed Ly49B. Ly49B was more consistently expressed on the subpopulation of these cells that were CD11b1high (gate R4, upper right panel of Fig. 4B). Collectively, these results demonstrate that in spleen and BM Ly49B is not expressed at detectable levels on any lymphocyte population but instead is found on multiple subpopulations of CD11b+ cells that coexpress a range of markers characteristic of monocytes, macrophages, and granulocytes.

Ly49B-expressing cells were readily detected in the peritoneum. In untreated C57BL/6 mice, ∼30% of peritoneal cells were Ly49B+, most of which were CD11b1high Gr11low, and F4/801high cells (Fig. 5A) that lacked 7/4, expressed Dectin-1, and displayed high forward light scatter and SSC (data not shown), a phenotype characteristic of macrophages. Within 3 h of injecting thioglycollate, a new population of Ly49B+ cells appeared that expressed low levels of Ly49B and was Gr11highF4/801low (Fig. 5B) with high levels of 7/4 and low or no Dectin-1, a phenotype characteristic of neutrophils. Over the next 24 h, the size of this Ly49Blow population increased while the F4/801highLy49B+ population virtually disappeared to be replaced by a third population of Ly49B+ cells that expressed low levels of Ly49B and Gr1, intermediate levels of F4/80 (Fig. 5C), and both 7/4 and Dectin-1 (data not shown).

A Untreated

B Thioglycollate 3h

C Thioglycollate 24h

D Untreated

E Thio 16h

FIGURE 5. Expression of Ly49B on peritoneal cells. Peritoneal cells from untreated mice or from mice injected i.p. with thioglycollate 3, 16, or 24 h earlier were costained with mAbs to Ly49B and CD11b, CD117, CCR3, Gr1, and F4/80. The data shown in A–C are representative of results from six experiments; those in D and E from two experiments.
shown). These cells have been identified by others as infiltrating monocytes (28, 30). A fourth population of peritoneal cells that expressed Ly49B was eosinophils identified by their characteristic FSC<sub>low</sub>SSC<sub>high</sub>Gr1<sup>−</sup>/H<sup>11002</sup>phenotype and by high expression of CCR3 (31). In normal animals, most such cells were Ly49B<sup>+</sup> (Fig. 5<sup>D</sup>), but in thioglycollate-treated animals, only a minority were (Fig. 5<sup>E</sup>). CD11<sup>+</sup><sup>high</sup> peritoneal mast cells were Ly49B<sup>−</sup> (Fig. 5<sup>D</sup>), as were CD11<sup>+</sup><sup>+</sup> mast cells generated by culturing BM cells with IL-3 (data not shown). The eosinophilic and mast cell nature of these populations was confirmed by cell sorting and cystospin preparations (see below).

In all of the above experiments, staining was done in the presence of an excess of blocking Ab against the CD16 and CD32 FcR, and in addition, isotype control Abs gave no detectable staining, making it extremely unlikely that the staining observed by the 1A1 anti-Ly49B mAb was nonspecific. However, to formally rule out this possibility, we took advantage of the fact that the 1A1 mAb lacks detectable reactivity against the BALB/c isoform of Ly49B (Fig. 1). As shown in Fig. 6<sup>A</sup>, whereas 1A1 stained a substantial proportion of cells in the R3 and R4 regions of SSC<sub>high</sub>CD11b<sup>−</sup>Gr1<sup>+</sup> BM cells from C57 mice, the corresponding populations from BALB/c mice were unstained, the histograms for 1A1 staining being completely superimposable on those for the isotype control mAb (Fig. 6<sup>A</sup>) and for the medium control (data not shown). Similarly, whereas 1A1 stained essentially all high scatter peritoneal cells of C57 mice, it showed no detectable staining of the corresponding cells of BALB/c mice (Fig. 6<sup>B</sup>).

