Review Article

Role of paired Ig-like receptor-B in the humoral immune response

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ABSTRACT

The Ig-like receptors provide positive and negative regulation of immune cells upon recognition of various ligands, thus enabling those cells to respond adequately to extrinsic stimuli. Murine paired Ig-like receptor (PIR)-A and PIR-B, a typical receptor pair of the Ig-like receptor family, are expressed on a wide range of cells in the immune system, such as B cells, mast cells, macrophages and dendritic cells, mostly in a pair-wise fashion. The PIR-A requires the homodimeric Fc receptor common γ chain for its efficient cell-surface expression and for the delivery of activation signaling. In contrast, PIR-B inhibits receptor-mediated activation signaling in vitro upon engagement with other activating-type receptors, such as the antigen receptor on B cells and the high-affinity Fc receptor for IgE on mast cells. Although the ligands for PIR-A and PIR-B remain unknown, recent studies on PIR-B-deficient mice have provided us with valuable insight into the physiological significance of PIR-B, particularly in its regulatory role in balancing the humoral immune response.

Key words: autoimmunity, B cell, dendritic cell, hypersensitivity, mast cell, Th2 response.

INTRODUCTION

Paired immunoglobulin (Ig)-like receptors (PIR) were first identified as those homologous to human Fc receptor (FcR) for IgA, FcαRI. In 1997, Hayami et al.1 reported the isolation of several cDNA clones coding for a novel molecule from a B10.A mouse macrophage cDNA library during the course of experiments to obtain a hypothetical murine counterpart of FcαRI. The gene product was initially designated as p91 due to the calculated molecular weight of the mature polypeptide backbone.1 A similar approach by Kubagawa et al.2 yielded a novel gene family, the members of which constitute a set of PIR-A and PIR-B genes in the BALB/c splenic library (Fig. 1). Because p91 and PIR-B were the most plausibly identical molecules based on their 98% sequence identity, the nomenclature has been standardized as PIR to avoid confusion.3

These initial studies could not detect PIR binding to IgA nor other Ig,1,2 suggesting that they are not receptors for Ig. Instead, PIR-A and PIR-B are now proposed as relatives or orthologs of human leukocyte Ig-like receptors (LILR), based on their similarities in structure, expression profiles and genomic localization.1,2,4–6 The analogy of PIR-B to the inhibitory isoform of LILR, namely LILRB, and the findings that constitutive phosphorylation of PIR-B in splenocytes was reduced in β2-microglobulin (β2m)-deficient mice7 led to the notion that PIR-B may recognize classical or non-classical major histocompatibility complex (MHC) class I molecule(s).6,7 The observation that human histocompatibility leukocyte antigen (HLA)-G, a non-classical MHC class Ib on fetal trophoblast cells, binds PIR-B8 supports this notion. However, there is no direct evidence for the interaction between PIR and MHC class I molecules. In the present review, the possible physiological significance of PIR will be discussed with particular reference to results from recent studies on PIR-B-deficient (PIR-B−/−) mice.9
The PIR-A and PIR-B receptors are expressed on various hematopoietic cell lineages, including B cells, mast cells, macrophages, granulocytes and dendritic cells (DC), mostly in a pair-wise fashion, but are not expressed on T and natural killer (NK) cells (Fig. 1).1,2,10 Amino acid sequences of PIR-A and PIR-B ectodomains are highly homologous (over 92% identity).1,2 The deduced structure of PIR-B is a type I transmembrane glycoprotein with six extracellular Ig-like domains, a hydrophobic transmembrane segment and an intracellular polypeptide with four immunoreceptor tyrosine-based inhibitory motifs (ITIM) or ITIM-like sequences (consensus: (I/L/V/S)XXYXX(L/V); Fig. 1). The PIR-B is highly homologous to the murine gp49B1 (31% homology at the amino acid level),11 human killer Ig-like receptors (KIR; 34%),12-15 the human FcαRI (29%),16 the bovine Fcγ2R (32%)17 and less homologous to human and mouse FcγRIIB (17%).18

In contrast, PIR-A molecules have six Ig-like extracellular domains, but contain unique pretransmembrane, transmembrane and short cytoplasmic sequences harboring no ITIM-like motifs (Fig. 1). In addition, their transmembrane domains contain a positively charged residue, Arg, which presumably is crucial for the association of the FcR common γ subunit (FcRγ), which itself is critical for expression on the cell surface and for delivery of the activation signal.3,10,19,20

