Adhesion of human T cells to antigen-presenting cells through SIRPβ2-CD47 interaction costimulates T-cell proliferation

Laura Piccio, William Vermi, Kent S. Boles, Anja Fuchs, Carey A. Strader, Fabio Facchetti, Marina Cella, and Marco Colonna

Signal-regulatory proteins (SIRPs) are transmembrane glycoproteins belonging to the immunoglobulin (Ig) superfamily that are expressed in the immune and central nervous systems. SIRPα binds CD47 and inhibits the function of macrophages, dendritic cells, and granulocytes, whereas SIRPβ1 is an orphan receptor that activates the same cell types. A recently identified third member of the SIRP family, SIRPβ2, is as yet uncharacterized in terms of expression, specificity, and function. Here, we show that SIRPβ2 is expressed on T cells and activated natural killer (NK) cells and, like SIRPα, binds CD47, mediating cell-cell adhesion. Consequently, engagement of SIRPβ2 on T cells by CD47 on antigen-presenting cells results in enhanced antigen-specific T-cell proliferation. (Blood. 2005;105:2421-2427)

Introduction

Signal-regulatory proteins (SIRPs) comprise a family of transmembrane glycoproteins expressed in the immune and central nervous system (CNS). SIRPs are characterized by 3 homologous extracellular immunoglobulin (Ig)-like domains (D1-D3) but have distinct transmembrane and cytoplasmic domains that transduce different signals. The prototypical member of the SIRP family, SIRPα, is expressed in macrophages, dendritic cells (DCs), granulocytes, neurons, and astrocytes. SIRPs binds CD47, integrin-associated protein, which is ubiquitously expressed and functions in cell adhesion and migration. SIRPα-CD47 binding, stimulation of cells with various growth factors, and cell-cell adhesion induce phosphorylation of immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in the cytoplasmic domain of SIRPα. Phosphorylated ITIMs recruit the SH2 domain-containing protein tyrosine phosphatases SHP-2 and SHP-1, which inhibit tyrosine kinase-coupled signaling pathways. Thus, SIRPα is an inhibitory receptor that modulates macrophage and DC function, as well as signaling pathways induced by growth factors and cell adhesion. In addition, SIRPα mediates cell-cell adhesion in the immune system and CNS, supporting fusion of macrophages; DC–T-cell interactions; migration of DCs, monocytes, and neutrophils; neurite extension and synapse formation. A second member of the SIRP receptor family, SIRPβ1, is also expressed in myeloid cells. However, it does not bind CD47 and lacks cytoplasmic ITIMs capable of recruiting phosphatases and mediating inhibitory signals. In fact, SIRPβ1 contains a single basic lysine residue within the hydrophobic transmembrane domain that mediates association with an adapter protein, DAP12 or KARAP, which contains a cytoplasmic tyrosine-based activating motif (ITAM). Thus, engagement of SIRPβ1 activates myeloid cells, leading to cytokine release, tyrosine phosphorylation, and calcium (Ca2+) mobilization.

A third member of the SIRP family, SIRPβ2, was recently identified. SIRPβ2 transcripts are variably expressed in many human tissues, including the brain, lung, and placenta, and are particularly abundant in liver. The predicted SIRPβ2 protein is highly homologous to SIRPα and SIRPβ1 in the extracellular domain, but lacks both cytoplasmic ITIMs and the transmembrane lysine required for association with DAP12. Thus, it is unclear whether and how SIRPβ2 mediates signaling; the ligand for SIRPβ2 is also unknown.

We investigated expression, specificity, and function of the SIRPβ2 protein and found that SIRPβ2 is quite distinct from SIRPα and SIRPβ1. SIRPβ2 is the only member of the SIRP family that is expressed on T cells, CD56 bright natural killer (NK) cells, and all activated NK cells. SIRPβ2 does bind CD47, albeit with less affinity than SIRPα. This interaction mediates cell-cell adhesion, rather than inhibitory signals. The adhesion mediated by contact of SIRPβ2 on T cells with CD47 on antigen-presenting cells (APCs) promotes antigen-specific T-cell proliferation and costimulates T-cell activation.