**FIGURE 6.** Comparative staining of cell populations from C57 and BALB/c mice by mAb 1A1. Spleen and peritoneal cells from C57 and BALB/c mice were stained in parallel: the histograms on the right show the staining of specific subpopulations, defined in the dot plots on the left, with anti-Ly49B (solid) or isotype control IgG1 (dotted) mAbs (for further details of staining see figure legends to Figs. 4 and 5).

**FIGURE 7.** Expression of Ly49B and Q on spleen and BM cells. A and B, Spleen cells and BM cells were stained with mAbs to Ly49B and Ly49Q or with both mAbs together. Lower plots, The scatter characteristics of the total population (left), Ly49B<sup>+</sup>Q<sup>+</sup> cells (center), and Ly49B<sup>−</sup>Q<sup>−</sup> cells (right). C, BM cells were costained with mAbs for Ly49B or Ly49Q together with mAbs for B220 and CD11c or CD4 and CD8. Plots show the expression of the latter four molecules on the three SSC- and Gr1-defined subpopulations of Ly49B<sup>−</sup> cells and on Ly49Q<sup>−</sup> cells. D, BM cells were costained with mAbs for Ly49B or Ly49Q together with mAbs for Gr1 and CD11c. Histograms show the staining of the CD11c/Gr1 populations defined by the R1 gate with anti-Ly49B or Ly49Q (solid line) and isotype control (dotted line) mAbs. All data shown are representative of results obtained in two to three experiments.
Ly49B IS EXPRESSED ON MYELOID CELLS

The finding that Ly49B was expressed on myeloid cells suggested that its pattern of expression might be similar to that of Ly49Q, which was recently reported to be expressed at high levels on DC and at lower levels on some other myeloid populations (13, 16, 32). However, as shown in Fig. 7A, containing spleen cells with mAbs to Ly49B and Ly49Q revealed that most of the cells that stained strongly for Ly49Q lacked detectable Ly49B, whereas most of the cells that expressed low levels of Ly49Q appeared to be Ly49B⁺. Conversely, the cells that expressed Ly49B showed at most only weak staining for Ly49Q. Similarly, in the BM, those cells that stained clearly for Ly49Q lacked detectable Ly49B whereas those that stained clearly for Ly49B expressed little or no detectable Ly49Q (Fig. 7B).

The largely distinct nature of most Ly49B⁻ and Ly49Q⁺ cells was confirmed in several ways. First, in both spleen and BM, Ly49B⁺ Q⁻ cells and Ly49B⁻ Q⁺ cells had different light scatter characteristics (Fig. 7, A and B). Second, whereas most Ly49Q⁺ cells in the BM expressed CD11c and B220, most Ly49B⁻ cells lacked these markers, although ~30% of the SSC™Ly49B⁻ cells were CD11c⁺ (Fig. 7C). Third, most BM Ly49Q⁺ cells expressed CD4 and many also expressed CD8, but very few Ly49B⁻ cells expressed CD4 or CD8 (Fig. 7C). Finally, direct gating on CD11c⁺ cells in BM showed that among those that were Gr1⁺, very few expressed Ly49B but most expressed Ly49Q; conversely, most of the CD11c⁺ cells that expressed low levels of Gr1 expressed Ly49B but little or no Ly49Q (Fig. 7D). In the spleen, Ly49B was rarely expressed on CD11c⁺ cells, whereas CD11c⁻ cells frequently expressed Ly49B (data not shown).

