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Role for CD47-SIRPα signaling in xenograft rejection by macrophages

Kentaro Ide[*§], Hui Wang[†§], Hiroyuki Tahara[*], Jianxiang Liu[‡], Xiaoying Wang[‡], Toshimasa Asahara[*], Megan Sykes[†], Yong-Guang Yang[†¶], Hideki Ohdan[*¶]

[*]Department of Surgery, Division of Frontier Medical Science, Programs for Biomedical Research, graduate School of Biomedical Science, Hiroshima University, Hiroshima 734-8551, Japan, [†]Bone Marrow Transplantation Section, Transplantation Biology Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02129, United States, [‡]Neuroprotection Research Laboratory, Department of Radiology and Neurology, Massachusetts General Hospital, and Program in Neuroscience, Harvard Medical School, Boston, MA 02129, United States.

Corresponding author:
Hideki Ohdan, MD, PhD. Department of Surgery, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University 1-2-3 Kasumi, Minami-Ku, Hiroshima, 734-8551, Japan. TEL: +81-82-257-5222, FAX: +81-82-257-5224, E-mail: hohdan@hiroshima-u.ac.jp and Yong-Guang Yang, MD, PhD. Bone Marrow Transplantation Section, Transplantation Biology Research Center, Massachusetts General Hospital, Harvard Medical School, MGH-East, Building 149, 13th Street, Boston, MA 02129, United States. TEL: 617-726-6959, FAX: 617-724-9892, E-mail: yongguang.yang@tbrc.mgh.harvard.edu

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Abstract

We have previously proven that human macrophages can phagocytose porcine cells even in the absence of antibody or complement opsonization, indicating that macrophages present a pivotal immunological obstacle to xenotransplantation. A recent report indicates that the signal regulatory protein (SIRP) α is a critical immune inhibitory receptor on macrophages, and its interaction with CD47, a ligand for SIRPα, prevents autologous phagocytosis. Considering the limited compatibility (73%) in amino acid sequences between pig and human CD47, we hypothesized that the interspecies incompatibility of CD47 may contribute to the rejection of xenogeneic cells by macrophages. In the present study, we have demonstrated that porcine CD47 does not induce SIRPα tyrosine phosphorylation in human macrophages, and soluble human CD47-Fc fusion protein inhibits the phagocytic activity of human macrophages toward porcine cells. In addition, we have verified that manipulation of porcine cells for expression of human CD47 radically reduces the susceptibility of the cells to phagocytosis by human macrophages. These results indicate that the interspecies incompatibility of CD47 significantly contributes to the rejection of xenogeneic cells by macrophages. Genetic induction of human CD47 on porcine cells could provide inhibitory signaling to SIPRα on human macrophages, providing a novel approach to preventing macrophage-mediated xenograft rejection.

Introduction

The ability to transplant pig organs into humans would resolve the current crisis in the supply of cadaveric human organs for the treatment of end stage organ failure (1, 2). Humans lack a functional α1, 3-galactosyltransferase (GalT) gene and therefore do not express Galα1,3Galβ1,4GlcNAc (Gal) carbohydrate residues, and produce abundant natural antibodies (Abs) to the Gal epitope (3). These anti-Gal Abs are a major barrier to xenotransplantation of pig organs into humans, because hyperacute rejection and acute humoral rejection are initiated by their binding to Gal determinants that are ubiquitously present on porcine cells. The genetically engineered GalT-knockout pigs that no longer express Gal epitopes appear promising in conferring protection against this type of xenograft rejection (4). Following such a significant step forward, the subsequent barriers of a vigorous cellular immune response to xenografts are currently a major concern. The survival of hearts from GalT-deficient pigs in baboons can be markedly prolonged under a chronic immunosuppressive regimen, but these are eventually rejected (5). A promising approach to conquering such inevitable rejection involves inducing tolerance of xenoreactive T cell in pig-to-human transplant by achieving mixed hematopoietic chimerism (7).

