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Biochemical Nature and Cellular Distribution of the Paired Immunoglobulin-like Receptors, PIR-A and PIR-B

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Summary

PIR-A and PIR-B, paired immunoglobulin-like receptors encoded, respectively, by multiple Pira genes and a single Pirb gene in mice, are relatives of the human natural killer (NK) and Fc receptors. Monoclonal and polyclonal antibodies produced against a recombinant PIR protein identified cell surface glycoproteins of ~85 and ~120 kD on B cells, granulocytes, and macrophages. A disulfide-linked homodimer associated with the cell surface PIR molecules was identified as the Fc receptor common γ (FcRγc) chain. Whereas PIR-B fibroblast transfectants expressed cell surface molecules of ~120 kD, PIR-A transfectants expressed the ~85-kD molecules exclusively intracellularly; PIR-A and FcRγc cotransfectants expressed the PIR-A/FcRγc complex on their cell surface. Correspondingly, PIR-B was normally expressed on the cell surface of splenocytes from FcRγc−/− mice whereas PIR-A was not. Cell surface levels of PIR molecules on myeloid and B lineage cells increased with cellular differentiation and activation. Dendritic cells, monocytes/macrophages, and mast cells expressed the PIR molecules in varying levels, but T cells and NK cells did not. These experiments define the coordinate cellular expression of PIR-B, an inhibitory receptor, and PIR-A, an activating receptor; demonstrate the requirement of FcRγc chain association for cell surface PIR-A expression; and suggest that the level of FcRγc chain expression could differentially affect the PIR-A/PIR-B equilibrium in different cell lineages.

Key words: Fc receptor γ chain • activating receptor • inhibitory receptor • dendritic cells • innate immunity

The paired immunoglobulin-like receptors (PIR)1-A and -B have been identified recently in mice on the basis of their homology with the human Fcα receptor (FcαR) (1, 2). PIR-A and PIR-B share sequence similarity with a gene family that includes human FcαR and killer inhibitory receptors (KIR), mouse gp49, bovine Fc receptor for IgG (FcγR), and the recently identified human Ig-like transcritps (ILT)/leukocyte Ig-like receptors (LIR)/ monocYTE/macrophage Ig-like receptors (MIR) (3–12). The Pira and Pirb genes are located on mouse chromosome 7 in a region syntenic with the human chromosome 19q13 region that contains the FcαR, KIR, and ILT/LIR/MIR genes (1, 4, 5, 9, 11–14). DNA sequences for PIR-A and PIR-B predict type I transmembrane proteins with similar ectodomains (>92% homology) each containing six Ig-like domains. However, PIR-A and PIR-B have distinctive membrane proximal, transmembrane, and cytoplasmic regions. The PIR-B protein, encoded by the Pirb gene (1, 13, 15), has a typical uncharged transmembrane region and a long cytoplasmic tail with multiple candidate immunoreceptor tyrosine-based inhibitory motifs (ITIMs). Recent studies have demonstrated the inhibitory function of the two most membrane-distal ITIM units in the PIR-B cyto-

H. Kubagawa and C.-C. Chen contributed equally to this work.

1Abbreviations used in this paper: CY, cytochrome; EC, extracellular domain; FcαR, Fc receptor for IgA; FcγR, Fc receptor for IgG; FcγRγc, Fc receptor common γ chain; ILT, Ig-like transcript; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibitory motif; KIR, killer inhibitory receptor; LIR, leukocyte Ig-like receptor; MIR, monocYTE/macrophage Ig-like receptor; PIR, paired Ig-like receptor; SPIT, solid-phase immunoisolation technique.
plasmic region (16, 17). The PIR-B inhibitory function is mediated through ITIM recruitment of the protein tyrosine phosphatase SH-P-1 (16, 17). Conversely, the predicted PIR-A protein has a short cytoplasmic tail and a charged arginine residue in its transmembrane region, suggesting possible association with transmembrane proteins containing immunoreceptor tyrosine-based activation motifs (ITAMs) to form a signal-transducing unit. In addition, the PIR-A receptors, which are encoded by multiple Pira genes, display sequence diversity in their extracellular regions.

In this study, monoclonal and polyclonal antibodies specific for common epitopes on PIR-A and PIR-B molecules were used to characterize these cell surface receptors and the cellular distribution of their expression in normal and Fc receptor common γ chain (FcγR)−/− deficient mice. The results indicate an essential role for PIR-A association with the FcγR for cell surface expression on B lineage, myeloid, and dendritic cells.