Expression and regulation of Ly49B and Q on cultured cells

To examine in more detail, the expression of Ly49B on myeloid cells, highly enriched populations of such cells were obtained by culture with appropriate cytokines. As shown in Fig. 8A, splenic macrophages derived by culture with M-CSF constitutively expressed substantial levels of both Ly49B and Ly49Q. By contrast, although BM macrophages derived by culture in M-CSF expressed Ly49Q, they lacked detectable expression of Ly49B. Exposure of these cells to LPS, γ-IFN, or α-IFN, however, induced expression of Ly49B but left Ly49Q expression largely unchanged. DC derived from BM cells by culture in GM-CSF or FL displayed a broad and largely monotypic expression of Ly49Q but expressed Ly49B on a distinct subpopulation of cells. The expression of Ly49B and Ly49Q on these cells was differentially affected by LPS and IFN. Thus, both γ-IFN and α-IFN markedly increased the expression of Ly49B, especially on the Ly49B-expressing subpopulation of FL-derived cells where it reached levels higher than on any other cell type studied. However, whereas α-IFN increased the expression of Ly49Q on GM and FL DC, γ-IFN generally did not. Similarly, although LPS increased the expression of Ly49B on FL-derived DC, it reduced the expression of Ly49B on GM-derived DC and generally reduced the expression of Ly49Q on both cells.

Comparative expression of Ly49B and Ly49Q

The data are representative of four to nine experiments with each cell type. For all PCRs, controls with no template were run and invariably gave no detectable product. C, GM DC were stained with mAbs to Ly49B or Ly49Q together with mAbs to B220, cII, CD11c, and F4/80. Fluorescence data are plotted over three decades (fluorescence values 1–1000), except for F4/80, which is plotted over four decades (fluorescence values 1–100,000). The region labeled A delineates a population of highly autofluorescent GM DC that lack B220 and cII. The results shown are representative of two experiments. D, Similar analysis of FL DC. Data are representative of five experiments.

FIGURE 8. Expression and regulation of Ly49B and Q on macrophages and DC. A, Macrophages (mph) derived by culturing spleen or BM cells in M-CSF and DC derived by culturing BM cells in either GM-CSF (GM DC) or FL (FL DC) for 5–7 days were cultured for an additional 2 days in medium containing LPS, γ-IFN, or α-IFN. They were then stained with the following reagents: isotype control (left dotted histogram), anti-Ly49B (solid histogram), and anti-Ly49Q (right dotted histogram). For clarity, only the isotype control for the Ly49B mAb is shown (the histogram for the Ly49Q isotype control was invariably in a similar position or further to the left). The numbers shown in each plot are the net median fluorescence values for Ly49B (upper numbers) and Ly49Q (lower numbers) calculated by subtracting the median fluorescence for isotype control from the median fluorescence for specific mAb. Median values are for the entire population except in the case of Ly49B expression on DC where they are for the subpopulation that expresses Ly49B. The data for spleen mph, BM mph, GM DC, and FL DC are representative of two, nine, nine, and six experiments, respectively. B, RT-PCR analysis. mph derived by culturing spleen or BM cells in M-CSF for 7 days, and J774 and RAW264 cells, were cultured for 1 day with Med, LPS, or γ-IFN, then RNA extracted. PCR was performed using the same primer sets as in Fig. 2. Each PCR contained cDNA equivalent to 2000 cells. The data are representative of four to nine experiments with each cell type. For all PCRs, controls with no template were run and invariably gave no detectable product. C, GM DC were stained with mAbs to Ly49B or Ly49Q together with mAbs to B220, cII, CD11c, and F4/80. Fluorescence data are plotted over three decades (fluorescence values 1–1000), except for F4/80, which is plotted over four decades (fluorescence values 1–10,000). The region labeled A delineates a population of highly autofluorescent GM DC that lack B220 and cII. The results shown are representative of two experiments. D, Similar analysis of FL DC. Data are representative of five experiments.
Analysis at the RNA level confirmed and extended these findings (Fig. 8B). Thus, cultured splenic macrophages clearly contained transcripts for Ly49B and Ly49Q. Unstimulated BM macrophages, despite their lack of detectable surface expression of Ly49B, also contained full-length Ly49B transcripts, most easily detected using the 5′ primer set. The level of full-length transcripts increased markedly following stimulation with LPS or γ-IFN. The macrophage lines J774, RAW264, and P388D1, all of BALB/c origin, were also examined. None of these cells showed detectable surface staining with the 1A1 anti-Ly49B mAb (data not shown) but, as exemplified by the results for J774 and RAW264 shown in Fig. 8B, contained full-length Ly49B transcripts (again most readily detected with the 5′ primers) whose levels sometimes increased and sometimes decreased in response to LPS or γ-IFN. Ly49Q transcripts were also present in these cells.

