Corrections

**BIOCHEMISTRY.** For the article “Bepridil opens the regulatory N-terminal lobe of cardiac troponin C” by Yu Li, Michael L. Love, John A. Putkey, and Carolyn Cohen, which appeared in number 10, May 9, 2000, of *Proc. Natl. Acad. Sci. USA* (97, 5140–5145), the authors note the following correction to the Acknowledgments. “We thank the staff at the Cornell High Energy Synchrotron Source for assistance with data collection.”

Correction published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.170295797. Text and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.170295797

**IMMUNOLOGY.** For the article “Paternal monoallelic expression of the paired immunoglobulin-like receptors PIR-A and PIR-B” by Ching-Cheng Chen, Vincent Hurez, J. Scott Brockenbrough, Hiromi Kubagawa, and Max D. Cooper, which appeared in number 12, June 8, 1999, of *Proc. Natl. Acad. Sci. USA* (96, 6868–6872), the authors note the following correction. The authors have learned that the Jackson and Bailey substrains of inbred BALB/c mice have undergone sequence variation in their Pir genes over the 60 years of independent breeding. The allelic determinants recognized by the 10.4 anti-PIR antibody are expressed in the BALB/cBy substrain but are not present in the BALB/cJ PIR sequence (GenBank accession no. AF288356). Mixed provenance in the BALB/c parents for the [C57BL/6 (male) × BALB/cJ (female)]F1 offspring versus [BALB/cBy (male) × C57BL/6 (female)]F1 offspring described in the article led to the erroneous conclusion of monoallelic PIR expression. The data in this article are otherwise correct. The authors apologize for the inconvenience that their incorrect conclusion may have caused other investigators.
Paternal monoallelic expression of the paired immunoglobulin-like receptors PIR-A and PIR-B

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Contribution by Max D. Cooper, April 19, 1999

ABSTRACT A diverse pattern of polymorphism is defined for the paired Ig-like receptors (PIRs) that serve as activating (PIR-A) and inhibitory (PIR-B) receptors on B lymphocytes, dendritic cells, and myeloid-lineage cells in mice. The monoclonal anti-PIR antibody 10.4 is shown to recognize an allelic PIR-A/PIR-B determinant on cells from BALB/c but not C57BL/6 mice. Other strains of inbred mice also can be typed on the basis of their expression of this PIR allelic determinant. Analysis of (BALB/c × C57BL/6) F1 hybrid offspring indicates that PIR molecules bearing the paternal PIR allotype are expressed whereas PIR-A and PIR-B molecules bearing the maternal allotype are not. The monoallelic expression of the polymorphic PIR-A and PIR-B molecules, and possibly of their human Ig-like transcript/leukocyte Ig-like receptor/monocyte/macrophage Ig-like receptor and killer cell inhibitory receptor relatives, may influence innate and specific immune responses in outbred populations.

Paired Ig-like receptors (PIRs) A and B are recently identified cell surface molecules with very similar extracellular regions comprised of six Ig-like domains, D1–D6 (1, 2). The invariant PIR-B molecules differ from the variable PIR-A molecules in that PIR-B possesses cytoplasmic immunoreceptor tyrosine-based inhibitory motifs that can recruit the protein tyrosine phosphatase SHP-1 to inhibit signal transduction (3–6). Conversely, the closely related PIR-A molecules all have a short cytoplasmic tail and a polar transmembrane region that facilitates association with homodimers of the Fc receptor common γ chain to form a cell activating receptor complex (7–9). The PIR-A and PIR-B molecules are expressed on B lymphocytes, dendritic cells, monocyte/macrophages, granuloctyes, and mast cells, where their expression is up-regulated during cellular maturation (9). The physiological ligands of PIR-A/PIR-B are still unknown, although it seems likely these receptors participate in the regulation of immune and inflammatory responses (1).

A single Pira gene encodes the invariant PIR-B molecules whereas multiple Pirb genes encode the different PIR-B molecules (refs. 1 and 10; data not shown). The Pira and Pirb genes belong to a subfamily of the Ig gene superfamily, members of which encode activating or inhibitory receptors (11, 12). This subfamily includes the human Ig-like transcripts (ILT) (13) [also known as leukocyte Ig-like receptors (LIR) (14), monocyte/macrophage Ig-like receptors (MIR) (15), and HM18 (16)], the killer cell inhibitory receptors (KIR) (17), and the Fcα receptor (18), the genes for which are located on human chromosome 1q13 (13, 15, 16, 19–21) in a region that is syntenic with the centromeric proximal region on mouse chromosome 7, where the Pira and Pirb genes reside (1).