Materials and Methods

Cell Preparation. Bone marrow cells were isolated from the femur by flushing with media, and the erythrocytes lysed in a 0.1 M ammonium chloride buffer solution at pH 7.4. Splenocytes were prepared by splenic disruption, gentle teasing, and density gradient centrifugation over Lympholyte-α-M (Accurate Chemistry & Science Corp.). Splenic B cells were enriched by depletion of M ac-1 macrophages and granulocytes and of CD3+ T cells by a panning method (18). Granulocytes were isolated from peritoneal exudates induced by prior injection of 0.4% (wt/vol) calcium caseinate (Spectrum Quality Products, Inc.).

Preparation of Rombinant PIR Protein. The PIR extracellular domains EC1 and EC2 were amplified by PCR using Taq DNA polymerase and the PIR-A cDNA as the template. The forward primer, 5′-GCACCGATCTCCCTCTTAAAGCCTATTCTCCAGA-3′, corresponds with the 5′ side of the EC1 (the BamHI site is underlined), and the reverse primer 5′-TATCGATAGCCGACAGGTCTTCGAAGTC-3′, with the 3′ side of the EC2 (the HindIII site being underlined). The ~570 bp products were purified by agarose gel electrophoresis, digested with BamHI and HindIII, and ligated into the pcDNA3 expression vector (Qiagen) which was used to transform LTK fibroblasts with the aid of lipofectin, and stable transfectants were selected by Geneticin (G418) exposure.

For cotransfection of a murine FcγR molecule with EC1 or EC2, the Fc receptor molecule was cloned into the PIR-A1–transfected fibroblasts, and the FcγR expression vector was cotransfected with the ~400 bp FcγR coding sequence from the original pcEXV-3 vector (21) into EcoRI-digested pcBlueScript II cloning vector. The pBlue-FcγR construct with a correct orientation was cotransfected with BamHI and XhoI to the pcDNA3.1/Hyg-FcγR constructs with a correct orientation was cotransfected with BamHI and XhoI to remove the FcγR fragment, before ligation into the pcDNA3.1/Hyg (+) expression vector. The resulting expression vector pcDNA3.1/Hyg-FcγR was then linearized with Avil and transfected with the aid of Superfect into LTK fibroblasts which had been already transfected with PIR-A1. Stable cotransfectants were selected by Geneticin (G418) and Hygromycin B.

Western Blot Analysis. B cells and myeloid cells (5 × 107 cells/sample) and PIR-A or PIR-B transfected LTK cells (105) were prepared in 500 μl of lysate buffer (1% NP-40 in 150 mM NaCl/50 mM Tris-HCl, pH 7.5, containing 5 mM EDTA, 20 mM β-mercaptoethanol, 0.1% sodium azide, 20 mM ε-aminocaproic acid, antipain [2 μg/ml], leupeptin [1 μg/ml], soybean trypsin inhibitor [100 μg/ml], aprotinin [2 μg/ml], 1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride, chymostatin [2.5 μg/ml], and pepstatin [1 μg/ml]). A solid-phase immunosolubilization technique (SPIT) (22) was used to identify anti-PIR reactive molecules in cell lysates precleared by centrifugation. In brief, 96-well
Figure 1. Cell surface reactivity of the 6C1 anti-PIR mAb. Mouse LTK cells transfected with the empty vector (A), LTK cells transfected with the PIR-B expression vector (B), M1 myeloblastoid cells (C), and WEHI3 myeloid cells (D) were incubated with 6C1 rat anti-PIR mAb (shaded histogram) or an isotype-matched control mAb (open histogram), before developing with PE-labeled goat antibodies to rat Ig. The stained cells were analyzed by flow cytometry.