Materials and methods

Cells

Human peripheral blood mononuclear cells (PBMCs) were separated from peripheral blood of healthy donors by Ficoll gradient centrifugation. CD56+ CD3− NK cells were separated from PBMCs by cell sorting and...
cultured in medium containing recombinant interleukin 2 (IL-2), phytohemagglutinin (PHA), and irradiated feeder cells. CD47-deficient Jurkat T cells (Jurkat-CD47)\(^2\) were kindly provided by Bill Frazier (Washington University School of Medicine, Saint Louis, MO).

**cDNAs and transfectants**

Full-length SIRP\(_x\) (NM_080792), SIRP\(_b\) (NM_006065), and SIRP\(_b\) (NM_018556) cDNAs were amplified by reverse transcriptase-polymerase chain reaction (RT-PCR), cloned into pCDNA3 (Invitrogen, Carlsbad, CA) or pMX,\(^3\) and transfected into the murine T-cell hybridoma BW (BW-SIRP\(_x\), BW-SIRP\(_b\), BW-SIRP\(_b\)). Expression of SIRPs on stably transfected cells was assessed by flow cytometry using monoclonal antibody (mAb) 14B,\(^4\)\(2\)

**SIRP-IgG fusion proteins**

We expressed the 2 membrane distal immunoglobulin domains (D1D2) of SIRP-IgG fusion proteins with the Fc portion of human IgG. SIRP cDNA fragments were amplified by PCR with the primer pairs indicated in Table 1 and cloned into pFLAG-CMV1 (Sigma, St Louis, MO) in frame with a cDNA fragment encoding the Fc portion of human IgG fusion proteins.\(^3\) SIRP-D1D2-IgG chimeric cDNAs were transiently expressed in 293 cells using Lipofectamine (Invitrogen) and secreted SIRP-IgG fusion proteins were purified from culture supernatant on protein A (Pharmacia Amersham, Uppsala, Sweden).

**Antibodies**

To obtain mAbs against SIRP\(_b\) (clone LSB1.50, mouse IgG1) and SIRP\(_b\) (clone LSB2.20, mouse IgG1), we immunized mice with SIRP\(_b\)-D1D2-IgG and SIRP\(_b\)-D1D2-IgG, respectively. We selected hybridomas that specifically stained BW-SIRP\(_b\) or BW-SIRP\(_b\). mAb 14B has been described.\(^2\) The mAbs against CD2, CD4, CD8, CD3, CD20, and CD56 are mouse IgG2a and IgG2b (Beckman-Coulter Immunotech, Birmingham, AL), followed by streptavidin conjugated with allophycocyanin (Molecular Probes, Eugene, OR).

**Immunohistochemistry and immunofluorescence**

Specimens from human tissues included reactive lymph nodes and thymuses removed for diagnostic purposes or during cardiac surgery. Cryostat sections of frozen specimens were air dried overnight at room temperature and fixed in acetone for 10 minutes before staining. SIRP\(_b\) was detected with mAb LSB2.20, followed by biotinylated anti-immunoglobulin multilinks secondary antibody (Biogenex, San Ramon, CA) and streptavidin-immunoperoxidase. In 2-color immunofluorescence, LSB2.20 was detected with fluorescein isothiocyanate (FITC)–conjugated isotype-specific antibody (Southern Biotechnology); CD3 (rabbit polyclonal; Dako, Glostrup, Denmark) and CD11c (LeuM5; BD Biosciences) were revealed with biotinylated secondary antibodies (Vector Laboratories, Burlingame, CA) when conjugated with BW transfectants. Alternatively, Jurkat and Jurkat-CD47\(_0\) cells were stained with Vibrant (Molecular Probes) and CFSE (Molecular Probes) when conjugated with BW transfectants. Alternatively, Jurkat and Jurkat-CD47\(_0\) cells were stained with Vibrant (Molecular Probes) and CFSE before conjugation. Various combinations of 2 of these cell types (2×10\(^5\) of each) were mixed, spun down, and incubated at 37°C for 30 minutes in the presence or absence of antibodies against SIRPs and CD47. After one wash, binding of fusion proteins to cells was detected by flow cytometry using a biotinylated mouse antihuman IgG-Fc (BD Biosciences) followed by streptavidin conjugated with allophycocyanin (Molecular Probes).