Although comparison of the available sequences of PIR extracellular portions from 129/Sv, B10.A and

**FIGURE 1**

Schematic structures of mouse and rat paired Ig-like receptor (PIR)-A and PIR-B and their close relatives, chicken CHIR (chicken PIR homologs) and human leukocyte Ig-like receptors (LILR) and killer Ig-like receptors (KIR). Upper boxes show ligands for them and bottom boxes show cellular distribution. Activating-type Ig-like receptors have a positively charged amino acid, Arg (R) or His (H), in their transmembrane domains, which is involved in their interaction with negatively charged Asp (D) residues of FcRγ or DAP12 homodimer.39,40 In contrast, inhibitory Ig-like receptors have several immunoreceptor tyrosine-based inhibitory motifs (ITIM) or ITIM-like sequences in their cytoplasmic portions important for SHP-1 recruitment upon tyrosine phosphorylation. GenBank accession numbers for PIR: U83172, AF040946040953 and AF041035 and AF041036 (as p91) and U96682–96693. The multiple PIR-A and single PIR-B genes locate near the centromeric region of mouse chromosome 7, syntenic to human LRC located in 19q13.4. Fluorescence in situ hybridization analysis of rat PIR genes identified the locus 1q21.1–21.3, which is syntenic to the mouse chromosome 7 centromeric region.5 MHC, major histocompatibility complex. Repertoires of KIR and LILR are more complicated than those illustrated here. For details for the KIR family, see http://www.ncbi.nlm.nih.gov/prow/guide/67966474_g.htm and http://www.gene.ucl.ac.uk/nomenclature/genefamily/kir.html; for LILR, see http://www.gene.ucl.ac.uk/nomenclature/genefamily/lilr.html

**STRUCTURAL OVERVIEW OF PIR**

The PIR-A and PIR-B receptors are expressed on various hematopoietic cell lineages, including B cells, mast cells, macrophages, granulocytes and dendritic cells (DC), mostly in a pair-wise fashion, but are not expressed on T and natural killer (NK) cells (Fig. 1).1,2,10 Amino acid sequences of PIR-A and PIR-B ectodomains are highly homologous (over 92% identity).1,2 The deduced structure of PIR-B is a type I transmembrane glycoprotein with six extracellular Ig-like domains, a hydrophobic transmembrane segment and an intracellular polypeptide with four immunoreceptor tyrosine-based inhibitory motifs (ITIM) or ITIM-like sequences (consensus: (I/L/V/S)XXYXX(L/V); Fig. 1). The PIR-B is highly homologous to the murine gp49B1 (31% homology at the amino acid level),11 human killer Ig-like receptors (KIR; 34%),12-15 the human FcαRI (29%),16 the bovine Fcγ2R (32%)17 and less homologous to human and mouse FcγRIIB (17%).18

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Although comparison of the available sequences of PIR extracellular portions from 129/Sv, B10.A and
BALB/c mice indicated a fairly high sequence similarity, multiple substitutions of amino acid residues were observed, especially in the first four extracellular domains. The polymorphic nature of PIR may indicate that the ligand(s) would also be polymorphic, similar to the situation for LILR and KIR, which have many polymorphic substitutions in their extracellular domains. The PIR genes were localized to the proximal end of the mouse chromosome 7, which is a syntenic position of human chromosome 19q13.3–13.4, harboring the leukocyte receptor cluster (LRC). In LRC, LILR and KIR genes have been mapped, supporting the notion that the PIR and LILR gene families are close relatives or orthologs.

**Expression and Gene Evolution**

Monoclonal and polyclonal antibodies to PIR identified cell-surface glycoproteins of approximately 85 and 120 kDa on B cells, granulocytes and macrophages. Using a fibroblast transfection experiment as well as FcRγ-deficient (FcRγ−/−) mice, it was shown that approximately 120 kDa PIR-B is normally expressed on the cell surface without any other subunits, whereas approximately 85 kDa PIR-A requires association with FcRγ for its cell surface expression. Interestingly, cell surface levels of PIR molecules on myeloid and B lineage cells increased with cellular differentiation and activation. Surface PIR levels are highest on marginal zone B cells and the B1 B cells express higher PIR levels than the B2 B cells.