In addition to T cells, the mononuclear phagocyte system plays a critical role in the vigorous cellular immune response toward xenografts. Macrophages mediate rapid rejection of porcine hematopoietic cells (8, 9) and pancreatic islets (10), which express little

or no Gal antigen (11). After infusion into baboons, these cells are avidly scavenged by primate macrophages and perhaps other reticuloendothelial system cells (including liver Kupffer cells and splenic red pulp macrophages) (12). Based on the fact that macrophages can be activated by pro-phagocytic signaling pathways through activating receptors, such as Fcγ and complement receptors, complement-mediated and Ab-mediated phagocytosis by macrophages could be a mechanism for targeting opsonized porcine cells. The use of genetically engineered pigs deficient for the GalT gene and/or transgenic for human complement regulatory proteins might overcome such obstacles (13, 14). However, we have recently reported that human reticuloendothelial (RE) macrophages can phagocytose porcine cells in the absence of antibody or complement-opsonization, and that removing Gal epitopes from porcine cells failed to prevent this phagocytosis (15). This suggests that regulation of macrophages in human recipients might be needed to achieve successful engraftment of porcine islet xenografts or marrow grafts used for tolerance induction even if GalT-deficient pigs were used. However, the long-term use of macrophage-depleting reagents is unlikely to be acceptable in the clinical setting, even if nontoxic drugs could be developed, because macrophages play a critical role in initiating immune responses toward pathogens. The elucidation of mechanisms for phagocytozing unopsonized porcine xenogeneic cells by human macrophages should provide further insights into the understanding of the robust xenoreactivity of macrophages, and may lead to the development of approaches for attenuating macrophage mediated xenograft rejection.

CD47, known as integrin-associated protein, is a ubiquitously expressed 50-kDa cell

surface glycoprotein, that serves as a ligand for signal regulatory protein (SIRP) α (also known as CD172a, SHPS-1), an immune inhibitory receptor on macrophages. CD47 and SIRPα constitute a cell-cell communication system (the CD47-SIRPα system) that plays important roles in a variety of cellular processes including cell migration, adhesion of B cells, and T cell activation (16-19). In addition, the CD47-SIRPα system is implicated in negative regulation of phagocytosis by macrophages. CD47 on the surface of several cell types (i.e. erythrocytes, platelets or leukocytes) can protect against phagocytosis by macrophages by binding to the inhibitory macrophage receptor SIRPα. The role of CD47/SIRPα interactions in the recognition of self and inhibition of phagocytosis has been illustrated by the observation that primary, wild-type mouse macrophages rapidly phagocytose unopsonized red blood cells (RBCs) obtained from CD47-deficient mice but not those from wild-type mice (20). It has also been reported that through its SIRPα receptors, CD47 inhibits both Fcγ and complement receptor mediated phagocytosis (21). Considering the limited compatibility (73%) in amino acid sequences between pig and human CD47 (22), we hypothesized that interspecies CD47 incompatibility might contribute to the rejection of xenogeneic cells by human macrophages.

Results

Porcine CD47 does not induce SIRPα tyrosine phosphorylation in human macrophages. In the CD47-SIRPα system, the ligation of SIRPα on macrophages by CD47 on target cells inhibits phagocytosis by promoting cytoplasmic domain phosphorylation of tyrosine and recruitment of Src homology (SH) 2 domain-containing protein tyrosine phosphatase (SHP)-1, which is the major regulator of phagocytic responses (23). In order to determine whether porcine CD47 can interact with human SIRPα, we assessed SIRPα tyrosine phosphorylation in a human macrophage cell line after contact with either porcine or human porcine RBCs. Western blotting revealed that the incubation of human macrophages with human RBCs resulted in detectable SIRPα tyrosine phosphorylation as expected (Fig. 1). Following incubation with porcine RBCs, SIRPα tyrosine phosphorylation was not induced in human macrophages above the level in control macrophages that were incubated with medium alone, indicating that porcine CD47 fails to induce SIRPα tyrosine phosphorylation in human macrophages.