**Immunoprecipitations**

Cells were surface labeled with 1 mCi (37 MBq) using the sulfosuccinimidyl-3-(4-hydroxyphenyl)propionate method. Labeled cells were lysed in 1% Triton X-100, 100 mM Tris (tris(hydroxymethyl)aminomethane)–HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA (ethylenediaminetetraacetic acid), 1 mM phenylmethysulfonfluoride (PMSF), 10 μg/mL aprotinin, and 10 μg/mL leupeptin. After overnight preclearing with protein G-Sepharose, lysates were incubated with mAb LSB2.20, mAb148, or isotype-matched control mAb at 4°C for 4 hours, and immune complexes were precipitated by addition of protein G-Sepharose for 1.5 hours. Precipitates were washed 3 times with lysis buffer, followed by a final wash with 10 mM Tris–HCl, pH 7.4, 15 mM NaCl, and then resuspended in reducing sample buffer. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis was performed according to a standard procedure. Gels were dried and exposed to autoradiography film (Amersham Pharmacia Biotech, Piscataway, NJ) for 2 to 5 days.

**Binding assay**

SIRP-IgGs (100 μg/mL) were incubated with various cells for 10 minutes at 37°C and 30 minutes on ice in the presence or absence of antibodies against SIRPs and CD47. After one wash, binding of fusion proteins to cells was detected by flow cytometry using a biotinylated mouse antihuman IgG-Fc (BD Biosciences) followed by streptavidin conjugated with allophycocyanin (Molecular Probes).

**Cell conjugations**

BW-SIRP\(_b\) was labeled with carboxy-fluoresceindiacetate-succinimidyl-ester (CFSE) (CFSE) (Molecular Probes). Jurkat and Jurkat-CD47\(_0\) cells were stained with anti-CD45-allophycocyanin (Beckman-Coulter Immunotech) when conjugated with BW transfectants. Alternatively, Jurkat and Jurkat-CD47\(_0\) cells were stained with Vibrant (Molecular Probes) and CFSE before conjugation. Various combinations of 2 of these cell types (2×10\(^5\) of each) were mixed, spun down, and incubated at 37°C for 30 minutes in the presence or absence of antibodies against SIRPs, CD47, or CD18 (HB203, mouse IgG1; American Type Culture Collection [ATCC], Manassas, VA). Conjugates were gently resuspended in a small volume of medium for flow cytometric analysis on a FACSCalibur (BD Biosciences).