Considering gene evolution of PIR, it is interesting to see PIR from other species. The LILR are human relatives of the PIR gene family are conserved in rats and mice. Of note, analysis of mRNA detected unpaired expression of rat PIR-A by B cells and rat PIR-B by NK cells, suggesting divergence of PIR regulatory elements during rodent speciation. Similarly, chicken PIR homologs (termed CHIR) were identified (Fig. 1). Although the activating type CHIR-A and inhibitory CHIR-B have only two Ig-like domains in their extracellular portion, other structural characteristics were similar to mouse and rat PIR-A and PIR-B. A basic histidine residue was located in the CHIR-A transmembrane region and two tyrosine residues embedded in the ITIM consensus sequences were present in the CHIR-B cytoplasmic portion. Coordinate expression of CHIR-A and CHIR-B mRNA was observed in B and T cell lines.

**PIR-A Activation Signaling**

Engagement of transfected PIR-A expressed on the rat mast cell line RBL-2H3 with antibodies elicited a calcium mobilization and degranulation response, indicating that PIR-A plays a role in the activation of mast cells. The molecule associating with the PIR-A transmembrane region was FcRγ and the FcεRI chain in RBL-2H3, in which two charged residues, Arg626 and Glu643, were pivotal in the activating function of PIR-A and its association with the FcR subunits. Events in the downstream signaling of FcRγ have been studied extensively in relation to FcεRI on mast cells and FcγRI/III on macrophages. However, the exact physiologic nature of PIR-A/FcRγ signaling has not been studied adequately because of a lack of information regarding the ligand for PIR-A.

**Inhibitory Signal Transduction via PIR-B**

Most of the inhibitory isoforms of Ig-like receptors exert their negative regulation of cells by recruiting SH2-containing tyrosine phosphatases SHP-1 and/or SHP-2 to their phosphorylated ITIM. Exceptionally, a unique inhibitory FcγR, namely FcγRIIB, recruits SH2-containing inositol 5-phosphatase SHIP-1 to the phosphorylated ITIM. The PIR-B is demonstrated to function by recruiting SHP-1 and SHP-2. In vitro mutation analysis of cytoplasmic tyrosine residues in the ITIM of PIR-B indicated that the tyrosine in the third ITIM plays the most crucial role in mediating the inhibitory signal for B cell receptor (BCR)-mediated cell activation as assessed by calcium mobilization and activation of nuclear factor of activated T cells (NF-AT). Also in vitro, synthetic phosphotyrosyl peptides corresponding to the third and fourth ITIM of PIR-B can bind SHP-1, SHP-2 and SHIP-1 in cell extracts from macrophages, as well as in a B cell line. However, the PIR-B-mediated inhibition was markedly reduced in SHP-1 and SHP-2 double-deficient DT40 chicken B cells, whereas this inhibition was unaffected in SHIP-deficient cells, strongly suggesting that PIR-B can negatively regulate BCR activation by redundant functions of SHP-1 and SHP-2, but not by SHIP-1 (Fig. 2). In addition, in the mast cell line RBL-2H3, coaggregation of transfected PIR-B with FcεRI induced the PIR-B to recruit SHP-1 but not SHP-2 or
Even in the absence of the coaggregation, weak constitutive association of the PIR-B cytoplasmic domain with SHP-1 was observed. The third and fourth ITIM tyrosine residues were crucial for inhibition of RBL-2H3 cell degranulation and calcium mobilization.

A reverse approach yielded an interesting observation about the preferential association of SHP-1 to PIR-B. SHP-1 was shown to associate with a 130 kDa tyrosyl-phosphorylated species, namely P130, in murine macrophages, suggesting that the P130 may be an SHP-1 regulator and/or substrate. Interestingly, P130 consisted of two transmembrane glycoproteins, namely PIR-B and the signal-regulatory protein (SIRP) family member brain immunoglobulin-like molecule with tyrosine-based activation motifs (BIT). Furthermore, PIR-B was hyperphosphorylated in macrophages from SHP-1-deficient motheaten viable mice, whereas it was hypophosphorylated in SHP-1-deficient motheaten macrophages, suggesting a model in which SHP-1 dephosphorylates specific sites on PIR-B while protecting other sites from dephosphorylation via its SH2 domains. The PIR-B also associates with two tyrosyl phosphoproteins and a tyrosine kinase activity.