Soluble human CD47-Fc fusion protein partially inhibits the phagocytic activity of human macrophages toward porcine cells. We next examined whether recombinant soluble human CD47-Fc fusion protein (which contains the extracellular domain of human CD47 fused to the Fc portion of human Ig), which itself drives an inhibitory signaling pathway to human macrophages through CD47-SIRPα interaction (24), could prevent the porcine cells from being phagocytosed by human macrophages. Carboxyfluorescein succinimidyl ester (CFSE)-labeled porcine lymphoblastoid cell line (LCL) cells were used

as targets and human RE macrophages were used as effectors in the phagocytosis assay, in which the macrophages engulfing target porcine cells could be identified by confocal microscopy and flow cytometric (FCM) analyses. Because of the limited availability of appropriate human samples, the phagocytic properties of human RE macrophages have not been extensively investigated. We isolated human RE macrophages from the perfusion effluents of liver allografts used in clinical liver transplantation. Freshly isolated human RE macrophages showed phagocytosis of porcine LCL cells in the absence of antibody and complement (Fig. 2), although unmanipulated peripheral monocyte/macrophages did not (data not shown). We have confirmed that porcine LCL cells express porcine CD47 by using mouse anti human CD47 (BRIC126), which cross-reacts with porcine CD47, by FCM analysis. Since their expression levels were somewhat lower that those on freshly isolated porcine peripheral lymphocytes or RBCs, we could not rule out the possibility that the porcine LCL cells express CD47 in concentrations lower than those necessary to produce a "do not phagocytosize". However, in our previous study, porcine peripheral lymphocytes and RBCs expressing intact porcine CD47 were robustly phagocytosed by human RE macrophages, indicating that normal porcine CD47-signaling is ineffective (15). Mature resident macrophages differentiate from circulating monocytes and occupy the peripheral tissues and organs. These macrophages can employ a broad array of antimicrobial effector mechanisms, including phagocytosis of the pathogen. Hence, the difference in phagocytotic activity against porcine cells between the peripheral monocyte/macrophages and RE macrophages is presumably derived from the differences in their maturation and anatomical environments. Nevertheless, in the presence of human CD47-Fc, the

phagocytic activity of human RE macrophages toward porcine cells was partially but significantly suppressed (Fig. 2).

Human CD47 expression on porcine cells radically reduced the susceptibility of these cells to phagocytosis by human macrophages. To further determine whether human CD47 expression on porcine cells could efficiently prevent their phagocytosis by human RE macrophages, we generated human CD47-expressing porcine cell lines by transfecting porcine cells with a human CD47-expressing plasmid. The human CD47-expressing plasmid was prepared by inserting full-length human CD47 cDNA into the expression vector pKS336. LCL cells were transfected with either pKS336-human CD47 or the empty plasmid, and stable cell lines were obtained by blasticidin S selection. Human CD47 expression on the transfected porcine LCL cells was confirmed by FCM analysis and RT-PCR (Fig. 3). The pKS336-human CD47 vector-transfected porcine LCL cells expressed human CD47 on their surface, resembling human PBMCs, whereas both untreated porcine LCL cells and the control vector-transfected porcine LCL cells tested negative for human CD47. These LCL cells were compared for effects on the phagocytic activity of human RE macrophages. The macrophages that phagocytosed the target cells could be identified as CD14 and CFSE double positive cells (Fig. 4). As expected, human RE macrophages did not phagocytose human PBMCs, even in an allogeneic combination. In contrast, human RE macrophages actively phagocytosed porcine LCL cells without opsonization. However, human CD47 expression on porcine cells radically reduced the susceptibility of these cells to phagocytosis by human RE macrophages. Compared with

the inhibiting effect of soluble human CD47-Fc on phagocytic activity of human RE macrophages toward porcine cells, that of human CD47 expression on target porcine cells was much more profound, probably reflecting more efficient CD47-SIRPα signaling via cell-cell contact. These results indicate that the interspecies CD47 incompatibility significantly contributes to the rejection of xenogeneic cells by macrophages and that the genetic manipulation of porcine cells for human CD47 expression could partially overcome such macrophage-mediated xenograft rejection.