Two monoclonal antibodies, 6C1 and 10.4, have been generated that recognize PIR-A and PIR-B molecules (9). In this study, we have observed that, although the 6C1 antibody appears to have universal PIR specificity, the 10.4 anti-PIR antibody recognizes a strain-specific allelic determinant on PIR-A and PIR-B molecules. When this allelic determinant was used to trace expression of the parental PIR-A and PIR-B alleles in F1 hybrid mice, we found that PIR molecules encoded by the paternal allele are expressed whereas PIR molecules for the maternal allele are not. These findings extend the principle of genomic imprinting to a multimember family of polymorphic receptors that may participate in innate and specific immune responses.

MATERIALS AND METHODS

Mice. Young Adult A/J, BALB/cJ, C3H, C57BL/6, DBA/1, DBA/2, NZB, SJL, [C57BL/6 (female) × BALB/cJ (male)] F1, and [BALB/cJ (female) × C57BL/6 (male)] F1 mice either were obtained from The Jackson Laboratory or were derived in our animal facility.

Antibodies. Rabbit anti-PIR D1/D2 polyclonal antibodies were prepared, and the 10.4 and 6C1 anti-PIR D1/D2 monoclonal antibodies were phycoerythrin-conjugated as described (9). FITC-conjugated monoclonal antibodies specific for the B220 (RA3–6B2) and Mac-1 (CD11b, M1/70) antigens were obtained from PharMingen. FITC-conjugated anti-IgMb (MB86) and biotin-conjugated anti-IgMα (RS3.1) antibodies were gifts from John F. Kearney (Univ. of Alabama at Birmingham).

Immunofluorescence Analysis. Bone marrow and spleen cell suspensions were stained with fluorochrome-labeled antibodies as described (9) and were examined with a FACS Calibur flow cytometry instrument (Becton Dickinson), and the data were analyzed by using WINMDI 2.7 software [The Scripps Research Institute Cytometry Software Page (http://facs.scripps.edu/software.html)].

Reverse Transcriptase–PCR and Nucleotide Sequence Analysis. To determine the nucleotide sequences of PIR D1/D2 region transcripts, total RNA was isolated from spleen or bone marrow samples by using the TRI Reagent (Molecular Research Center, Cincinnati), converted to first-strand cDNA with oligo(dT)12–18 primers and RNase H– reverse transcriptase (Superscript II, GIBCO/BRL) and amplified with a set of primers: the forward primer 5′-TCCCTAACGCTATCCTCA-

Abbreviations: PIR, paired Ig-like receptors; ILT, Ig-like transcripts; KIR, killer cell inhibitory receptors; LIR, leukocyte Ig-like receptors; MIR, monocyte/macrophage Ig-like receptors.

Data deposition: The sequences reported in this paper have been published in the GenBank database [accession nos. AF148878 (A/J), AF148879 (SJL), and AF148880 (C57BL/6)].

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Preparation of Recombinant PIR Proteins and Enzyme-Based Immunoassay. Recombinant PIR proteins corresponding to mutated and nonmutated versions of the first two amino-terminal extracellular domains (D1 and D2) were produced in Escherichia coli (9). Plastic microtiter plates (Costar) coated with the PIR D1/D2 recombinant proteins (10 μg/ml) were incubated sequentially with varying concentrations of purified 6C1 or 10.4 mAbs, alkaline-phosphatase labeled goat anti-rat Ig antibodies (1 μg/ml; Southern Biotechnology Associates), and the p-nitrophenyl phosphate substrate. The product of the enzyme reaction was measured by light absorbance at 405 nm by using a Titertek Multiskan Plus ELISA reader (ICN).

Immunoblot Analysis of PIR Proteins. PIR proteins in whole cell lysates of splenocytes were isolated with the 6C1 and 10.4 anti-PIR antibodies, were resolved on SDS/PAGE, and then were transferred onto nitrocellulose membranes and immunoblotted with rabbit anti-PIR antibodies as described (9).