**T-cell assays**

The CD4\(^+\) SIRP\(_b^+\) T-cell clone V\(_B^3\)\(_P^3\) was generated from the peripheral blood of a healthy donor and selected for expression of T-cell receptor (TCR)–V\(_B^3\). This clone (5×10\(^5\)) was incubated with irradiated B-lymphoblastoid cells RPMI 8866 (10\(^3\); kindly provided by Bice Perussa, Philadelphia, PA) that had been pulsed for 2 hours with serial dilutions of *Staphylococcus* enterotoxin E (SEE). Anti-CD47 mAb (B6H12), anti-SIRP\(_b\) (LSB2.20), or control mouse IgG was added to T\(_B^\)-cell cocultures as indicated. After 48 hours, T-cell proliferation was measured by standard \(^3\)H-thymidine incorporation assay. Mixed lymphocyte cultures (MLCs) were performed by incubating 10\(^3\) PBMCs from a healthy donor (responder) with graded numbers of allogeneic immature DCs (stimulators). Culture supernatants were collected after 4 days and interferon γ (IFN-γ) was measured by cytometric bead array (BD Biosciences). T-cell proliferation was measured by standard \(^3\)H-thymidine incorporation assay. For costimulation assays, CD4\(^+\) T cells were purified from human peripheral blood by anti-CD4 magnetic microbeads (Miltenyi Biotec, Auburn, CA) and plated on serial dilution of mAb anti-CD3 (OKT3; ATCC) and 50 μg/mL mAbs against SIRP\(_b\), CD28 (Beckman-Coulter Immunotech), or control IgG1 (anti-CD19; technology). Sections were examined with a fluorescence microscope Olympus BX60, equipped with a DP-70 Olympus digital camera (Olympus, Melville, NY). Images were acquired using analySIS Image Processing software (Soft Imaging System GmbH, Münster, Germany).

**Table 1. Oligonucleotide primers for construction of SIRP-IgG fusion proteins**

<table>
<thead>
<tr>
<th>SIRP(_x)-D1D2 forward</th>
<th>5′-TAGTACAGAGTGTGATGGCAGCAGGACGACGG-3′</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIRP(_b)-D1D2 reverse</td>
<td>5′-TAGTACAGAGTGTGATGGCAGCAGGACGACGG-3′</td>
</tr>
<tr>
<td>SIRP(_b)-D1D2 forward</td>
<td>5′-TAGTACAGAGTGTGATGGCAGCAGGACGACGG-3′</td>
</tr>
<tr>
<td>SIRP(_b)-D1D2 reverse</td>
<td>5′-TAGTACAGAGTGTGATGGCAGCAGGACGACGG-3′</td>
</tr>
<tr>
<td>Human IgG-Fc forward</td>
<td>5′-TAGTACAGAGTGTGATGGCAGCAGGACGACGG-3′</td>
</tr>
<tr>
<td>Human IgG-Fc reverse</td>
<td>5′-TAGTACAGAGTGTGATGGCAGCAGGACGACGG-3′</td>
</tr>
</tbody>
</table>

Restriction sites are underlined.

---

From www.bloodjournal.org by guest on August 30, 2017. For personal use only.
Results

**SIRPβ2 is expressed on CD4⁺ T cells, CD8⁺ T cells, CD56bright NK cells, and all activated NK cells**

To obtain a SIRPβ2-specific mAb we immunized mice with a recombinant protein consisting of the 2 membrane-distal IgG domains of SIRPβ2 fused to the Fc portion of human IgG (SIRPβ2-D1D2-IgG). Hybridoma supernatants were selected for their ability to stain BW transfectants expressing full-length SIRPβ2 (BW-SIRPβ2). The mAb LSBB.20 stained BW-SIRPβ2, but not BW-SIRPβ1 or BW-SIRPβ3, demonstrating absolute specificity for SIRPβ2 (Figure 1). mAb 148, which recognizes SIRPα and SIRPβ1, also stained BW-SIRPβ2 and hence has a broad specificity for all SIRPs (Figure 1). Using mAb LSBB.20, we evaluated the cellular distribution of SIRPβ2 in PBMCs. SIRPβ2 was detected on all T cells, including CD4⁺ and CD8⁺ T cells, as well as a few CD20⁺ cells, which may correspond to a B-cell subset. SIRPβ2 was not expressed on NK cells directly isolated from blood, with the exception of CD56bright NK cells in most donors. However, it was up-regulated on all NK cells upon activation in vitro with IL-2, feeder cells, and PHA (Figure 2). SIRPβ2 was also expressed on several T and NK cell lines, including Jurkat and NK92 (data not shown). In contrast, monocytes, DCs, and granulocytes did not express SIRPβ2 (data not shown).