Discussion

It has been reported that CD47-SIRPα interactions exhibit limited cross-species reactivity probably because of species-specific posttranslational modifications of CD47 such as glycosylation, i.e. CD47 on pig but not mouse, cow, or rat RBCs bind the recombinant extracellular dominant of human SIRPα1 (25). In addition, we have recently demonstrated that pig CD47 does not interact with mouse SIRPα (26). However, it remained to be elucidated if pig CD47-human SIRPα interactions could deliver a signal inhibiting engulfment or not. In the present study, we have proven that porcine CD47 fails to induce SIRPα tyrosine phosphorylation in human macrophage-like THP-1 cells, and does not inhibit engulfment by human macrophages toward porcine cells.

While macrophages can be activated by pro-phagocytic signaling pathways through activating receptors, such as Fcγ and complement receptors, their phagocytic activity is also controlled by the signal strength of immune inhibitory receptors (21). Besides Fcγ and complement receptors, it has been postulated that lectin-mediated carbohydrate binding provides activating signals to macrophages without opsonization (27). A recent study has shown that Galectin-3, a ~30-kDa lectin composed of a terminal carbohydrate recognition domain, which is responsible for the specific recognition of β-galactose (Galβ(1-3/4) Glc NAc) and an N-terminal domain, is expressed in human macrophages (28). This lectin has been shown to be a receptor for xenoantigens, including not only α-Gal but also N-glycolylneuraminic acid, which is expressed on porcine cells. Galectin-3 binds to porcine cells much more strongly than to human cells. GalT-deficient pigs may not

eliminate macrophage-mediated rejection because removal of the GalT enzyme could leave many of the N-acetyllactosamine structures uncapped, as seen in GalT deficient mice (29). It is not yet clear if inhibitory signals such as CD47-SIRPα will override all activating signals delivered to macrophages by xenoantigens. In this study, the CD47-SIRPα inhibitory signal appeared to overcome whatever activating signal for macrophage phagocytosis was being delivered. Although inhibitory signaling seems to be predominant, elimination of xenoantigen-induced activating signals may also be required to completely abolish phagocytic activity of human macrophages toward porcine cells, consistent with our finding that human CD47 expression on porcine cells markedly reduced but did not completely eliminate the susceptibility of these cells to phagocytosis by human RE macrophages. (7)

In the present study, soluble human CD47-Fc fusion protein partially inhibits the phagocytic activity of human RE macrophages toward porcine LCL cells. However, the reason for the solubility of CD47-Fc not being a comparably effective blocker against macrophage phagocytosis to the recombinant expression of human CD47 can only be speculated. Either the cross-linking required for CD47 signaling or CD47 have to be expressed on the same cells for the protective effect against phagocytosis. In order to address these possibilities, we have attempted mixing human RBCs expressing intact human CD47 with LCL cells to examine if this will prevent phagocytosis. Even when human RE macrophages were constantly in contact with human RBCs, they continued to display unabated phagocytotic activity against porcine LCL cells (Fig 5, which is published

as supporting information on the PNAS web site), suggesting that CD47 expression on the target cells is critical for the complete protective effect. It might be possible that CD47-Fc nonspecifically binds to the cellular membrane of LCL cells and provides a signal that partially protects these cells against phagocytosis by RE macrophages.

It is well known that innate immune responses mediated by monocytes/macrophages may drive and shape the process of adaptive immunity. Phagocytic activities of macrophages are a first line of defense against invading infectious microbes, and the phagocytosed macrophages can present antigen derived from such foreign pathogens to T cells. It is likely that these mechanisms also take place in xenotransplantation from phylogenetically distant species. Therefore, specific elimination of phagocytic activity of human macrophages toward porcine cells by genetically inducing human CD47 might also attenuate subsequent T cell immune responses against porcine antigens, while maintaining normal responses against other pathogens.