RESULTS

Alloreactivity of the 10.4 Anti-PIR Antibody. The 10.4 and 6C1 anti-PIR monoclonal antibodies were produced by heterohybridomas derived by fusion of a nonproducer mouse plasmacytoma with lymph node cells from rats hyperimmunized with a recombinant D1/D2 protein, in a region of sequence identity between BALB/c PIR-B and PIR-A1 molecules (1, 9). Variable patterns of reactivity were observed when the two antibodies were used for immunofluorescence assessment of cells from different strains of inbred mice. Although both anti-PIR antibodies reacted with myeloid and B lineage cells from BALB/c mice, only the 6C1 antibody was found to react with cells from C57BL/6 mice (Fig. 1). Because human KIR and certain ILT/LIR/MIR relatives of the mouse PIR molecules have been shown to have binding specificity for different MHC class I alleles, we examined the expression of the 10.4 allotype in inbred strains of mice with a variety of MHC haplotypes to determine whether expression of the 10.4 determinant is related to the MHC haplotype. The 10.4 anti-PIR antibody was found to react with cells from BALB/c, A/J, and SJL mice and not with the other inbred strains (Table 1). The expression pattern of the 10.4 PIR allelic determinant in this panel of inbred mice thus appeared to be unrelated to the MHC haplotype. The 6C1 anti-PIR antibody by contrast was reactive with the PIR-A and PIR-B molecules in all mouse strains tested.

Analysis of PIR-A and PIR-B Polymorphism. As a first step in examining the basis for the selective 10.4 reactivity with different strains of mice, we determined the nucleotide sequences of the six extracellular Ig-like domains (D1–D6) for PIR-B between each of the inbred strains, and the 10.4–C57BL/6 strain, then compared these extracellular sequences with those determined previously for the BALB/c (10.4+) and B10.A (10.4−) mice (1, 2). This analysis confirmed the presence of an invariant PIR-B sequence in each strain. It also indicated differences in the nucleotide sequences (96–99% homology) for PIR-B between each of the inbred strains, except for the B10.A and C57BL/6 sequences, which were identical. Because the nucleotide variations noted between the 10.4+ A/J and SJL strains were silent, they shared an identical amino acid sequence. The only differences in the PIR-B extracellular regions among these 10.4+ strains of mice (BALB/c, A/J, SJL) were two amino acid residues in D3. The nucleotide homology between the extracellular PIR-B sequences for 10.4+ and 10.4− strains was ≈96%. With regard to the possible location of the 10.4 D1/D2 allelic determinant, several D1 sequence differences were evident between the 10.4+ and 10.4− strains whereas, except for position 97, the amino acid sequences for PIR-B D2 were virtually identical in all strains.

We extended this analysis to PIR-A by sequencing the D1/D2 region of seven PIR-A clones from the 10.4− strain, C57BL/6. These sequences were slightly different from their counterpart BALB/c PIR-A sequences and, for both strains, D2 exhibited greater sequence variation than D1 (Fig. 2B). The

Table 1. Strain distribution of the 10.4 PIR allelic determinant

<table>
<thead>
<tr>
<th>10.4 anti-PIR reactivity*</th>
<th>Inbred mouse strain</th>
<th>MHC haplotype</th>
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<tbody>
<tr>
<td>Positive</td>
<td>A/J</td>
<td>a</td>
</tr>
<tr>
<td>Positive</td>
<td>BALB/c</td>
<td>d</td>
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<td>Positive</td>
<td>SJL</td>
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<tr>
<td>Negative</td>
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<td>Negative</td>
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<tr>
<td>Negative</td>
<td>NZB</td>
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*Tested by immunofluorescence assay.
D2 variability in the different PIR-As was concentrated to three regions of the sequences that were predicted to be on loops between the β-sheets by comparative modeling [SWISS-MODEL (http://www.expasy.ch/swissmod)] based on the structure of a KIR relative (27).