To better understand the diverse patterns of expression of Ly49B and Q on cultured BM-derived cells, costaining was performed with a range of macrophage and DC markers. Cultured BM macrophages uniformly expressed CD11b, Gr1, F4/80, and Dec-205, with little or no 7/4, and no detectable B220, MHC class II (cII), or CD11c (data not shown). GM-CSF DC also lacked expression of B220 but showed heterogeneous expression of cII, CD11c, and F4/80 (Fig. 8C). Despite the complexity of GM-CSF-derived cells, Ly49B and Ly49Q were expressed in an essentially identical manner, both molecules being largely absent from cII+ cells but present on the majority of CD11c+ and F4/80+ cells. Indeed, the expression of Ly49B and Ly49Q on GM-CSF-derived DC was essentially confined to cells that were B220−, cII+, CD11c+, and F4/80+. By contrast, FL-derived DC differed in several ways: they were smaller, a higher proportion expressed CD11c and cII (Fig. 8D), a lower proportion expressed Gr1 (data not shown), and many expressed B220 (Fig. 8D). Remarkably, whereas most Ly49B+ FL DC resided within a B220−cII+CD11c+F4/80+ population, most Ly49Q+ cells resided within a B220−cII+CD11c+F4/80− population, and a substantial minority resided in a second subpopulation that expressed high levels of B220.

Taken together, the data presented in this section demonstrate that Ly49B and Q are expressed on multiple subpopulations of in vitro-derived myeloid cells, that these two molecules can be expressed independently of each other, and that they are subject to differential regulation by bacterially derived and endogenous immunoregulators.

**Morphological and histological identification of Ly49B-expressing cells**

Fig. 9A shows the typical morphology of cells present in various cell populations that expressed or lacked Ly49B. More than 80% of splenic CD11b+Gr1high cells that uniformly expressed Ly49B had strikingly segmented nuclei with pale featureless cytoplasm, many with the classical morphology of neutrophils (Fig. 9A-a). By contrast, ~70% of splenic CD11b+Gr1low/intLy49B+ cells were macrophage-like cells with generally oval or indented nuclei and substantial vacuolar cytoplasm (Fig. 9A-b). Approximately 15% were a smaller version of this with less cytoplasm (most likely monocytes), whereas an additional ~15% had the highly segmented nuclei and pink cytoplasmic granules characteristic of eosinophils (lower right cell in Fig. 9A-b). Cells in the SSChigh CD11b+Gr1high population of BM cells, ~50% of which expressed Ly49B, had a variable neutrophilic appearance (Fig. 9A-c), most having less heavily segmented nuclei than the subpopulation of these cells that expressed the highest level of CD11b (gate R4 upper right panel in Fig. 3), which had a similar mature neutrophilic appearance to the corresponding population in spleen (Fig. 9A-a). By contrast, cells in the SSCintCD11b+Gr1high population that expressed CD117 but lacked Ly49B were noticeably smaller and frequently had nonsegmented nuclei with a central hole (Fig. 9A-d). These findings indicate that as granulocytes mature, increasing in size and granularity, losing CD117, and gaining increasingly fragmented nuclei they also gain expression of Ly49B. By contrast, the SSChighCD11b+Gr1low/int BM cells that expressed Ly49B had a monocyte/macrophage morphology (Fig. 9A-e). The CD11b+Gr1 F4/80+Ly49B+ cells in normal peritoneal washings were large heavily vacuolated macrophages (Fig. 9A-f). The CD11b+Ly49B− population of normal peritoneal cells also included macrophage-like cells, but these were generally smaller, less vacuolated, and with less contorted nuclei (Fig. 9A-g). Approximately 90% of the Gr1highLy49Blow cells from the peritoneum of thioglycollate-treated mice were typical neutrophils (Fig. 9A-h), whereas ~90% of the Gr1highLy49B+ cells had a monocyte/macrophage appearance (Fig. 9A-i). By contrast, the SSChighFS-C5highGr1low/int peritoneal cell population comprised predominantly eosinophils (Fig. 9A-j).