Figure 2. Analysis of cell surface and intracellular PIR molecules. Viable splenocytes from normal adult BALB/c mice were radiolabeled with 125I and solubilized in 0.5% NP-40. The cleared membrane lysates were incubated in wells precoated with 6C1 anti-PIR or an isotype-matched control mAb. The bound materials were resolved on SDS-PAGE using 10% acrylamide under nonreducing (not shown) and reducing conditions (A) or digested with or without N-glycanase before SDS-PAGE analysis (B). The same ~85 and ~125-kD PIR molecules were observed in splenocytes, purified splenic B cells, and granulocytes (C) as well as the X16C8.5 B cell and WEHI3 macrophage cell lines (D). In contrast, the 85-kD band was expressed in PIR-A transfected fibroblasts, while the ~120-kD band was expressed by the PIR-B transfectants (D). In the experiments shown in C and D, NP-40 lysates of splenocytes, purified B cells granulocytes, the LTK cells transfected with empty vector or PIR-A1 or PIR-B expression vectors, the X16C8.5 B cell line, and the WEHI3 macrophage cell line were incubated in wells precoated with 6C1 anti-PIR mAb or isotype-matched control mAb (not shown). The bound PIR molecules were separated in SDS-PAGE under nonreducing conditions, transferred onto membranes, and identified with rabbit anti-PIR antiserum and enzyme-labeled goat anti-rabbit Ig antibody before visualization by enhanced chemiluminescence.
beled with PE, FITC, CY, or biotin (Southern Biotechnological Associates). Stained cells were analyzed with a FACScan® flow cytometry instrument (Becton Dickinson).

**Results**

Generation of Monoclonal and Polyclonal Anti-PIR Antibodies. Recombinant PIR protein containing the two NH₂-terminal Ig-like extracellular domains, EC1 and EC2, in the PIR-A1 molecule was produced in E. coli. The PIR-A1 EC1/EC2 recombinant protein was selected to immunize rats because of its sequence identity with PIR-B (1). 1 d before fusion of regional lymph node cells to produce hybridomas, the immunized rats were boosted with viable WEHI3 myeloid cells to enhance the possibility of obtaining antibodies that recognize epitopes on native PIR molecules. 33 hybridoma clones produced antibodies that reacted by ELISA with the recombinant PIR-A1 EC1/EC2 protein. Cell surface immunofluorescence analysis indicated that one of the antibodies, 6C1 (γ1κ), was reactive with PIR-B–transfected LTK fibroblasts and the WEHI3 myeloid cell line (Fig. 1). This mAb was unreactive with mock-transfected LTK fibroblasts and the M1 myeloblastoid cell line that lack PIR-A and PIR-B transcripts. Binding of the 6C1 antibody to the WEHI3 cells was inhibited by the recombinant PIR-A1 EC1/EC2 protein in a dose-dependent manner. The recombinant PIR-A1 EC1/EC2 protein was also used to produce a rabbit antiserum, the specificity of which was indicated by ELISA and by Western blot assays of the native PIR-A and PIR-B proteins described below.

Biochemical Characteristics of the PIR-A and PIR-B Molecules. When iodinated cell surface proteins on splenocytes, B cell lines, and macrophage cell lines were examined by SDS-PAGE, the anti-PIR antibodies identified two major species of ~85 and ~120 kD with minor bands of slightly lower molecular masses (Fig. 2 A). Slightly higher molecular masses were evident under reducing conditions, ~90 and ~125 kD, respectively, consistent with predicted intradisulfide linkages of the six Ig-like extracellular domains. N-glycanase treatment reduced their apparent molecular masses by 10–15 kD in keeping with the presence of five to six potential N-linked glycosylation sites in the PIR-A and PIR-B molecules (Fig. 2 B). Two-dimensional electrophoretic analysis under nonreducing and reducing conditions confirmed that the ~85 and ~120-kD proteins identified on splenocytes by the anti-PIR antibodies did not exist as covalently linked molecules (not shown). B cells purified from the spleen and granulocytes purified from peritoneal exudates also expressed ~85 and ~125-kD molecules (Fig. 2 C). These findings indicate that cell surface PIR molecules are glycoproteins of ~85 and ~120 kD, and that both molecular species are expressed by clonal B and myeloid cells.

Since the monoclonal and polyclonal antibodies recognize epitopes present on both PIR-A and PIR-B molecules, mouse LTK cells transfected either with PIR-A1 or PIR-B cDNAs were used for discrimination of the PIR molecules. A major band of ~120 kD, together with a faint band of ~97 kD, was immunoprecipitated by the 6C1 anti-PIR mAb from lysates of the PIR-B transfectants, and not from lysates of mock transfectants probed by immunoblotting with the rabbit anti-PIR antibodies (Fig. 2 C). Conversely, the anti-PIR mAb precipitated a major band of ~85 kD and a minor band of ~70 kD from cell lysates of the PIR-A1 transfectants. The two major bands of 85 and 120 kD were also identified by the 6C1 mAbs and the rabbit anti-PIR antibodies in normal splenocytes, B cell, and myeloid cell lines, all of which express Pira and Pirb.