This interstrain comparison of PIR-B (D1-D6) and PIR-A (D1yD2) sequences thus revealed two types of diversity. The first type was represented by allelic variations that were concentrated in the Ig C2-like D1 domain. The second type of diversity featured variable sequence motifs in the V-like D2 domain of PIR-A molecules, and these were conserved in different inbred strains. For example, the extended PSYDR-A-F-N-F-F-A motif was observed in PIR-A sequences of both BALBc and C57Bl6 strains, as was the N-R-M-N-F motif (Fig. 2B). A third extended motif, SQSY-R-Q-V-I-G, in the BALBc PIR-A3 sequence was not seen among the limited panel of C57Bl6 sequences. The predicted surface location of these extended sequence motifs raises the possibility of their potential role in ligand-binding specificity.

Analysis of the 10.4 Allelic Determinant. To refine the definition of the allelic determinant recognized by the 10.4 alloreactive antibody, the 10.4 and 6C1 antibodies were tested for their capacity to bind PIR D1/D2 recombinant proteins corresponding to the first and second Ig-like extracellular domains (D1 and D2) of PIR-B (●), PIR-A1 (■), -A2 (○), -A3 (▲), -A4 (●), and -A6 (□) was assessed by ELISA. (B) Amino acid sequence comparison of the D1 and D2 domains of six different BALB/c cDNA clones (PIR-B and -A1 to -A6) and nine different C57BL/6 clones (C57Bl2 to C57Bl15). The C57BL/6 sequences lack the beginning of D1 and the end of D2 because of the position of the PCR primers. Amino acid identity with the PIR-B sequence (top line) is indicated by dashes (–), and changes are indicated by boldface letters. The D1 Trp residue at position 19 is highlighted in gray to indicate its importance in the likely determinant recognized by the 10.4 antibody.

D2 variability in the different PIR-As was concentrated to three regions of the sequences that were predicted to be on loops between the β-sheets by comparative modeling [swiss-model (http://www.expasy.ch/swissmod/SWISS-MODEL.html)] based on the structure of a KIR relative (27).

This interstrain comparison of PIR-B (D1-D6) and PIR-A (D1/D2) sequences thus revealed two types of diversity. The first type was represented by allelic variations that were concentrated in the Ig C2-like D1 domain. The second type of diversity featured variable sequence motifs in the V-like D2 domain of PIR-A molecules, and these were conserved in different inbred strains. For example, the extended PSYDR-A-F-N-F-F-A motif was observed in PIR-A sequences of both BALB/c and C57BL/6 strains, as was the N-R-M-N-F motif (Fig. 2B). A third extended motif, SQSY-R-Q-V-I-G, in the BALB/c PIR-A3 sequence was not seen among the limited panel of C57BL/6 sequences. The predicted surface location of these extended sequence motifs raises the possibility of their potential role in ligand-binding specificity.

PIR Allelic Expression Pattern in F1 Hybrid Mice. An unanticipated inheritance pattern of the 10.4 PIR allele was observed when F1 hybrid mice derived by breeding 10.4 BALBc mice with the 10.4 C57Bl6 mice were examined (Fig. 3). In [BALB/c (male) × C57BL/6 (female)] F1 mice, the PIR molecules on B cells and myeloid lineage cells expressed the 10.4 BALB/c determinant as expected (Fig. 3, bottom row). In contrast, the 10.4 determinant was not found on bone marrow cells from [C57BL/6 (male) × BALB/c (female)] F1 mice, despite the fact that both parental IgM alleles were expressed and PIR-A/PIR-B expression could be verified with the 6C1 antibody (Fig. 3, third row). This pattern of monoallelic PIR expression was seen in all F1 offspring. To examine the possibility that the 10.4 reactive PIR molecules on cells in

![Figure 2](https://www.nature.com/articles/nature01091/figures/fig2.pdf)
Fig. 3. Immunofluorescence analysis of the inheritance pattern of the 10.4 PIR allotope expression. Bone marrow cells from C57BL/6, BALB/c, [C57BL/6 (male) × BALB/c (female)] F1, and [BALB/c (male) × C57BL/6 (female)] F1 mice were stained with a combination of either the 6C1 (Left) or the 10.4 (Middle) anti-PIR antibodies with the anti-B220 antibody. Splenocytes were stained with anti-IgM a and the anti-B220 antibody to verify the genetic background of the mice.