Histological analysis revealed that in spleen and lymph node, Ly49B-expressing cells were most abundant in the red pulp and medulla, respectively, and were only occasionally found in the white pulp and paracortex (Fig. 9, B and C). At higher magnifications Ly49B− cells were seen to vary in size, intensity of labeling, and nuclear morphology, consistent with Ly49B being expressed on multiple cell populations. The majority of Ly49B− cells was macrophage-like, although cells with a polymorphonuclear appearance were also labeled (inset arrow). Ly49B+ cells were found scattered in thymus cortex and medulla but in dual-labeling experiments (data not shown) did not colocalize with CD11c, suggesting they were not DCs. Ly49B+ cells were prominent in the lamina propria of gut villi and also within the dome region of the Peyer’s patch where they were identified as the autologofluorescing macrophages in this region and not the CD11c+ DCs (data not shown). Ly49B was also clearly expressed on at least some alveolar macrophages in the lung and Kupffer cells in the liver.

Remarkably, the distribution of Ly49B labeling in lymphoid tissue was different from that seen using a series of other myeloid cell markers (Fig. 9C). In the splenic red pulp, the Ly49B− cells appeared to represent a subpopulation of the more numerous F4/80+ macrophages, whereas within the marginal zone and T and B cell areas, a few Ly49B+ cells but no F4/80+ cells could be seen. The distribution of Ly49B was more similar to that of Dectin-1 (27), which is also expressed on a subpopulation of red pulp macrophages, but Dectin-1+ cells were more numerous within the marginal zone and T cell areas due to the expression of Dectin-1 on a proportion of DC and macrophages at these sites. Ly49B labeling was similar to that seen with macrosialin (CD68), a molecule generally considered to be a pan-macrophage-specific marker (29, 33), but again, fewer cells in the white pulp expressed Ly49B than macrosialin. The pattern of Ly49B labeling was very different to that of sialoadhesin, a member of the siglec family expressed on specialized macrophages within the spleen marginal zone and lymph node subcapsular sinus (34) (Fig. 9C). It was also different to that seen with anti-CD11c, cells staining strongly for CD11c being concentrated in the marginal zone and around the central arterioles where Ly49B− cells were rare.

In lymph nodes (Fig. 9C), the anti-Ly49B mAb labeled intensely large cells within the medullary region, which appeared to represent a subpopulation of both F4/80+ cells and macrosialin+ cells. Unlike F4/80, however, some Ly49B+ cells encroached upon the paracortex and some were present in the B cell follicles, perhaps representing the tangible body macrophages that are also
positive for macrosialin and Dectin-1. In lymph node, the patterns of Ly49B and Dectin-1 expression were more distinct than in the spleen. First, the expression of Ly49B on the medullary macrophages appeared greater than Dectin-1, and second, Dectin-1 was clearly expressed within the paracortex among the T cells and also within the B cell follicle where Ly49B expression was rare. As in the spleen, Ly49B expression in lymph node appeared very different to that of sialoadhesin, which is largely restricted to the subcapsular sinus macrophages and a few cells in the follicles, and CD11c, which is prominently expressed throughout the paracortex with only occasional weak staining elsewhere. CR-Fc, a chimeric protein containing the cysteine-rich domain of the mannose receptor that binds to sialoadhesin and other molecules on marginal zone metallophilic macrophages and follicular macrophages (17, 35), was used in two-color staining with mAb 1A1. These experiments demonstrated that in the marginal zone of the spleen and the subcapsular sinus of the lymph node Ly49B and CR-Fc staining identified largely distinct subpopulations of cells and that cells expressing high levels of Ly49B and CD11c were largely distinct from each other in spleen, lymph node, and gut (Fig. 9D).