In conclusion, we have demonstrated a lack of cross-reactivity between a major macrophage inhibitory receptor between pig and human. This discovery provides further insight into on understanding of the robust xenoreactivity of macrophages, and may lead to the development of approaches for attenuating macrophage mediated xenograft rejection, i.e., the genetic manipulation of porcine cells for human CD47 expression could provide a novel approach for preventing macrophage-mediated xenograft rejection.

Materials and Methods

Cell cultures. All cells were maintained at 37°C under a humidified atmosphere of 5% CO_2 in air. A porcine lymphoblastoid cell line (LCL) was kindly provided by Dr. Christene Huang (Harvard Medical School, Boston, USA.). Cells were cultured in RPMI1640 containing 10% fetal calf serum (FCS) with 5 μM 2-mercaptoethanol (2-ME) (Katayama, Osaka, Japan), 10% HEPES buffer (Gibco, NY), and 100 IU/ml penicillin-100 μg/ml streptomycin (Gibco). CHO-Ras-hCD47 cells producing human CD47-Fc were kindly provided by T. Matozaki (Gunma University, Gunma, Japan) (17). CHO-Ras-hCD47 cells were cultured in αMEM (Sigma) supplemented with 2 mM L-glutamine, 10 mM HEPES and 10% FCS and 100 IU/ml penicillin-100 μg/ml streptomycin and 500 μg/ml Geneticin and 500 μg/ml Zeocin. The human CD47-Fc fusion protein was then purified from such culture supernatants by column chromatography on recombinant Protein G (Amersham Pharmacia Biotech). Human IgG, Fc fragment (Chemicon) was used as control. The human macrophage cell line THP-1 (ATCC, Manassas, VA) was cultured in RPMI1640 containing 10% FCS with 5 μM 2-ME, 10% HEPES buffer, and 100 IU/ml penicillin-100 μg/ml streptomycin. Differentiation of THP-1 cells was achieved in 100 ng/mL phorbol myristate acetate (PMA) for 2 days and confirmed by attachment of these cells to tissue-culture plastic.

Expression vectors and transfection to LCL cells. The entire coding region of the CD47 cDNA was PCR-amplified from reverse-transcribed human lymphocyte cDNA with primers (sense) 5'-TGGACTCGACCATGTGGCCCCTGGTAGC-3' and (antisense)

5’-GGAGCGGCCGCCTATTATTCATCATTCATC-3’. Amplified PCR product was digested with *Xho*I/*Not*I and cloned into pKS336 vector (kindly provided by Dr. M. Saijo, National Institute of Infectious Diseases, Tokyo, Japan.) (30), which had been predigested with the same restriction endonucleases (Fig. 6, which is published as supporting information on the PNAS web site) and the sequence was verified using an ABI PRISM 3100 Sequencer (PE Applied Biosystems). LCL cells were transfected with pKS336-hCD47 or pKS336 (control transfection) using DMRIE-C Reagent as described by the manufacturer (Invitrogen). Stable transfectants were selected by culturing in RPMI containing blasticidin S (Invitrogen). Cell surface expression of CD47 was confirmed by immunofluorescence labeling and FACS analysis using anti-human CD47 mAbs (B6H12, BD PharMingen or BRIC126,).

Reverse transcription PCR analysis. Total RNA was isolated from human peripheral blood mononuclear cells (PBMC), porcine LCL and transfected porcine LCL according to standard procedures using ISOGEN (NIPPON GENE CO., LTD.), and cDNA was synthesized with a First-Strand cDNA Synthesis Kit (Amersham Biosciences Corp.). The primers used for the detection of human CD47 were 5’-TATACTCCTGTTCTGGGGAC-3’ and 5’-TGGTATACACGCCGCAATAC-3’. As a control for the presence of amplifiable RNA, GAPDH primers were utilized (sense; 5’-TATACTAATGTTCTGGGGAC-3’ and antisense; 5’-TGGTATACACGCCGCAATAC-3’). Amplified PCR products were analyzed by agarose gel (10%) containing ethidium bromide.