Analysis of SIRPβ2 expression in human lymph nodes revealed that SIRPβ2 is mainly present in the paracortical T-cell area (Figure 3A), whereas only a few SIRPβ2⁺ cells were observed in the mantle and germinal center of B-cell follicles (Figure 3A). Two-color immunofluorescence analysis showed that these SIRPβ2⁺ cells coexpress CD3 and therefore correspond to T cells (Figure 3B-C). Examination of the paracortical area at high magnification revealed clustering of SIRPβ2⁺ T cells around interdigitating DCs, which did not express SIRPβ2 (Figure 3D). In the human thymus SIRPβ2⁺ lymphocytes were primarily located in the medulla, whereas no expression was detected on the majority of cortical thymocytes (Figure 3E). Thus, SIRPβ2 is selectively expressed on mature T lymphocytes that have undergone thymic selection.

We have previously shown that mAb 148 detects SIRPα and SIRPβ1 on monocytes, granulocytes, and DCs. Because mAb 148 recognizes SIRPβ2 on transfected cells (Figure 1), one would expect this mAb to stain peripheral T cells, as does mAb LSBB.20. However, we found that mAb 148 does stain monocytes, granulocytes, and DCs, but not T cells or Jurkat cells. This unexpected discrepancy between the staining patterns of mAbs LSBB.20 and 148 suggests that the SIRPβ2 protein expressed on T cells and activated NK cells may differ significantly from that expressed on BW transfectants, possibly due to cell-specific posttranslational modifications. On the other hand, SIRPβ2 may be expressed as an alternatively spliced form that lacks one of the predicted domains of the protein. Accordingly, analysis of SIRPβ2 transcripts by RT-PCR revealed that T-cell mRNA includes not only a SIRPβ2 full-length transcript, but also 2 alternatively spliced forms that lack either one or 2 membrane-proximal Ig domains (GenBank accession nos. AY748247, AY748248, and NM_080816). To investigate if mAbs 148 and LSBB.20 detect different isoforms of SIRPβ2, we compared 148 and LSBB.20 immunoprecipitates from BW cells transfected with SIRPβ2. Moreover, we analyzed the biochemical characteristics of SIRPβ2 in a mutated Jurkat cell line, which lacks CD47 (Jurkat-CD47⁻). This T-cell line expresses high levels of SIRPβ2, which are detected by LSBB.20 but not 148. In LSBB.20 immunoprecipitates, SIRPβ2 appeared as a broad cluster of approximately 45- to 50-kDa proteins, most likely reflecting heterogeneous glycosylation (Figure 4). Moreover, LSBB.20 immunoprecipitates included a sharp approximately 30-kDa protein, which may correspond to the alternatively spliced form of SIRPβ2 that lacks the membrane-proximal Ig domain and contains only one site for N-linked glycosylation (AY748247 and AY748248). In contrast, the mAb 148 only detected a major about 50-kDa protein (Figure 4). Thus, T cells express
isoforms of SIRPβ2 that are preferentially recognized by the SIRPβ2-specific mAb LSB2.20 rather than the anti-SIRP mAb 148, which may explain why SIRPs were not previously detected on T cells and NK cells.

**SIRPβ2 is a receptor for CD47**

To investigate whether SIRPβ2 expressed on T cells and activated NK cells recognizes CD47 we tested the ability of a SIRPβ2 IgG fusion protein to bind the T-cell line Jurkat, which expresses CD47, and Jurkat-CD470 by flow cytometry. SIRPβ2-D1D2-IgG bound Jurkat but not Jurkat-CD470; the binding was totally blocked by mAb B6H12 and 36-61.3, which recognize CD47, and partially inhibited by mAbs 148 and LSB2.20, which recognize SIRPβ2, corroborating the specificity of binding (Figure 5). Importantly, binding of SIRPβ2-D1D2-IgG to Jurkat consistently yielded a lower median fluorescence intensity than did binding of SIRPα-D1D2-IgG in flow cytometry, suggesting that the affinity of SIRPβ2 for CD47 is lower than that of SIRPα (Figure 5). Of note, mAb 148 completely abrogated binding of SIRPα-D1D2-IgG to Jurkat but only partially reduced that of SIRPβ2-D1D2-IgG, confirming its preferential recognition of SIRPα versus SIRPβ2. These results demonstrate that SIRPβ2 is a receptor for CD47, although it probably binds with lower affinity than SIRPα.