**Functional analysis of Ly49B**

To examine the association of Ly49B with intracellular signaling molecules, RNK cells were transfected with a Ly49B construct bearing a C-terminal FLAG tag, and lysates from these cells were precipitated with anti-FLAG mAb. Western blots with Abs to SHP-1, SHP-2, and SHIP-1 revealed that each of these phosphatases was associated with Ly49B (Fig. 10A). In the case of SHP-1 and SHP-2, this association was only seen when cells were incubated with pervanadate before immunoprecipitation, implying that the association of SHP-1 and SHP-2 with Ly49B was dependent on tyrosine phosphorylation of substrate molecules. By contrast, SHIP-1 was strongly associated with Ly49B in untreated cells, and this association was lost following incubation with pervanadate.

To examine the possible functional consequences of these associations, cloned lines of RNK cells transfected with native...
Ly49B or Ly49B bearing a C-terminal HA tag were examined for their ability to kill FcR-bearing cells in the presence or absence of appropriate Abs. An example of the data obtained is shown in Fig. 10B. RNK cells uniformly expressing Ly49B at levels similar to those shown in Fig. 1 efficiently killed P388D1 targets, but inclusion in the assays of the IA1 mAb at concentrations that gave satiation binding to Ly49B caused no significant enhancement or inhibition of killing. Similar results were obtained with the FcR-bearing targets J774 and A20, with two other anti-Ly49B mAbs of the expected sizes. B, RNK cells transfected with Ly49B having a C-terminal HA tag were preincubated with saturating levels of mAb IA1 (■) or medium (□) before the addition of Cr-labeled P388D1 cells.

Discussion

The data presented in this report have revealed for the first time that, unlike most members of the Ly49 family, Ly49B is not normally expressed at detectable levels on NK cells but instead is expressed on multiple subpopulations of myeloid cells present in both lymphoid and nonlymphoid tissues. Among the myeloid cells present in cell suspensions obtained from the lymphoid tissues of normal animals, Ly49B expression was generally quite low, requiring the use of strong fluorochromes for clear visualization. However, following culture with inflammatory cytokines or bacterial products, the expression of Ly49B frequently increased, especially in the presence of other myeloid-specific molecules, confirming and extending the growing view that myeloid cells, and macrophages in particular, exist in numerous subpopulations that presumably have distinct functions in the immune system. Heterogeneity was also apparent in flow cytometric analysis of cell suspensions, Ly49B being expressed on multiple distinct CD11b- and Gr1-defined subpopulations of cells in the spleen, BM, and peritoneum. These subpopulations were dominated by cells with the morphological characteristics of granulocytes and monocytes/macrophages. The expression of Ly49B in myeloid cells was confirmed by flow cytometric and PCR analysis of macrophage cell lines and of highly enriched myeloid populations derived by expansion of BM progenitors with myelopoietic cytokines. PCR analysis also revealed the presence of Ly49B transcripts in NK cells and T cells, particularly CD8+ T cells, suggesting that these cells may under certain circumstances express Ly49B at the cell surface. In the case of NK cells, surface expression, albeit at a low level, was found on immature NK cell lines grown in IL-2 and on mature NK cells exposed to the inflammatory cytokine combination of IL-12 and IL-18. These findings are in line with previous reports that populations containing immature NK cells expanded from BM precursors of embryonic stem cells expressed Ly49B transcripts, although the possibility that these originated from contaminating myeloid cells would now need to be considered.