Isolation of human reticuloendothelial (RE) macrophages. Human RE macrophages were isolated as described previously (15). In brief, the mononuclear cells were isolated from the perfusion effluents of liver allografts for clinical liver transplantation by gradient centrifugation with Separate-L (Muto Pure Chemicals Co., Ltd, Tokyo, Japan). Human RE macrophages were purified using Monocyte Isolation Kit II (Miltenyi Biotec, Bergisch Gladbach, Germany) and a magnetic activated cell sorting (MACS) system in accordance with the manufacturer's instructions. The purity of $CD14^+$ macrophages was confirmed by flow cytometric (FCM) analysis immunofluorescence using FACSCalibur (Becton Dickinson, Mountain View, CA). More than 95% of the cells demonstrated positivity for the CD14 antigen. Without any pre-culture, freshly isolated human RE macrophages were immediately subjected to the phagocytosis assays**.** Ethical approval for this study was obtained from the Ethics Committee at The Hiroshima University Hospital. Informed consent was obtained from all donors for participation in this study.

Immunoprecipitation and immunoblotting. Differentiated THP-1 cells (2×10^6) were incubated in serum-free medium for 12h before experiments and rinsed once with PBS. Then, 2×10^7 human or porcine RBCs, which were suspended in 2 ml of serum-free Iscove's modified DMEM (IMDM) supplemented with 2 mM sodium pervanadate (Sigma), were added into the macrophage cultures, and incubated in a 37°C water bath for 30 min. The cells were lysed in 0.4ml of lysis buffer [50mM Tris-HCl (pH 7.5), 150mM NaCl, 1% NP-40, 1mM phenylmethylsulfonyl fluoride (PMSF), 1% protease inhibitor cocktail (Sigma) and 2mM sodium pervanadate]. For immunoprecipitation, the lysates were

mixed with mouse anti-human SIRPα antibodies and a 50% slurry of protein G–Sepharose beads by rotation at 4°C for 2 hrs. Precipitated proteins were separated by 10% SDS-PAGE, followed by blotting to a nitrocellulose membrane. Rabbit immunoaffinity purified anti-phosphotyrosine IgG (Upstate, Charlottesville VA) and goat anti-rabbit HRP-conjugated IgG (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) were used as primary and secondary antibodies, respectively.

Phagocytosis assay. Target cells were stained with the fluorescent dyes 5/6-carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Inc., Eugene, OR) according to the manufacturer's protocol. After CFSE-labeling, dead cells were removed from the target cells using a Dead Cell Removal Kit (Miltenyi Biotec) and MACS system in accordance with the manufacturer's instructions. CFSE-labeled target cells (4×10^5) were incubated with human RE macrophages (4×10^5) in 96-well polystyrene tissue culture plates (BD Labware, Franklin Lakes, NJ) in the absence of antibodies and complement factors. In some experiments, various volumes of autologus human RBCs were added. The macrophages engulfing target porcine cells could be identified as CFSE-labeling cells by flow cytometric (FCM) analyses. This was also confirmed by confocal microscopy (Fig. 7, which is published as supporting information on the PNAS web site). The cells were harvested at the indicated times and stained with allophucocyanin (APC)-conjugated mouse anti-human CD14 (M5E2) (BD Pharmingen) prior to flow cytometric analysis. Assays were performed in triplicate and repeated on at least three different days, using different macrophage donors.

Footnotes

Author contributions: K.I., H.W., X.W., T.A., M.S., Y-G.Y. and H.O. designed research; K.I., H.W., H.T. and J.L. performed research; K.I., H.W., Y-G.Y. and H.O. analyzed data; K.I., M.S., Y-G.Y. and H.O. wrote the paper.