**SIRPβ2-CD47 interaction mediates cell-cell adhesion**

Because SIRPβ2 lacks a cytoplasmic domain with known signaling motifs or a transmembrane residue allowing association with DNAX activation protein 12 (DAP12)/killer cell activating receptor-associated protein (KARAP), its involvement in inhibitory or activating signaling is unlikely. Accordingly, we observed that antibodies against SIRPβ2 alone do not activate or inhibit NK cell–mediated lysis of Fc receptor–positive target cells in redirected cytotoxicity assays (data not shown). Given this, we hypothesized that SIRPβ2 may be involved in cell-cell adhesion rather than inhibitory or activating signaling.

To test this, we mixed BW cells transfected with SIRPβ2 (BW-SIRPβ2) with Jurkat or Jurkat-CD470. After 30 minutes of incubation at 37°C we measured formation of conjugates by 2-color flow cytometry. Under these conditions, BW-SIRPβ2 made abundant conjugates with Jurkat but not with Jurkat-CD470 (Figure 6A). Conjugate formation was partially blocked by anti-SIRP and anti-CD47 antibodies, confirming the specificity of the interaction. In contrast, antibodies against CD18 (Figure 6A) or CD11a (data not shown) did not significantly block cell conjugation, suggesting that SIRPβ2-CD47 interaction mediates cell conjugation by a mechanism that is independent of leukocyte function–associated molecule-1 (LFA-1). To corroborate that SIRPβ2-CD47 interaction...
mediates cell-cell adhesion, we compared the ability of Jurkat, which expresses both CD47 and SIRPβ2, and Jurkat-CD47\(^{\text{top panel}}\), Jurkat and Jurkat-CD47\(^{\text{bottom panel}}\), and Jurkat-CD47\(^{\text{alone (middle panel). Jurkat-CD47 cells form fewer conjugates with itself than when mixed with Jurkat, and fewer than Jurkat forms with itself. Jurkat and Jurkat-CD47 were stained with Vibrant and CFSE before conjugation. Notably, Jurkat-CD47 cells express higher levels of CD47 than Jurkat cells (top histograms, gray profiles). This high expression of SIRPβ2 may allow Jurkat-CD47\(^{\text{cells to form as many conjugates with Jurkat cells as Jurkat forms with itself.}}\}

**Discussion**

Here we demonstrate that SIRPβ2 is a unique member of the SIRP receptor family; it is the only SIRP that has been detected on T cells and activated NK cells. Despite considerable homology among the SIRPs, previously established anti-SIRP antibodies failed to detect SIRPβ2 on T cells and NK cells. Accordingly, the newly generated mAb LSB2.20 specific for SIRPβ2 preferentially detected posttranslational modifications or alternative spliced forms of SIRPβ2 that

T-cell proliferation was strongly inhibited by the anti-CD47 antibody and partially inhibited by the anti-SIRPβ2 mAb, consistent with the established abilities of anti-CD47 and anti-SIRPβ2 antibodies to block SIRPβ2-CD47 interactions (Figures 5 and 6A). Similarly, mAbs against SIRPβ2 and CD47 inhibited T-cell proliferation and T-cell secretion of IFN-γ triggered by allogeneic immature DCs in mixed lymphocyte reactions (Figure 7B-C), indicating that SIRPβ2-CD47 interaction is important in promoting not only T-cell proliferation but also cytokine secretion.