![Figure 3](image-url) Figure 3. Association of PIR-A with FcRγc. (A) Assessment of cell surface PIR-A expression on LTK fibroblasts transfected with PIR-A alone (left) or with PIR-A and FcRγc (right). Viable cells were incubated with fluorochrome-labeled 6C1 anti-PIR (shaded histogram) or isotype-matched control mAb (open histogram). (B) Assessment of PIR expression in FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice. Splenocytes from FcRγc-deficient versus normal mice.
Further by the analysis of FcR
\[ \text{cell surface expression of PIR-A molecules was examined} \]
\[ \text{(Fig. 3 A). The apparent requirement of FcR} \]
\[ \text{structs expressed readily detectable PIR-A on the cell} \]
\[ \text{surface (Fig. 3 A). While splenocytes from the wild-type mice ex-} \]
\[ \text{pressed the PIR antigen at variable levels that correlated with} \]
\[ \text{Gr-1 expression levels (Fig. 4, first row). Examination of the morphological features of isolated PIR} \]
\[ ^{i} / \text{Gr-1}^{i} \text{and PIR} \]
\[ ^{i} / \text{Gr-1}^{i} \text{fractions confirmed the increase in PIR- and Gr-1 ex-} \]
\[ \text{pressions.} \]

**PIR-A A ssociation with FcRγc.** The PIR-A protein has a short cytoplasmic tail without recognizable functional motifs, but the presence of a charged amino acid, arginine, in the transmembrane region suggested its potential association with another transmembrane protein (1). Inferential evidence in support of this possibility was provided by studies of the cell surface proteins expressed by PIR-A and PIR-B fibroblast transfectants. A major band of \( \sim 120 \text{kD} \) was identified on the cell surface of PIR-B transfected LTK\(_2\) cells by the 6C1 antibodies (not shown), whereas the \( \sim 85 \) and \( \sim 70-\text{kD} \) molecules expressed by PIR-A1 transfectants (see Fig. 2 C) were identified only intracellularly. Cell surface PIR-A molecules were not identified by the anti-PIR mAb on PIR-A transfectants, thereby implying that PIR-A requires companion molecules, not present in fibroblasts, to reach the cell surface. Accordingly, two-dimensional analysis of the proteins precipitated with the 6C1 anti-PIR antibody from iodinated cell surface proteins on splenocytes revealed an associated homodimer composed of relatively small disulfide-linked subunits (\( \sim 10 \text{kD} \), not shown). Immunoblots of the cell surface PIR complex separated under reducing conditions by SDS-PAGE identified these associated proteins as FcRγc (Fig. 3 B).

The requirement of FcRγc association for the cell surface expression of PIR-A was examined by fibroblast cotransfection experiments. While PIR-A producing transfectants failed to express this product on the cell surface, fibroblasts cotransfected with the PIR-A and FcRγc constructs expressed readily detectable PIR-A on the cell surface (Fig. 3 A). The apparent requirement of FcRγc for cell surface expression of PIR-A molecules was examined further by the analysis of FcRγc chain-deficient (FcRγc\(^{-/-}\)) mice. While splenocytes from the wild-type mice expressed both PIR-A and PIR-B on the cell surface, only the PIR-B was identified on the cell surface of splenocytes in FcRγc\(^{-/-}\) mice (Fig. 3 B). This selective impairment of PIR-A cell surface expression in FcRγc\(^{-/-}\) mice was not attributable to a lack of PIR-A production, since the 6C1 mAb identified both the 85-kD PIR-A and the 120-kD PIR-B molecules within FcRγc\(^{-/-}\) splenocytes. Therefore, these findings indicate that FcRγc chains are required for the cell surface expression of PIR-A molecules.

**Immunofluorescence Analysis of the Cellular Distribution of PIR Expression.** When bone marrow cells from adult BALB/c mice were incubated with the fluorochrome-labeled 6C1 anti-PIR mAb, \( \sim 80\% \) of the nucleated cells were stained. Morphological analysis of isolated PIR\(^+\) cells indicated that most were myeloid cells, ranging from immature myeloblasts to mature granulocytes, whereas lymphocytes represented a minor constituent. When differential immunofluorescence analysis for other cell surface differentiation antigens was conducted to further characterize the PIR\(^+\) cells, bone marrow cells bearing the Gr-1 and Mac-1