The authors declare no conflict of interest.

[§]K.I. and H.W. contributed equally to this work.

[¶]To whom correspondence should be addressed. E-mail: hohdan@hiroshima-u.ac.jp or yongguang.yang@tbrc.mgh.harvard.edu

Abbreviations

Abs	antibodies
CFSE	Carboxyfluorescein succinimidyl ester
FCM analyses	flow cytometric analyses
Gal	Galα1,3Galβ1,4GlcNAc
GalT	α1, 3-galactosyltransferase
LCL	lymphoblastoid cell line
PBMC	peripheral blood mononuclear cell
RE macrophage	reticuloendothelial macrophage
SHP-1	Src homology 2 domain-containing protein tyrosine phosphatase-1
SIRPα	signal regulatory protein α

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Figures Legends

Fig. 1. SIRPα tyrosine phosphorylation in human macrophages was induced by incubation with human red blood cells but not porcine red blood cells (RBCs). Differentiated THP-1 cells were incubated with human or porcine RBCs at 37°C in a water bath for 30 min. The cells were lysed and the lysates were mixed with mouse anti-human SIRPα antibodies and 50% slurry of protein G–Sepharose beads by rotation at 4°C for 2 hrs. Precipitated proteins were separated by 10% SDS-PAGE, followed by blotting to a nitrocellulose membrane. Rabbit immunoaffinity purified anti-phosphotyrosine IgG and goat anti-rabbit HRP-conjugated IgG were used as primary and secondary antibodies, respectively. Porcine RBCs alone (lane 1), human RBCs alone (lane 2), THP-1 cells incubated in medium alone (lane 3), or human macrophage cell line THP-1 cells incubated with porcine (lane 4) or human (lane 5) RBCs are shown. IP, immunoprecipitation; IB, immunoblotting; anti-pY, anti-phosphotyrosine.

Fig. 2. Recombinant soluble human CD47-Fc partially protected the porcine cells from phagocytosis by human macrophages. Human RE macrophages were incubated with 10μg human IgG, Fc fragment (open bars) or 10μg recombinant soluble human CD47-Fc fusion protein (closed bars) for 5 min before the addition of CFSE-labeled porcine lymphoblastoid cell line (LCL) cells. The macrophages that had phagocytosed the LCL cells were identified as CFSE and CD14 double-positive cells by FCM analysis. Data shown are normalized to the level of phagocytosis of LCL cells in the absence of Fc

fragment. Data are the means ± SD for three separate experiments.

Fig. 3. Expression of human CD47 on a transfected porcine lymphoblastoid cell line (LCL) was confirmed by FCM analysis and RT-PCR. (A) Representative histograms obtained by FCM analysis for human PBMCs, untreated porcine LCL cells, pKS336-transfected porcine LCL cells and pKS336-human CD47-transfected porcine LCL cells are shown. Thin and bold lines represent staining with isotype control and anti-human CD47 mAb, respectively. (B) Expression of human CD47 on transfected porcine LCL was confirmed by RT-PCR using a specific pair of primers. Lane 1, human PBMC; Lane 2, non-transfected porcine LCL; Lane 3, pKS336-transfected porcine LCL; Lane 4, pKS336-human CD47-transfected porcine LCL. GAPDH was used as a DNA loading control.

Fig. 4. Human CD47-expressing porcine lymphoblastoid cell line (LCL) cells attenuate phagocytosis by human reticuloendothelial (RE) macrophages. (A) CFSE-labeled human PBMC, porcine LCL cells, pKS336-transfected porcine LCL cells and pKS336-human CD47-transfected porcine LCL cells were incubated with RE macrophages for 4 h at 37 °C. Macrophages counterstained with APC-conjugated anti-human CD14 and phagocytosis of CFSE-labeled targets were measured by FCM analysis. (A) Representative FCM profiles are shown. Upper left quadrant: region of non-phagocytosing macrophages. Upper right quadrant: region of phagocytosing macrophages. Lower right quadrant: region of residual targets. Percentages of total cells in each region are shown. (B) Phagocytic activity was

calculated by the following formula: phagocytic activity = (percentage of upper right quadrant: region / percentage of upper left quadrant: region + percentage of upper right quadrant: region) × 100. Data are the means ± SD for four separate experiments.