Of particular interest and importance in this study was the relationship between the expression of Ly49B and Ly49Q, which was recently reported to be expressed on myeloid cells. Our studies showed that among freshly prepared spleen and BM cells Ly49B and Ly49Q were most prominently expressed on distinct cell types, Ly49B-expressing cells mostly lacking high levels of CD11c, B220, CD4, and CD8, whereas Ly49Q+ cells mostly expressed these markers, which is consistent with previous work demonstrating that Ly49Q was most strongly expressed on cells characterized as plasmacytoid DC (16, 32). Some Ly49B+ cells present in fresh cell preparations appeared to express low levels of Ly49Q, and Ly49Q was clearly coexpressed with Ly49B on various subpopulations of cultured myeloid cells. Importantly, however, Ly49B and Ly49Q expression on these cells was found to be regulated independently. Histological data showed that Ly49B was not prominently expressed, if at all, on the classical DC populations in the T cell-rich areas of splenic white pulp and lymph node paracortex. Flow cytometric analysis, however, showed that Ly49B was expressed on the CD11c-low cells present in suspensions of fresh spleen and BM cells, and also on subpopulations of CD11c+ cells expanded from BM progenitors using GM-CSF or FL, some of which also expressed B220. Such CD11c+ cells are generally termed DC, although their relationship to DC in vivo is unclear given that they lack expression of the CD4 and CD8 markers frequently found on fresh DC.

The demonstration that Ly49B is expressed primarily on myeloid cells raises important questions concerning its function. Whereas most mouse Ly49 receptors show >90% amino acid identity with each other, Ly49B displays only ~50% homology with other family members, including Ly49Q, and contains 20 additional aa at its C terminus. It also displays only ~45% homology with most members of the rat Ly49 family but shows 63% homology with rat Ly49-i8 (which also has a C-terminal extension), suggesting that these two molecules are functional orthologs and share a common and possibly unique function in murine species. Despite this, significant sequence variability exists within the mouse, the C57 (GenBank accession no. AK017140) and 129 (GenBank accession no. AF395446) strain sequences (which are identical) differing by 12 aa from the BALB/c (GenBank accession no. AF253057) and CBA (GenBank accession no. MMU10304)
sequences (also identical). All but one of these differences reside in the carbohydrate recognition domain (CRD) and collectively would explain the recognition of the C57/129 isoform but not the BALB/c isoform by the 1A1 mAb. Remarkably, one of these differences involves the cysteine at position 251 that is conserved in virtually all animal C-type lectins (38, 39). In the C57/129 isoform, this is replaced by arginine, thereby precluding the formation of a conserved disulfide bond, found in all CRD crystal structures that have been solved, with the preceding conserved cysteine (C238 in Ly49B). The only other animal C-type lectins known to lack this conserved cysteine are the mouse Clr molecules (40) and their human ortholog LLT1 (41) that are encoded in the NKRP1 region (58). Among these are Cys152, Leu166, Ser214, and Gln216 that are Tyr, Trp, Trp, and Trp, respectively, in contexts (59), and SIRPα, which through its recognition of CD47 expressed on healthy RBC and lymphocytes prevents their phagocytosis (60), and via its interaction with surfactants may prevent nonspecific inflammatory responses in the lung (61). All of these molecules belong to the Ig superfamily. However, several ITIM-bearing members of the C-type lectin family, all encoded in the NK gene complex, such as DCIR (CLECSF6) (62), MICL (63), and Ly49Q, are widely expressed in myeloid cells. It will clearly be of considerable importance for understanding the role of myeloid cells in various disease states to formally establish whether and under what circumstances Ly49B (and these other ITIM-bearing C-type lectin molecules) inhibits myeloid cell function. Initial attempts to reveal inhibitory activity using reverse Ab-dependent cellular cytotoxicity assays were unsuccessful, but negative results in such assays have been reported previously with receptors of known inhibitory capability (see Discussion in Ref. 64), and alternative approaches will need to be explored. Additional priorities for future work include the determination of the three-dimensional structure of Ly49B and identification of its ligands.

Note added in proof. The Ly49B transcript sequence containing a novel additional exon has been deposited in GenBank (accession no. EF025059).

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Disclosures

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