![Figure 4. Immunofluorescence analysis of cell surface PIR expression.](Image) Bone marrow (BM), spleen (SP), and peritoneal lavage (PEC) cells from adult BALB/c mice were incubated first with aggregated human IgG to block FcγR, then stained with a combination of PE-labeled 6C1 anti-PIR and FITC-, CY-, allophycocyanin-, or biotin-labeled (and CY-labeled streptavidin) mAbs with the following specificity: Mac-1 and Gr-1 for myeloid lineage cells (first row); CD43 and B220 for pro-B/pre-B cell compartment (second row); CD19 and IgM for B lineage cells (third row); CD3 and DX5 for T and NK cells, and CD19 or Mac-1 for B cells and macrophages (fourth row); B220, CD21, and CD23 for marginal zone (MZ), follicular (FO), and newly formed (NF) B cells (fifth row); CD19, CD5, and Mac-1 for B1 and B2 subpopulations (sixth row). Staining of cells with light scatter characteristics of myeloid cells or small lymphoid and larger mononuclear cells was analyzed by flow cytometry. In the bottom two rows, B220\(^{-}\) or CD19\(^{-}\) B cells were examined for expression of the indicated cell surface antigens. The cell populations indicated by boxes in contour plots were examined for their expression of PIR molecules (solid line) versus background staining with an isotype-matched control mAb (dashed line). MFI indicates mean fluorescence intensity.
pression with progression of granulocyte differentiation. The B-lineage cells in bone marrow expressed PIR at lower levels relative to the myeloid cells, and most of the PIR+/B-lineage cells (CD19+) were IgM+ B cells; pro-B were PIR negative while pre-B cells were found to express PIR at low levels (Fig. 4, second and third rows), indicating a gradual increase in PIR expression as a function of B-lineage differentiation. Bone marrow–derived, IL-3–induced mast cells (c-kit+) also expressed PIR on their cell surface. PIR proteins were not detected on erythroid lineage cells (Ter119−) in the bone marrow. CD3+ thymocytes from newborn and adult BALB/c mice were PIR negative. In striking contrast, marginal zone B cells (B220+/CD19+, M ac-1+, FcyRII/III−) expressed PIR from relatively low to high levels (Fig. 5).

When subpopulations of splenic B cells were evaluated, the 6C1-identified PIR molecules in highly variable levels. When subpopulations of splenic B cells were evaluated, the marginal zone B cells (B220−, CD21high, CD23low−) were found to express higher PIR levels than newly formed (B220−, CD21−, CD23−) and follicular B cells (B220+, CD21med, CD23+) (Fig. 4, fifth row). Moreover, the B1 subpopulations of B cells in peritoneal lavage expressed higher levels of PIR than did the B2 cells (Fig. 4, sixth row). Consistent with this suggestive evidence that B cell activation may enhance PIR expression, cell surface levels of PIR were upregulated by ~33% after LPS stimulation of splenic B cells, and macrophage PIR expression was similarly enhanced by LPS stimulation. Splenic dendritic cells (MHC class II+, CD11c+, CD19+, M ac-1+, CD8+/−, FcyRII/III−), like thymic dendritic cells, were found to express relatively low to relatively high levels of PIR (Fig. 5).

Cell Surface Expression Levels of PIR-B in Fcγ−/− mice. Since cell surface expression of PIR-A was selectively impaired in FcRγc-deficient mice, PIR-B expression relative to total PIR-A/PIR-B expression could be estimated by immunofluorescence comparison of the cells from FcRγc-deficient and wild-type mice. Cell surface PIR-B expression alone was thereby found to increase in the FcRγc-deficient mice as a function of both myeloid and B lineage differentiation (Fig. 6).

Discussion

The monoclonal and polyclonal anti-PIR-A/PIR-B antibodies described here define the cell surface PIR-A and PIR-B receptors as glycoproteins of ~85 and ~120 kD, respectively. The ~120-kD estimate for PIR-B agrees with that obtained using a rabbit antiserum against a p91/PIR-B cytoplasmic peptide (2). In the present studies, the coordinate or paired expression of PIR-A and PIR-B by B and myeloid cells suggested by transcriptional analysis (1) was confirmed by demonstration of both molecules on representative clonal cell lines. Given the finding of splice variants among PIR-A cDNAs (1), we anticipated considerable size heterogeneity of the PIR-A proteins. Contrary to this expectation, the cell surface PIR-A and PIR-B receptors were relatively homogeneous in size, and the predominant protein species were physically indistinguishable in different cell types (B cells versus macrophages) and cell sources (cell lines versus splenocytes). The products of splice variant PIR-A cDNAs thus appear to comprise only a minor fraction of the total PIR-A pool. The PIR-A and PIR-B cDNA sequences indicate an additional free Cys residue in their ectodomains (1), thus suggesting the possible existence of disulfide-linked dimers of the PIR-A and PIR-B molecules. However, disulfide linkage of these molecules was not evident in either one- or two-dimensional gel electrophoresis analyses. It is still theoretically possible that cell surface PIR-A and PIR-B molecules could form noncovalent associations with functional consequence.