Fig. 1

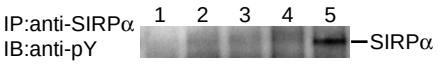


Fig.2

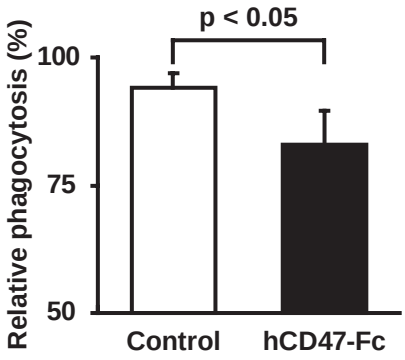


Fig. 3

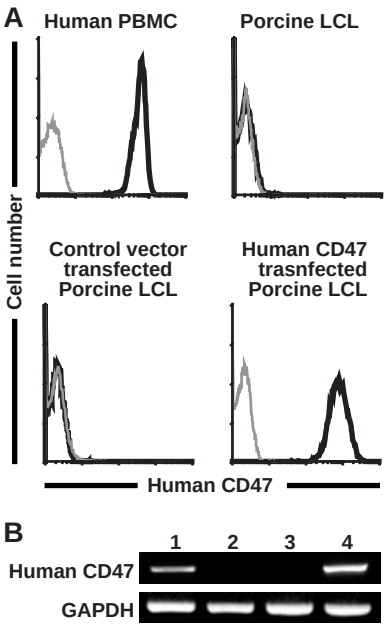
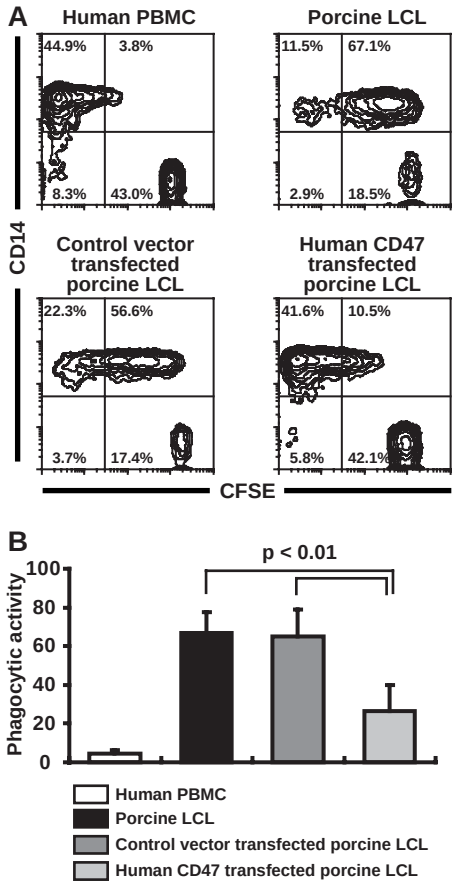

A
Human PBMC
Porcine LCL
Control vector transfected Porcine LCL
Human CD47 trasnfected Porcine LCL
Cell number
Human CD47
B
1
2
3
4
Human CD47
GAPDH

Fig. 4


A
Human PBMC
Porcine LCL
44.9%
3.8%
8.3%
43.0%
11.5%
67.1%
2.9%
18.5%
CD14
Control vector transfected porcine LCL
Human CD47 transfected porcine LCL
22.3%
56.6%
3.7%
17.4%
41.6%
10.5%
5.8%
42.1%
CFSE
B
p < 0.01
Phagocytic activity
100
80
60
40
20
0
Human PBMC
Porcine LCL
Control vector transfected porcine LCL
Human CD47 transfected porcine LCL