Irrespective of the cell activation status, PIR-B molecules in macrophages and B cells are constitutively phosphorylated and PIR-B in splenocytes was constitutively associated with SHP-1 and Lyn. In Lyn-deficient mice, PIR-B tyrosine phosphorylation was greatly reduced. The ligation of PIR-B on chicken DT40 cells inhibits the BCR-induced tyrosine phosphorylation of Igα/Igβ, Syk, Btk and PLC-γ2 (Fig. 2). The constitutive association of SHP-1 with PIR-B suggests that the inhibitory function of PIR-B may not necessarily require coligation of BCR and PIR-B by any ligand (Fig. 2). This speculation was shown to be the case in splenic B cells from PIR-B−/− mice, as described below.

**PHYSIOLOGICAL ROLE OF PIRS**

Human PIR relatives, the LILR, which share 50–60% sequence similarity with PIR, bind classical or non-classical MHC class I alleles. Constitutive tyrosine phosphorylation and constitutive association with the SHP-1 and Lyn protein tyrosine kinase imply that the PIR-B molecules are constitutively ligated with self ligands, such as MHC class I. The PIR-B tyrosine phosphorylation status was examined in β2m-deficient, transporter associated with antigen presentation 1 (TAP1)-deficient and MHC class II-deficient mice. The level of PIR-B tyrosine phosphorylation is reduced by approximately 50% in β2m-deficient mice, but is not significantly altered in TAP1- or MHC class II-deficient mice, suggesting that non-classical MHC class I or class I-like molecules are more likely candidates for native PIR ligands.

Although the ligand(s) for PIR is, at present, unidentified, the PIR may modulate inflammatory and immune responses by constitutive engagement with the putative ligand(s). Recent PIR-B−/− mice have provided us with insight into the physiological significance of PIR in the immune response, especially in humoral immunity.

**DOMINANT EXPRESSION OF PIR-B**

Because an available monoclonal antibody to PIR, namely 6C1, recognizes both PIR-A and PIR-B, it was not known which receptor is dominantly expressed on various cell surfaces. Given that the deletion of one receptor does not influence the expression of the other, PIR-B deficiency will tell us the surface expression of PIR-A by flow cytometry with 6C1, whereas cells devoid
of FcRγ will enable us to estimate PIR-B expression. Comparison of flow cytometric data on cells from either PIR-B−/− or FcRγ−/− mice revealed the more dominant expression of PIR-B than PIR-A on splenic macrophages, splenic DC and bone marrow-derived cultured mast cells and exclusive expression of PIR-B on splenic B cells. The consistent observations from either PIR-B or FcRγ deletion suggested that the PIR-B deletion did not largely alter PIR-A expression in these cells and vice versa. Thus, PIR-A and PIR-B surface expression is characteristic for each cell type, at least in its resting state, and the suppression of these cells by dominantly expressed PIR-B may have a physiological importance, such as in maintaining their resting state.

**PIR-B−/− B CELLS ARE HYPERRESPONSIVE**

The PIR-B molecules in macrophages and B cells are constitutively phosphorylated. Thus, deletion of PIR-B may induce B cell hypersensitivity upon BCR ligation, similar to that seen in CD22-deficient mice. In fact, PIR-B−/− splenic B cells showed significantly enhanced proliferation upon anti-BCR F(ab’)2 stimulation. When stimulated with anti-BCR whole IgG antibodies after blocking with anti-FcγRIIB monoclonal antibody, the enhanced PIR-B−/− B cell proliferation was more pronounced due to masking of an inhibitory effect by FcγRIIB. This indicates that the inhibitory effects by PIR-B and FcγRIIB are additive, possibly because of the fact that PIR-B uses the SHP-1 cascade for inhibition whereas FcγRIIB uses SHIP. These results indicate that PIR-B−/− B cells are hypersensitive to stimulation via BCR ligation. Consistent with this observation, PIR-B−/− mice showed a higher IgM response against T-independent antigens trinitrophenyl (TNP)-Ficoll and TNP-lipopolysaccharide (LPS). The enhanced response upon TNP-LPS challenge suggested that the PIR-B inhibitory effect could also be exerted independently of BCR ligation. Enhanced tyrosine phosphorylation of cellular proteins in PIR-B−/− B cells even in the resting state indicated the constitutive activation of PIR-B−/− B cells. Thus, PIR-B may downregulate BCR signaling by interacting with any unidentified ligand. A PIR-B deficiency may generally render B cells active and hyperresponsive to stimulation via BCR. However, serum IgM levels were not increased in naïve PIR-B−/− mice. In addition, antidouble-stranded DNA antibodies were not detected in adult PIR-B−/− mice, in contrast with CD22-deficient mice, in which hyper-IgM and autoantibody production is evident.