Intriguingly, the anti-PIR mAb recognized the PIR-B cell surface receptor on PIR-B transfectants, whereas PIR-A cell surface molecules could not be detected on PIR-A1 producing transfectants. This finding implied the require-
ment for additional membrane-bound protein(s) for PIR-A cell surface expression, a possibility that was also suggested by the presence of a charged Arg residue in the transmembrane region of the predicted PIR-A1 protein (1). There are well documented precedents for the noncovalent association of Ig-like receptor chains containing such charged transmembrane regions with another membrane-bound protein for cell surface expression and/or function. These include (a) the association of ligand-binding α chain of several Fc receptors (FceR, FcyRI, FcyRIII, FcαR) with signal transducing subunits (β, γ, or ζ); (b) the TCR/CD3 complexes; and (c) the killer activating receptor/dendritic associated protein-12 or killer activating receptor associating protein complexes (20, 26–38). In these examples, the associated proteins typically contain ITAMs in their cytoplasmic domains (31, 39–42). Our immunoprecipitation analysis indicated that the ITAM-containing FcγR is associated with PIR molecules present on the cell surface. The implication that the FcγR is essential for PIR-A cell surface expression was confirmed in fibroblast cotransfection studies and by the selective expression of PIR-B molecules on B cells and myeloid cells in FcγR-deficient mice. In studies reported since submission of this manuscript, a cell activation for the PIR-A/FcγR complex has been demonstrated in rat mast cell line and chicken B cell line transfected with constructs for chimeric proteins containing the PIR-A transmembrane and cytoplasmic regions (43, 44).

The restricted expression of PIR molecules by B and myeloid lineage cells indicated by our immunofluorescence analysis is consistent with previous analyses of PIR-A and PIR-B transcripts (1, 2). Cell surface PIR expression was found to increase as a function of cellular differentiation in both cell lineages, indicating that the PIR family is primarily involved in mature cell function. The levels of PIR-A/PIR-B expression by splenic B cells were remarkably variable. Examination of the different splenic subpopulations indicated that the expression levels were highest on marginal zone B cells, which appear to be primed cells (45). Correspondingly, the B1 subpopulations of B cells expressed higher PIR levels than did the B2 subpopulation. The suggestion that activated B cells may express higher PIR levels was supported by the observation that LPS stimulation enhanced B cell and myeloid cell expression of the PIR molecules, in keeping with the presence of potential binding sites for the LPS-IL-6-dependent DNA-binding protein in the promoter region of the Pirb gene (15).

In addition to B cells and myeloid lineage cells, the dendritic cells in the thymus and spleen, defined as MHC class II+CD11c+CD8−/−CD4+CD19−Mac-1+, were also found to express the PIR molecules on their surface. This finding suggests that the variable PIR-A molecules and invariant PIR-B molecules could be involved in self/non-self discrimination or antigen presentation. The human Ig-like ILT/LIR/MIR receptors, which share 50–60% sequence similarity with mouse PIR, are also expressed by B cells, monocyte/macrophages, and dendritic cells, but, unlike the murine PIR molecules, these human relatives may also be expressed by N K cells and a subpopulation of T cells (9, 11, 46). Some members of the human ILT/LIR/MIR family have been shown to bind classical or nonclassical MHC class I alleles (10, 46–48). Although the ligand or ligands for the PIR molecules are presently unknown, their pattern of expression on myeloid, lymphoid, and dendritic cells suggests that, like other Ig-like cell surface receptors, they may coligate with other activation/inhibitory systems to modulate inflammatory and immune responses. In this regard, these results suggest that the PIR-A requirement for FcγR association could result in differential regulation of the PIR-A/PIR-B equilibrium as a function of cellular activation or differentiation pathway.

Figure 6. PIR expression in FcγR-deficient and wild-type mice. Bone marrow cells from Fcγ-deficient (thick line) and wild-type mice (thin line) were stained with 6C1 anti-PIR or control (dotted line) mAb, and the stained cells were analyzed as described in the legend for Fig. 4. Only the wild-type mice control staining is illustrated.
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