To further investigate the T-cell stimulatory function of SIRPβ2, we determined whether engagement of SIRPβ2 can enhance activation of CD4\(^{+}\)T cells in the presence of serial dilution of a TCR ligand. The anti-SIRPβ2 mAb enhanced the proliferation of peripheral blood CD4\(^{+}\) T cells in the presence of suboptimal concentration of anti-CD3 (Figure 7D). Remarkably, ligation of SIRPβ2 was almost as effective as the engagement of CD28 in costimulating T-cell proliferation. Thus, we conclude that SIRPβ2-CD47 interaction enhances superantigen-dependent T-cell proliferation and has a critical role as an accessory costimulatory molecule on T cells.

**Figure 6. Conjugate formation between cells expressing SIRPβ2 and CD47.** (A) BW-SIRPβ2 transfectants make conjugates with Jurkat but not with Jurkat-CD47\(^{\text{top panel}}\). Conjugation is partially blocked by anti-CD47 and anti-SIRP antibodies, whereas no significant inhibition is observed with an antibody against CD18 (\(\mu\)2 integrin). Percentages of conjugates are indicated next to the upper right quadrants. (B) The frequency of conjugation between Jurkat alone (top panel), Jurkat and Jurkat-CD47\(^{\text{bottom panel}}\), and Jurkat-CD47\(^{\text{alone (middle panel). Jurkat-CD47 forms fewer conjugates with itself than when mixed with Jurkat, and fewer than Jurkat forms with itself. Jurkat and Jurkat-CD47 were stained with Vibrant and CFSE before conjugation. Notably, Jurkat-CD47 cells express higher levels of CD47 than Jurkat cells (top histograms, gray profiles). This high expression of SIRPβ2 may allow Jurkat-CD47\(^{\text{cells to form as many conjugates with Jurkat cells as Jurkat forms with itself.}}\).**

**Figure 7. SIRPβ2-CD47 interaction enhances superantigen-mediated T-cell-mediated proliferation and costimulates T-cell activation.** (A) The CD4\(^{+}\) SIRPβ2\(^{\text{Vβ3+}}\) T-cell clone Vβ3\(^{\text{P345}}\) was incubated with irradiated B lymphoblastoid cells RPMI 8866 that had been pulsed with serial dilutions of SEE (ng/mL). Anti-CD47 (\(\bigcirc\)), anti-SIRPβ2 (\(\bigtriangleup\)), or control mouse antibodies (\(\bigtriangleup\)) were added to T/B-cell cocultures as indicated. T-cell proliferation was measured by \(^{3}\)H-thymidine incorporation assay. (B-C) Blockade of SIRPβ2-CD47 interaction with mAbs partially inhibits T-cell proliferation and IFN-γ production triggered by allogeneic immature DCs in MLCs. Symbols represent same as in panel A; in addition, \(\bigtriangleup\) indicates medium, and \(\bigtriangleup\), 148. (D) CD4\(^{+}\) T cells purified from peripheral blood were plated on serial dilution of anti-CD3 antibody (µg/mL) in the presence of fixed amounts of mAbs against SIRPβ2 (\(\bigtriangleup\)), CD28 (\(\bigtriangleup\)), or control IgG (\(\bigtriangleup\)). T-cell proliferation was measured after 72 hours as described.
may occur in T cells and NK cells, creating unique epitopes undetected by previously established anti-SIRP antibodies.