**PIR-B DEFICIENCY LEADS TO TH2-SKEWED RESPONSE**

The Th2-prone humoral responses were augmented in PIR-B−/− mice upon immunization with T-dependent antigens in terms of both interleukin (IL)-4-rich and interferon-γ-poor cytokine profiles and enhanced IgG1 and IgE production. At least one of the mechanisms for the Th2-skewed responses in PIR-B−/− mice was the immature phenotype of DC (Fig. 3). Flow cytometric analysis of surface markers, such as MHC class II, CD80 and CD86, on bone marrow-derived cultured DC before and after antigen loading revealed that the DC from PIR-B−/− mice were immature. The production of IL-12, a Th1-polarizing cytokine, was greatly diminished upon antigen loading of DC from PIR-B−/− mice. Successful adoptive transfer of a Th2-prone response by PIR-B−/− DC into wild-type mice demonstrated that the impaired maturation of DC was responsible for the skewing.

What is the mechanism for the impaired maturation of DC in PIR-B deficiency? To test the possibility that intracellular signaling may differ between PIR-B−/− DC and wild-type cells, bone marrow DC were stimulated with granulocyte–macrophage colony stimulating factor (GM-CSF), an inducer cytokine for DC development, and were examined for their protein tyrosine phosphorylation profile. The PIR-B was tyrosine phosphorylated in resting wild-type DC similar to that found in resting

![Fig. 3 Possible mechanism for the Th2-skewed humoral response in paired Ig-like receptor (PIR)-B−/− mice. Granulocyte–macrophage colony stimulating factor-induced dendritic cells (DC) from PIR-B−/− bone marrow cells show immature phenotype in terms of their lower expression of major histocompatibility complex class II, CD80 and CD86 and lower interferon (IL)-12 production after antigen loading. Because IL-12 is a Th1-polarizing cytokine, its less-abundant production by DC will lead to poor naive T (T0) to Th1 differentiation and, as a result, Th2-prone differentiation could occur. wt, wild type; CTL, cytotoxic T lymphocyte.](image-url)
B cells. The tyrosine phosphorylation of PIR-B was augmented upon GM-CSF stimulation, indicating that PIR-B is involved in the cytokine signaling. After GM-CSF stimulation, the GM-CSF receptor common β chain was tyrosine phosphorylated, but the time-course of the phosphorylation in PIR-B–/– DC was more transient than that in wild-type cells. Thus, PIR-B deficiency leads to altered phosphorylation profiles of GM-CSF signaling, which yields immature DC. Thus, analysis of PIR-B phosphorylation profiles of GM-CSF signaling, which wild-type cells. Thus, PIR-B deficiency leads to altered  

**Future PIR research**

Collectively, identification of the ligand(s) for PIR-A and PIR-B and its characterization should be the next step. Given that the classical or non-classical MHC class I molecules could, in fact, interact with PIR-B, then PIR-B should be a novel, ubiquitously expressed negative regulator for immune cells, such as B cells and DC. Various immune cells may have adopted recognition of self-MHC class I molecules as a common strategy to inhibit cellular activation. Further analysis of PIR-B–/– mice will provide us with much more information about the physiological role of PIR-B and pathophysiological aspects of its involvement in infection, hypersensitivity and autoimmune diseases. Similarly, we should make more detailed analysis of FcγR–/– mice in terms of the influence of loss of PIR-A expression. The FcγR–/– mice exhibit pleiotrophic effector cell defects in IgG- or IgE-mediated immune responses,25 such as the disappearance of the phagocytic activity of macrophages and unresponsiveness to IgE-mediated hypersensitive responses due to the loss of FcγRI and FcγRIII expression, but we do not know whether the phenotypes of FcγR–/– mice involve the effect of PIR-A deficiency or not. Detailed analysis of FcγR–/– mice may suggest a possible ‘activating’ function of PIR-A in light of the speculation that PIR-B is a constitutive inhibitor for many cells in the immune system.

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**References**


38 Magram J, Connaughton SE, Warrier RR et al. IL-12-deficient mice are defective in IFNγ production and type 1 cytokine responses. Immunity 1996; 4: 471–81.