[C57BL/6 (male) × BALB/c (female)] F1 mice could simply be modulated by interaction with an unknown ligand, an immunoblot analysis of PIR molecules in lysates of splenocytes from the bi-directional (BALB/C × C57BL/6) F1 hybrids and both parent strains was performed by using the 6C1 and 10.4 anti-PIR antibodies. The PIR molecules produced by all of the mice were precipitated by the 6C1 anti-PIR antibody, whereas 10.4-reactive PIR molecules were identified only in BALB/c and the [BALB/c (male) × C57BL/6 (female)] F1 mice (Fig. 4). This analysis thus confirmed the exclusive production of PIR-A and PIR-B molecules bearing the paternal allelic marker.

DISCUSSION

The diverse PIR-A molecules pair with immunoreceptor tyrosine-based activation motif-containing Fc receptor common motifs would be on adjacent loops between the very closely related C57BL/6 and B10.A strains. The allospecificity of the 10.4 anti-PIR antibody allowed us to examine the parental origin of PIR-A and PIR-B molecules produced by BALB/c and C57BL/6 mice by native PIR-A and PIR-B molecules produced by BALB/c mice was confirmed by their immunoprecipitation with the 10.4 antibody.

Monoallelic PIR-A and PIR-B Expression. The allospecificity of the 10.4 anti-PIR antibody allowed us to examine the expression of the parental alleles in F1 mice. In an analysis of the offspring derived from cross-breeding of the 10.4 BALB/c and 10.4 C57BL/6 mice, we observed exclusive expression of the paternal PIR-A and PIR-B molecules. The
maternal PIR-A and PIR-B alleles were not expressed at any stage in the differentiation of B-lineage and myeloid-lineage cells. This analysis thus provides direct evidence for the monoallelic expression of PIR-B and some of the PIR-A molecules. The subset of BALB/c PIR-A molecules for which we currently lack an allelic marker are also likely to be expressed in a monoallelic fashion. Monoallelic expression has not been observed previously for cell surface receptors that are expressed in a monoallelic fashion. Monoallelic expression has been demonstrated for this cluster of mouse genes, as well as for the genomic imprinted Peg3/Pw1 gene, which is a region syntenic with the mouse chromosome 7 region where the Pira and Pirb genes are located on mouse chromosome 7 in a region near the centromere that includes the paternally expressed gene 3 [Peg3 (29)], the human homologue of which, Pw1, is involved in the tumor necrosis factor–NF-κB activation pathway leading to inflammatory and immune responses. However, the Pira and Pirb genes are not expressed previously for cell surface receptors that may be involved in inflammatory and immune responses.

The nearest relatives of the murine PIR-A/PIR-B family of activating and inhibitory receptors, the inhibitory KIR relatives have been shown to be MHC class I alleles (12, 13). The nearest relatives of the murine PIR-A/PIR-B family of activating and inhibitory receptors, the inhibitory PIR-B family of which appears to be dominant, could predispose hematopoietic cells to an increased susceptibility to leukemia because tumor development has been noted after monoallelic mutations of other imprinted genes that affect cell growth (31). Other potential physiological consequences of the monoallelic expression of polymorphic PIR-A and PIR-B molecules are less obvious, given the unknown nature of their ligands. However, the ligands for their human KIR relatives have been shown to be MHC class I alleles (12, 17). The nearest relatives of the murine PIR-A/PIR-B molecules are the human ILT/LIR/MIR molecules, some of which have been shown to recognize MHC class I alleles (22–26). This raises the possibility that the PIR-A/PIR-B molecules also may bind MHC class I molecules.

The genes encoding the KIR and ILT/LIR/MIR receptor families reside on human chromosome 19q13, together with the genomic imprinted Peg3/Pw1 gene, in a region syntenic with the mouse chromosome 7 region where the Pira and Pirb genes reside. Given that monoallelic expression has been demonstrated for this cluster of mouse genes, as well as for the human Peg3/Pw1 gene, it seems reasonable to predict that the ILT/LIR/MIR and KIR genes will be imprinted. If so, the monoallelic expression of these receptors in outbred populations could influence maternal–fetal relationships and perhaps other innate and specific immune responses. Elucidation of the mechanism for the monoallelic expression of the PIR-A/PIR-B molecules therefore could also provide information relevant to the physiological roles of their ILT/LIR/MIR and KIR relatives.

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