Remarkably, SIRPβ2 can bind CD47, providing T cells and NK cells with a cell surface molecule capable of interacting with CD47. Because SIRPβ2 lacks a cytoplasmic domain with known signaling motifs or a transmembrane residue allowing association with DAP12/KARAP. SIRPβ2 does not deliver activating or inhibitory signals on its own. SIRPβ2-CD47 interaction mediates strong cell-cell adhesion and supports T cell-APC contact, enhancing antigen presentation and consequent T-cell proliferation and cytokine secretion. In contrast, we did not detect a significant effect of SIRPβ2-CD47 interaction on CD8 T-cell– or NK cell–mediated cytotoxicity (data not shown). This discrepancy may reflect the differential impact of SIRPβ2 on these disparate functions. T-cell proliferation requires sustained activation of T cells, and therefore SIRPβ2-CD47 interactions may significantly contribute to this process by stabilizing T cell-APC binding. In contrast, SIRPβ2-CD47 adhesion may be dispensable for the more transient interactions that mediate T-cell– and NK cell–mediated cytotoxicity. Further insight might be provided by investigating the behavior of SIRPβ2 in the formation and stabilization of T-cell synapse.

SIRPβ2 enhanced T-cell proliferation induced by suboptimal concentration of T-cell receptor ligand. Whether SIRPβ2 acts as a costimulator similar to CD28 or synergizes with TCR signaling by other mechanisms is presently unknown. Interestingly, it has been shown that engagement of CD47 with some antibodies also results in augmentation of T-cell activation and that the costimulatory function of CD47 depends on its capacity to induce cell spreading. Thus, SIRPβ2 may facilitate T-cell activation by a similar mechanism. It is also possible that SIRPβ2-CD47 interaction promotes other functions of T cells and NK cells dependent on cell-cell adhesion, such as attachment to endothelial cells and transmigration into lymph nodes or peripheral tissues, as previously reported for SIRPs-CD47.

The characterization of SIRPβ2 in this study provides strong evidence for structural and functional diversity of the SIRP receptors. To date, 3 SIRPs have been characterized, each with a different affinity for CD47 and distinct signaling properties. Whereas SIRPα4,7 and SIRPβ2 (this study) bind CD47, a soluble form of SIRPβ1 encompassing the 2-membrane distal IgG domains does not (data not shown). We showed that the binding of SIRPα to CD47 is stronger than that of SIRPβ2. These differences in specificity may depend on the diversity of SIRP extracellular domains. Moreover, 15 distinct SIRP cDNAs have been reported in the literature2 and 2 additional SIRP loci, called protein tyrosine phosphatase nonreceptor type substrate 1-like 2 (PTPN1L2) and PTPN1L3, have been annotated in the National Center for Biotechnology Information (NCBI) database. Thus, SIRP family diversity may be even broader than presently known, due to the additional SIRP genes and, possibly, polymorphisms of the SIRPα, SIRPβ1, and SIRPβ2 genes as well. Similar mechanisms of diversification have been observed for other immune gene loci, particularly those encoding KIRs, LILRs, and Ly49s.6,37,38 What selective pressure is responsible for evolution and diversification of SIRP molecules? One clue to this question is provided by the observation that poxviruses encode homologues of CD47.39 Thus, it is possible that the SIRP diversity reflects a sort of arms race between the host and poxviruses, in which viruses try to exploit or disrupt endogenous SIRP-CD47 interactions to elude immune responses, whereas the host counteracts this viral strategy by changing the specificity and function of endogenous SIRPs.

**Acknowledgments**

We thank Susan Gilfillan and Bill Frazier for critically reading the manuscript; Francesca Gentili (supported by Fondazione Beretta, Brescia, Italy) for performing immunohistochemical analysis.

**References**


Adhesion of human T cells to antigen-presenting cells through SIRPβ2-CD47 interaction costimulates T-cell proliferation

Laura Piccio, William Vermi, Kent S. Boles, Anja Fuchs, Carey A. Strader, Fabio Facchetti, Marina Cella and Marco Colonna

Updated information and services can be found at:
http://www.bloodjournal.org/content/105/6/2421.full.html

Articles on similar topics can be found in the following Blood collections
- Cell Adhesion and Motility (790 articles)
- Immunobiology and Immunotherapy (5504 articles)
- Signal Transduction (1930 articles)

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml