의학석사학위논문

이종 면역반응에서 인간 CD200과 CD47의 대식세포 억제능 연구

Suppression of human macrophages by human CD200 and human CD47 during xenogeneic immune response

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Abstract

Suppression of human macrophages by human CD200 and human CD47 during xenogeneic immune response

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Background: CD200 and CD47 are inhibitory proteins expressed on a variety of cells and they bind to their receptors to exert inhibitory functions. CD47 suppresses the phagocytic activity of macrophages through SIRPα ligation and CD200-CD200R interaction induces the intracellular signaling of CD200R and promotes immune modulation such as suppression of cell adhesion and cytokine release, thus

enhancing engraftment and immune tolerance. The aim of this study is to compare the suppressive activity of human CD200 and human CD47 against human macrophages and assess the additive effects of both molecules in the interaction of porcine endothelial cells (PECs) and human macrophages.

Materials and Methods: Porcine endothelial cell lines that stably overexpress human CD200 or CD47 were established by human CD200 or CD47 containing recombinant lentivirus construction followed by viral transduction. Human macrophages were incubated with recombinant PECs to evaluate suppressive effect of human CD200 and CD47 on cytotoxicity, phagocytosis, secretion of pro-inflammatory cytokines and proliferation of human macrophages during in the xenogeneic immune reaction.

Results: The present study demonstrated that overexpression of both human CD200 and human CD47 in PECs suppressed the xenogeneic immune response of human macrophages. Human CD200 and CD47 inhibited cytotoxicity such as cell necrosis and early and late apoptosis and suppressed phagocytosis of human macrophages. Also, human CD200 and CD47 negatively controlled pro-inflammatory cytokine secretion and proliferation of human macrophages in response to xenogeneic stimulation. Furthermore, the co-expression of human CD200 and human CD47 had additive effects in suppressing macrophage activation in response to xenogeneic stimulation.

Conclusion: both human CD200 and human CD47 suppressed the activation of human macrophages in response to xenogeneic stimulation. Moreover, the suppressive activity of the co-expression of human CD200 and human CD47 was more effective against macrophage activation than that of the expression of either CD200 or CD47 alone. Therefore, the generation of double-transgenic pigs with human CD200 and human CD47 could be a promising approach for improving xenograft survival.

Keywords: Xenotransplant, Macrophage, CD200, CD47, porcine

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Introduction

Despite successful outcomes after transplantation, donor shortage is still the biggest obstacle in organ transplantation. As an alternative solution to address organ shortage, pig-to-human xenotransplantation has been investigated, and some barriers such as hyperacute xenograft rejection have been overcome.

The xenogeneic immune response consists of three stages of xenograft rejection including hyperacute rejection, acute vascular rejection, and cellular xenograft rejection. (1-3) Hyperacute rejection is caused by a high titer of natural antibodies against the α-1,3-Gal xenoantigen (α-gal). These α-gal epitopes exist on the surface of the vascular endothelium of all species except humans and primates. (1) Acute vascular rejection, which is also known as delayed xenograft rejection, is a second barrier that is associated with the endothelial cell damage, thrombosis, and infiltration of the grafts with mononuclear cells. The next obstacle to xenotransplantation is cellular xenograft rejection. Cellular xenograft rejection involves T cells or innate immune cells and triggers a stronger immune response than allogeneic cellular rejection. (3)

Among the various innate and adaptive immune cells involved in xenograft rejection, macrophages play an important role in xenograft rejection. (4) In the early stage following xenotransplantation, graft-infiltrating macrophages respond to ischemia-reperfusion injuries by destroying nearby tissues and mediating T cell

immune responses. (5) Macrophages are believed to function as a key mediator of acute vascular rejection, involving donor endothelial cell damage with mononuclear cell infiltration, where macrophages are activated by xenoreactive antibody-dependent cellular cytotoxicity. (6, 7) Moreover, macrophages contribute to cellular xenograft rejection by either direct toxicity or amplifying T cell-mediated immune responses. (8) Macrophages can be activated by MCP-1 and IFN-γ, which are produced by activated T cells, and infiltration of macrophages to the graft tissue leads to further T cell activation. (9) Macrophages are also activated by either galectin-3 binding to α-gal or toll-like receptor ligation. When these macrophage receptors bind to xenoantigens, macrophages secrete TNFα, IL-1, and nitric oxide and exert a cytotoxic effect on donor tissues. (10)

Considering the important roles of macrophages in xenograft rejection, macrophage activation needs to be regulated to improve xenograft survival. CD47 is an inhibitory molecule that is expressed on a variety of cells and binds to the receptor CD172a (signal regulatory protein, SIRPα) on myeloid cells such as macrophages and dendritic cells. CD47 suppresses the phagocytic activity of macrophages through SIRPα ligation and subsequent intracellular signaling. SIRPα contains two immunoreceptor tyrosine-based inhibitory motifs (ITIMs) with phosphatase activity in its cytoplasmic region (SHP-1 and SHP-2). Several xenograft transplantation studies have been conducted with CD47. In a study, the death of only CD47 knock-out cells occurred when the cells from wild-type mice

and CD47 knock-out mice were co-transplanted; thus, CD47 was first proposed as an inhibitory molecule. (11) Furthermore, during allogeneic hematopoietic cell transplantation, the blood cells of CD47 knock-out mice were rapidly eliminated from wild-type mice (12), and the level of the CD47-SIRPα interaction was found to have a positive correlation with phagocytosis inhibition. (13) In regard to species incompatibility, porcine CD47 could not interact with the SIRPα of mouse macrophages, whereas porcine cells with transgenic expression of mouse CD47 could avoid phagocytosis by mouse macrophages. (14) In addition, porcine cells with human CD47 demonstrated decreased *in vitro* phagocytosis by human macrophages. (15) The survival of pig skin tissue has been extended following administration of porcine cells with human CD47, and the transgenic expression of human CD47 has enhanced engraftment in a murine model of pig-to-human hematopoietic cell transplantation. (16, 17) All these findings reveal the contribution of CD47 interspecies incompatibility to xenograft rejection and suggest the beneficial role of species-specific CD47 expression for a successful xenograft engraftment.

Similar to CD47, CD200 is one of the inhibitory molecules involved in the regulation of macrophage pro-inflammatory immune responses to maintain body homeostasis. Therefore, they can be target molecules for macrophage inhibition in the xenogeneic immune response. Although both CD200 and CD47 are expressed on various cells, their receptors are restricted to myeloid cells such as macrophages

and monocytes. A short cytoplasmic tail of CD200 and CD47 might indicate the absence of intracellular signaling; however, their receptors, CD200R and SIRPα, have longer cytoplasmic domains that enable intracellular signaling after binding with their ligands. In the CD200-CD200R interaction, ligation of CD200 to CD200R induces the intracellular signaling of CD200R and promotes immune modulation such as suppression of cell adhesion and cytokine release, thus enhancing engraftment and immune tolerance. Unlike other inhibitory receptors, the intracellular signaling of CD200R is not associated with ITIMs but with three tyrosine residues. (18) Among the tyrosine residues of CD200R, the distal residue has a NpxY domain that binds to the phosphotyrosine-binding domain of Dok1, Dok2, and Shc. (19) Dok proteins phosphorylate Ras GTPase (RasGAP) and SH2-containing inositol phosphatase (SHIP) proteins, leading to the inhibition of mitogen-activated protein kinase (MAPK) and NF-κB. Inhibition of MAPK and NF-κB suppresses the secretion of pro-inflammatory cytokines (IL-1, IL-6, and TNFα) and increases the secretion of anti-inflammatory cytokines (IL-10 and TGF-β). (20) In addition to direct cell-to-cell inhibition, the CD200-CD200R interaction has an indirect inhibitory effect on T cells by inducing regulatory T cells and activating indoleamine 2, 3-dioxygenase (IDO), thus suppressing antigen-presenting cells and T cells. (21)

The immunomodulatory effects of CD200 have been studied in various transplantation models. CD200-Fc infusion can extend allograft survival in rat-to-

mouse models of skin and kidney transplantation (22), and the CD200-Fc-induced suppression of mouse skin allograft rejection may be interfered by synthesized N-terminal proteins of CD200 and CD200R. (23) Genetic manipulation of CD200 or CD200 receptor also has demonstrated the important role of the CD200-CD200R interaction in allograft survival. Wild-type recipients require both CD200 and CD200R1 expression on graft tissues for prolongation of allograft survival, and in comparison with wild-type grafts, CD200 transgenic grafts stimulate antigen-specific cytotoxic T lymphocytes to a lesser degree in draining lymph nodes. (24) Furthermore, grafts with transgenic expression of CD200R can increase allograft survival in CD200 transgenic recipients. Prolonged allograft survival by CD200 overexpression in recipients is associated with elevated levels of regulatory T cells and increased IL-35 and IDO expression. (25-28) In studies using CD200R knock-out recipients, allograft survival was not increased in CD200 transgenic recipients. (29, 30) These data suggest that the CD200-CD200R interaction plays an important role in the suppression of allograft rejection. Furthermore, CD200-Fc also prolonged xenograft survival in rat-to-mouse models of islet, skin, and kidney xenotransplantation. (22, 31) However, no studies have investigated whether the CD200-CD200R interaction can suppress the xenogeneic immune response in discordant xenogeneic interactions beyond concordant xenogeneic interactions.

Therefore, it would be interesting to compare the effect of human CD200 overexpression in porcine endothelial cells (PECs) with that of human CD47

overexpression in PECs on the xenogeneic immune response of human macrophages. Here, we compared the suppressive activity of human CD200 and human CD47 against human macrophages and assessed the additive effects of both molecules in the interaction of PECs and human macrophages. We also compared the suppressive effects of the transmembrane and secretory forms of both molecules.

Materials and Methods

Target gene preparation

The commercial plasmid vector was obtained from the Harvard Plasmid Database (Clone ID: HsCD00417195 – human CD200, HsCD00326795 – human CD47). The human inserts (CD200 and CD47) of the plasmid vectors were acquired by PCR amplification using a forward primer encoding BamHI (CAAGGATCCGATGGAGAGGCTGGTGATCAGG) and a reverse primer encoding EcoRI (GCAGAATTCTTAGGGCTCTCGGTCCTGATTCC) for CD200 (Figure 1A) as well as a forward primer encoding BamHI (CAAGGATCCGATGTGGCCCCTGGTAGCGGCG) and a reverse primer encoding xbal (CCCTCTAGATCAGTTATTCCTAGGAGGTTGTAT) for CD47 (Figure 1B). The human immunoglobulin G_4 ($hIgG_4$) Fc fragment (672 bp) was kindly provided by Prof. Junho Chung (Seoul National University, Seoul, Republic of Korea).

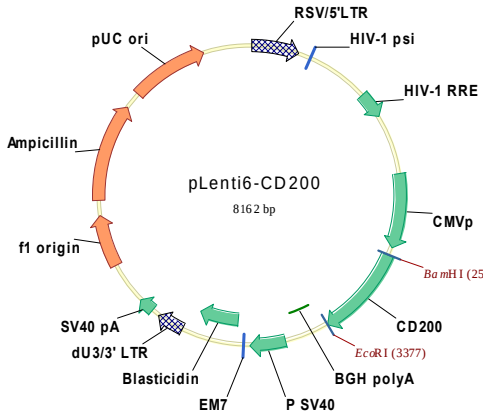
Recombinant lentivirus production

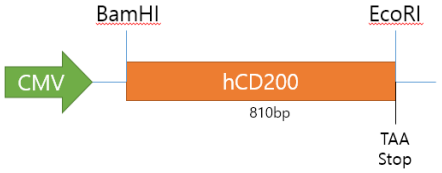
Lentiviruses containing the target proteins or mock vector were produced by transient co-transfection of a three-plasmid system (Figure 1C). HEK293 cells were transiently co-transfected with the target plasmid, packaging plasmid, and envelope plasmid using calcium phosphate transfection buffer. The virus-containing cell culture media was collected 3–4 days after co-transfection, filtered through a 0.45-μm filter, and centrifuged at 50,000 × *g* for 2 h. The virus particle pellet was resuspended by adding an appropriate volume of Hanks' Balanced Salt Solution.

Viral transduction

PECs were seeded at a density of 2×10^5 cells per well in complete DMEM media 24 h prior to lentiviral transduction. Viral transduction was performed by adding the lentivirus in 5 mL of cell culture media, and cells were incubated (37°C, 5% CO_2) for 10 days (Figure 1C). Culture media (5 mL) was added 24 h after transduction. The cells were cultured in 10 mL of fresh media and passaged every other day.

A





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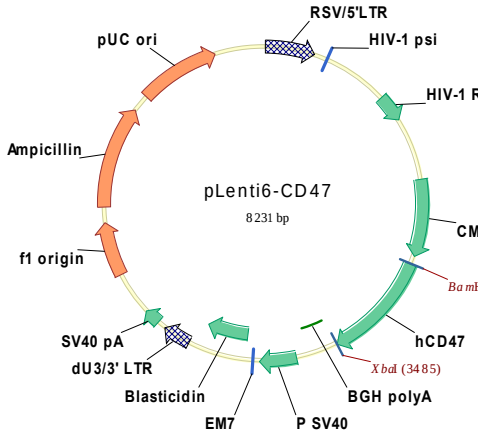
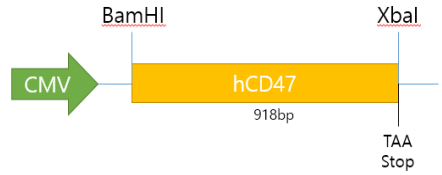
CD200-R EcoRI	GCAGAATTCTTAGGGCTCTCGGTCCTGATTCC

B





pLenti-Fg-CD47	
CD47-F BamHI	CAAGGATCCGATGTGGCCCCTGGTAGCGGCG
CD47-R (918) XbaI	CCCTCTAGATCAGTTATTCCTAGGAGGTTGTAT

C

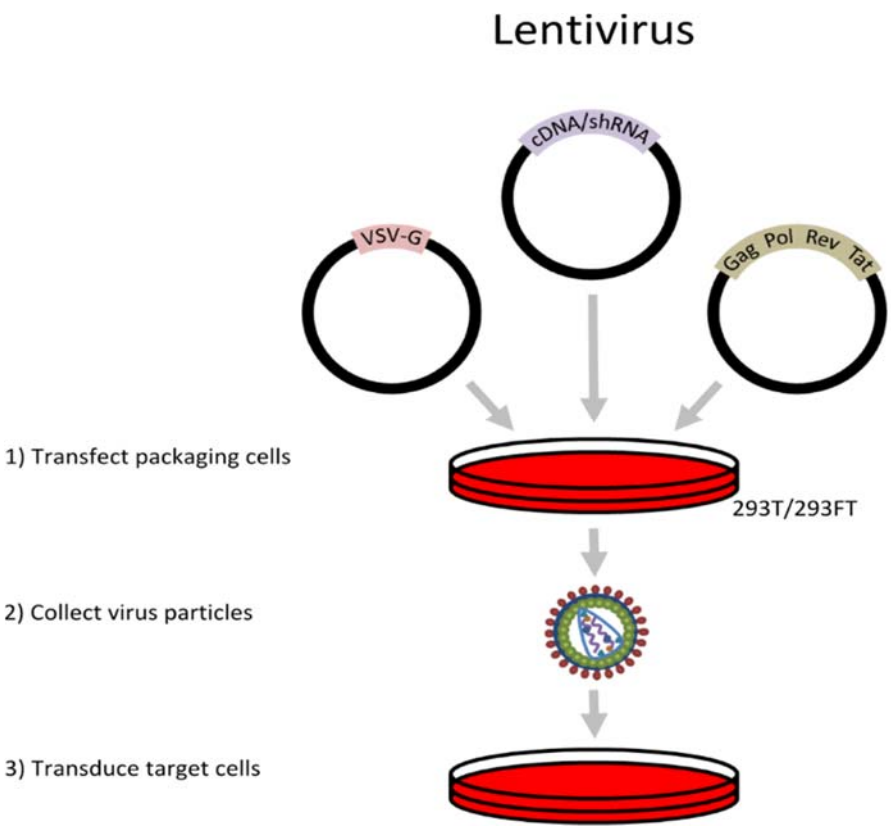

Lentivirus
cDNA/shRNA
VSV-G
Gag Pol Rev Tat
1) Transfect packaging cells
293T/293FT
2) Collect virus particles
3) Transduce target cells

Figure 1.

Figure 1. Lentiviral construction and establishment of porcine endothelial cell lines with stable human CD200 or CD47 overexpression

(A–B) Schematic map of (A) pLenti6-CD200 and (B) pLenti6-CD47. The CMVp promoter is followed by inserts of the BamHI-to-EcoRI fragment and BamHI-to-Xbal fragment for pLenti6-CD200 and pLenti6-CD47, respectively. (C) Establishment of a PEC line with human CD200 or CD47 overexpression. Recombinant lentiviruses were produced by co-transfection of the target plasmid, packaging plasmid, and envelope plasmid on the HEK293 cells and harvested by collecting the virus-containing cell culture media. Then, porcine endothelial cells were transduced with purified recombinant lentiviruses. After viral transduction, the cells were passaged and cultured for the stable expression of target molecules. PEC, porcine endothelial cell. CMVp, human cytomegalovirus promoter; HIV-1RRE, HIV-1 Rev response element; LTR, long-terminal repeat.

Human macrophage preparation

Human monocytes were separated from the peripheral blood of healthy donors using Ficoll-Hypaque gradient and Percoll gradient. The blood was layered onto Ficoll (1.077) and centrifuged at 800 × *g* for 20 min at 4°C. Then, the peripheral blood mononuclear cell (PBMC) layer was harvested, washed twice with cold phosphate-buffered saline (PBS), and resuspended in cold PBS. PBMCs were again layered onto 50% Percoll (1.065) and centrifuged at 800 × *g* for 20 min at 4°C. The human monocyte layer was harvested, washed twice with cold PBS, and cultured for 4 h at 37°C for cell attachment. After 4 h, the supernatant was discarded, and the attached cells were washed with cold PBS. After overnight incubation, adherent human macrophages were harvested.

Cell culture

All cells were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air and were cultured in RPMI-1640 media containing 10% heat-inactivated fetal bovine serum, 50 μM of 2-mercaptoethanol (2-ME), 20 mM of HEPES buffer, and 1% antibiotics (1 unit/mL penicillin and 1 mg/mL streptomycin).

Cytotoxicity assay

PECs with overexpression of target molecules were seeded in 96-well plates (1 × 10^5/well). Subsequently, human macrophages were added to each well (2 × 10^5/well). Both cells were incubated together for 6 h to induce the apoptosis or necrosis of PECs. Then, the cell mixtures were harvested, stained with Annexin V and 7-aminoactinomycin D (7-AAD), and analyzed by flow cytometry. Overall, cellular viability was also assessed by lactate dehydrogenase (LDH) assay (Sigma, St Louis, Mo, USA).

Phagocytosis assay

PECs were stained with a violet fluorescent dye according to the manufacturer's protocol. Labeled PECs (1 × 10^5/well) were incubated with $CD14^+$ human macrophages (2 × 10^5/well) in 96-well plates for 2 h at 37°C. Then, the cell mixtures were harvested and stained with anti-human CD14. In flow cytometric analysis, the proportion of $CD14^+$ $violet^{int}$ double-positive cells among $CD14^+$ cells was calculated to evaluate the phagocytic function of human macrophages.

Cytokine assay

Human macrophages (2×10^5/well) were mixed with PECs (1×10^5/well) and incubated for 24 h in 96-well plates. After incubation, the supernatants were collected, and the secretion levels of human pro-inflammatory cytokines such as IL-1β, IL-6, and TNFα were measured using enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, San Diego, CA, USA) according to the manufacturer's protocol.

Proliferation assay

PECs were stained with Violet Cell Tracker and irradiated at 100 rad. Irradiated and labeled PECs (1×10^4) were seeded in 96-well plates and incubated for 2 h. Human macrophages were labeled with carboxyfluorescein succinimidyl ester (CFSE). Then, CFSE-labeled macrophages were added to each well (2×10^5) and incubated with PECs for 72 h.

Statistics

Continuous variables were compared by t-test, and P values less than 0.05 were considered statistically significant. Statistical analyses were performed using GraphPad Prism 7.

Results

Sequence similarity between CD200 and CD47 of human and porcine

When amino acid sequence of porcine CD47 was compared with that of human CD47 using Clustal Omega program (EMBL-EBI, Hinxton, UK), sequence identity and similarity were 73.1% and 79.67%, respectively (Figure 2A). Sequence identity and similarity between human and porcine CD200 were 82.8% and 86.6%, respectively (Figure 2B). Sequence homology of CD200 between human and porcine seems to be better than CD47.

A

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MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKWKF
MWPLVVVVLLGSAYCGSAQLIFNITKSVEFTVCNTTVTIPCFVNNMEAKNISELYVKWKF
*****..:***** ******:** *******.** **.*****.****:* :*:******

KGRDIYTFDGALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTELT
KGKDIFIFDGAQHISKPSEAFPSSKISPSELLHGIASLKMDKRDAV--IGNYTCEVTELS
**:**: **** : *.    * *:**. *:**:* ******* ***   **********:

REGETIIELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALL
REGETIIELKRRFVSWFSPNENILIVIFPILAILLFWGQFGILTLKYKSSYTKEKTIFLL
********** *.*****************:********** ****:*.  .**** **

VAGLVITVIVIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYYVFSTAIGLTSFVIA
VAGLMLTIIVIVGAILFIPGEYSTKNACGLGLIVIPTAILILLQYCVFMMALGMSSFTIA
****::*:*********:***** *** ******  *.*****:* **  *:*::**.**

ILVIQVIAYILAVVGLSLCIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQ
ILILQVLGHVLSVVGLSLCVSECTPVHGPLLISGLGIIALAELLGLVYMKCVASDHKTIQ
**::**:.::*:*******:: * *:*********.*:***:******** ***::****

PPRNN   → Homo sapiens CD47
PPRNN   → Sus scrofa CD47
*****
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B

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MERLVIRMPFCHLSTYSLVWVMAAVVLCTAQVQVVTQDEREQLYTPASLKCSLQNAQEAL
MERLVFRSFFCHLSTFQLIWFMAAGMLCRAQ--VVTQDVRERLNTPASLRCSLQNPQEVL
*****:*  ******:.*:*.*** :** **  ***** **:* *****:***** **.*

IVTWQKKKAVSPENMVTFSENHGVVIQPAYKDKINITQLGLQNSTITFWNITLEDEGCYM
IVTWQKIKAVSPENMITFSKNHGVVVQPAYKDKINITQLGLMNSTITFWNTTLEDEGCYK
****** ********:***:*****:*************** ******** ********

CLFNTFGFGKISGTACLTVYVQPIVSLHYKFSEDHLNITCSATARPAPMVFWKVPRSGIE
CLFNTFGAGKISGIACLTLSVQPTVSLHYKLSEDQLNITCSANARPAPMISWKISGSGIE
******* ***** ****: *** ******:***:*******.******: **:  ****

NSTVTLSHPNGTTSVTSILHIKDPKNQVGKEVICQVLHLGTVTDFKQTVNKGYWFSVPLL
NSTEILVHPNGTTSVTSILQIKDPKSQVGKEVICQVLHLGTVTDYRETVNKGFWFSVPLL
***  * ************:*****.******************:::*****:*******

LSIVSLVILLVLISILLYWKRHRNQDREP → Homo sapiens CD200
LSIVSLVILLVLISILLYWKRRRNQDREP → Sus scrofa CD200
**********************:*******
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Figure 2.

Figure 2. Comparison of amino acid sequences between human and porcine CD200 and CD47

(A) When amino acid sequence of porcine CD47 was compared with that of human CD47, sequence identity and similarity were 73.1% and 79.6%, respectively. (B) When amino acid sequence of human and porcine CD200 were compared, its identity and similarity were 82.8% and 86.6%, respectively (Figure 2B). Sequence homology between human and porcine CD47 and CD200 were compared using Clustal Omega program (EMBL-EBI, Hinxton, UK).

Expression of transmembrane and secretory forms of human CD200 in PECs

In comparison with control PECs transduced with the mock vector, PECs transduced with lentiviruses containing the transmembrane form of human CD200 (hCD200-PECs) had a much higher expression level of human CD200 (Figure 3A). In contrast, the CD200 expression in PECs transduced with lentiviruses containing the secretory form of human CD200 (hCD200-Fc-PECs) was slightly higher than that in control PECs (Figure 3B).

Expression of transmembrane and secretory forms of human CD47 in PECs

The expression level of human CD47 in PECs transduced with the transmembrane form human CD47 (hCD47-PECs) or secretory form human CD47 (hCD47-Fc-PECs) was compared with PECs transduced with the mock vector. Similar to the expression level of CD200 in hCD200-PECs and hCD200-Fc-PECs, the expression level of CD47 in hCD47-PECs was much higher than in control PECs (Figure 3C), and it was slightly higher in hCD47-Fc-PECs than in control PECs (Figure 3D).

Co-expression of human CD200 and human CD47 in PECs

The target gene expression levels in PECs transduced with lentiviruses containing both human CD200 and human CD47 were analyzed by flow cytometry. Most of the hCD200/hCD47-PECs had a high expression level of both CD200 and CD47 (Figure 3E). hCD200-Fc/hCD47-Fc-PECs also expressed CD200 and CD47 simultaneously, albeit in low levels (Figure 3F).

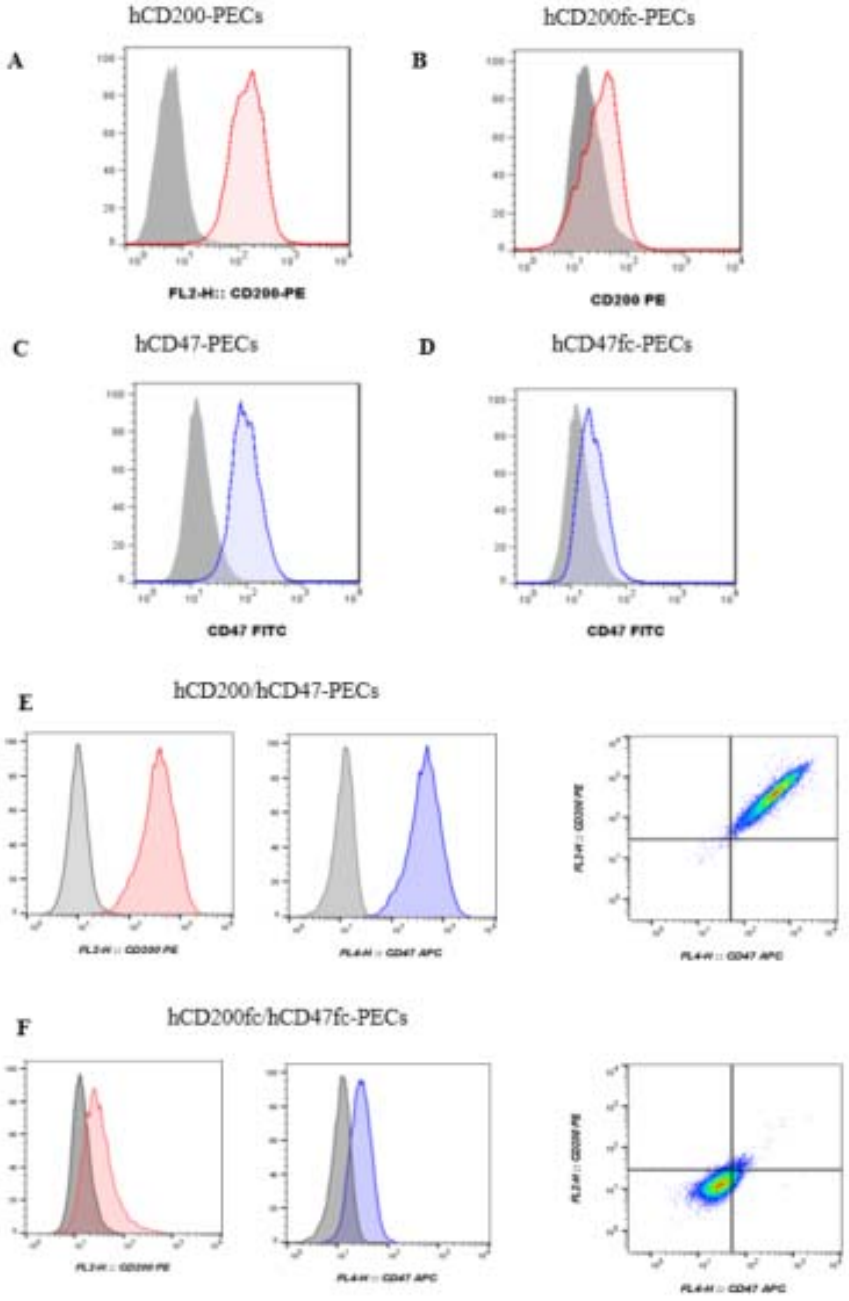
hCD200-PECs
A
FL2-H:: CD200-PE
hCD200fc-PECs
B
CD200 PE
C
hCD47-PECs
CD47 FITC
D
hCD47fc-PECs
CD47 FITC
E
hCD200/hCD47-PECs
F
hCD200fc/hCD47fc-PECs

Figure 3.

Figure 3. Expression of human CD200 and human CD47 in porcine endothelial cells

The expression levels of human CD200 and human CD47 in lentivirus transduced PECs were analyzed by flow cytometric analysis. (A–D) Both (A) CD200 and (C) CD47 were highly expressed in hCD200-PECs and hCD47-PECs, respectively; however, (B) the expression of CD200 in hCD200-Fc-PECs and (D) CD47 in hCD47-Fc-PECs was only slightly higher than their corresponding expression in control PECs with the mock vector. (E) hCD200/hCD47-PECs that were simultaneously transduced with lentivirus-CD200 and lentivirus-CD47 had a high expression level of both CD200 and CD47. (F) In contrast, hCD200-Fc/hCD47-Fc-PECs that were transduced with lentiviruses containing the secretory form of CD200 and CD47 had a weak expression level of both CD200 and CD47, and they appeared as the $CD200^{low}CD47^{low}$ population. PEC, porcine endothelial cell.

CD14 expression in human macrophages prepared from PBMCs

We prepared human macrophages from PBMCs by adhesion. Flow cytometric analysis showed that most of the macrophages (95.5%) expressed CD14 (Figure 4).

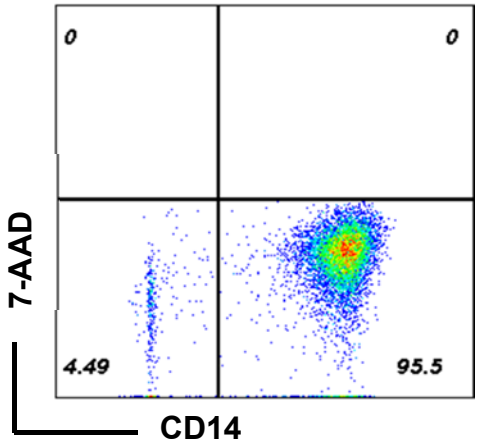

Figure 4. High expression level of CD14 in human macrophages

Human macrophages were prepared from human peripheral mononuclear cells by adhesion. Over 95% of the human macrophages had a high level of CD14 expression.

Suppression of the cytotoxicity of human macrophages by overexpression of human CD200 and human CD47 in PECs

In LDH assays, both CD200 and CD200-Fc suppressed the cytotoxicity of human macrophages against PECs, and the suppressive effect of CD200 was higher than that of CD200-Fc (Figure 5A). CD47 also demonstrated strong suppressive effects on macrophage cytotoxicity, whereas CD47-Fc suppressed the cytotoxicity of human macrophages in the xenogeneic immune response to a lesser degree (Figure 5B). Simultaneous staining of Annexin-V and 7-AAD showed that both CD200 and CD200-Fc in PECs suppressed the early and late apoptosis of PECs by human macrophages, and the suppressive effect of the transmembrane form was better than that of the secretory form (Figure 5C, 5E). Although both CD47 and CD47-Fc suppressed the cytotoxic activity of human macrophages against PECs, there was no difference in suppressive activity between the transmembrane and secretory forms (Figure 5D, 5F). Overall, both human CD200 and human CD47 suppressed the cytotoxic activity of human macrophages against PECs.

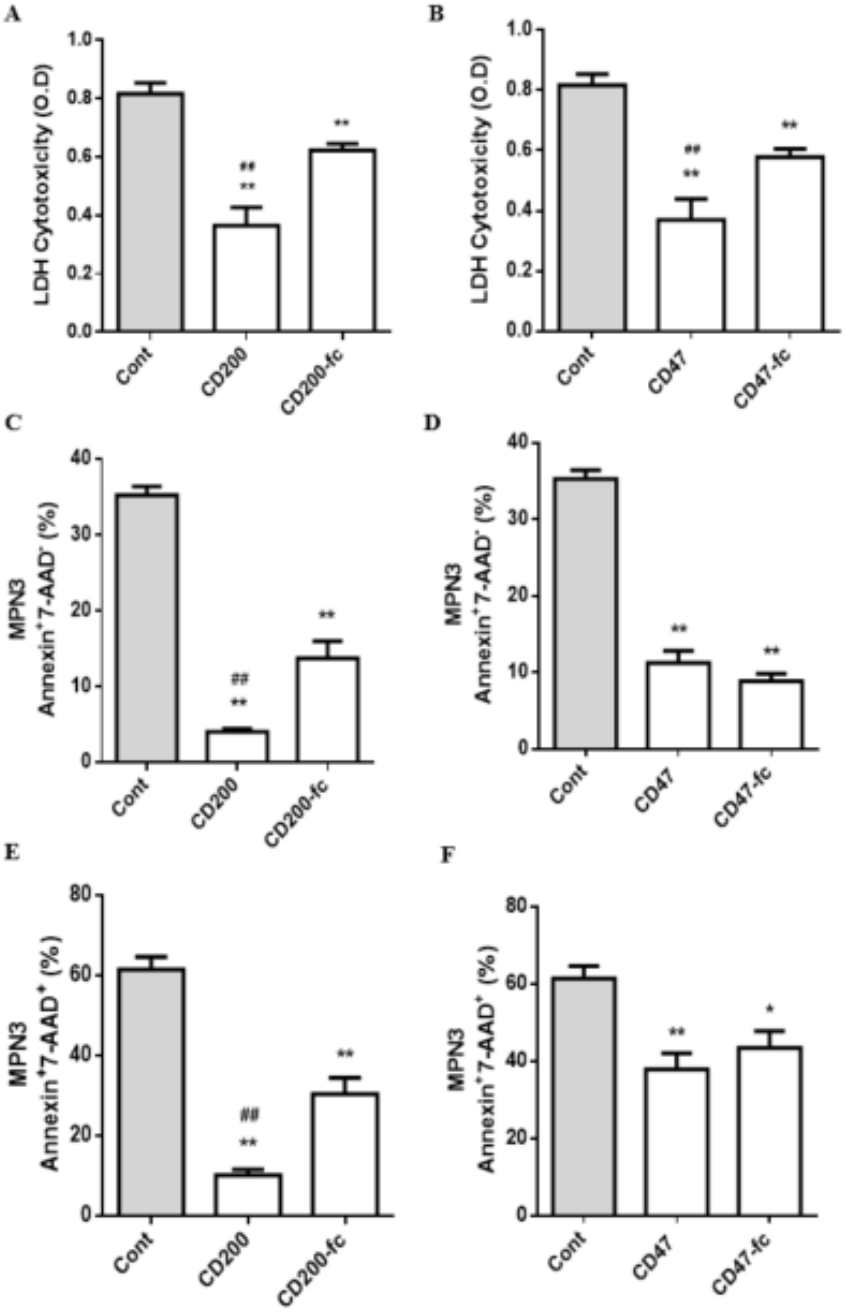
A
LDH Cytotoxicity (O.D)
1.0
0.8
0.6
0.4
0.2
0.0
##
**
**
Cont
CD200
CD200-fc
B
LDH Cytotoxicity (O.D)
1.0
0.8
0.6
0.4
0.2
0.0
##
**
**
Cont
CD47
CD47-fc
C
MPN3
Annexin+7-AAD- (%)
40
30
20
10
0
##
**
**
Cont
CD200
CD200-fc
D
MPN3
Annexin+7-AAD- (%)
40
30
20
10
0
**
**
Cont
CD47
CD47-fc
E
MPN3
Annexin+7-AAD+ (%)
80
60
40
20
0
##
**
**
Cont
CD200
CD200-fc
F
MPN3
Annexin+7-AAD+ (%)
80
60
40
20
0
**
*
Cont
CD47
CD47-fc

Figure 5.

Figure 5. CD200 and CD47 suppression of the cytotoxic activity of human macrophages against porcine endothelial cells

(A) The cytotoxic activity of human macrophages against PECs was suppressed to a greater extent by CD200 than by CD200-Fc in LDH assays. (B) The cytotoxic activity of human macrophages against PECs was suppressed to a greater extent by CD47 than by CD47-Fc in LDH assays. (C) The early apoptosis (Annexin-V^+/7-AAD^-) of PECs was reduced to a greater extent by CD200 than by CD200-Fc in flow cytometric analysis. (D) The early apoptosis (Annexin-V^+/7-AAD^-) of PECs was reduced to a similar degree by CD47 and CD47-Fc in flow cytometric analysis. (E) The late apoptosis (Annexin-V^+/7-AAD^+) of PECs was reduced to a greater extent by CD200 than by CD200-Fc in flow cytometric analysis. (F) The late apoptosis (Annexin-V^+/7-AAD^+) of PECs was reduced to a similar degree by CD47 and CD47-Fc in flow cytometric analysis. $^*P < 0.05$, $^{**}P < 0.01$ compared with the control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the secretory forms. LDH, lactate dehydrogenase; PEC, porcine endothelial cell.

Suppression of the phagocytic activity of human macrophages by overexpression of human CD200 and human CD47 in PECs

The phagocytic activity of human macrophages against PECs was suppressed to a lesser degree by CD200 than by CD47 (Figure 6). However, phagocytosis was suppressed to a similar degree by CD200-Fc and CD47-Fc (Figure 6). Taken together, both human CD200 and human CD47 suppressed the phagocytic activity of human macrophages against PECs.

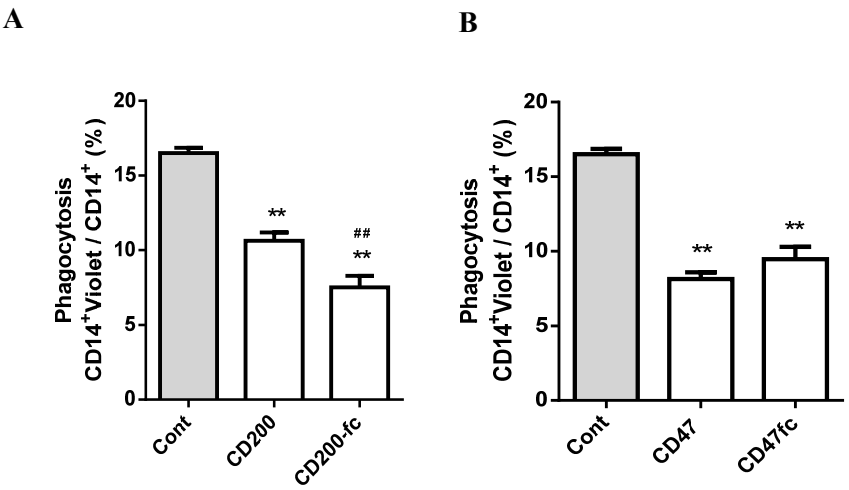

Figure 6.

Figure 6. CD200 and CD47 suppression of the phagocytic activity of human macrophages against porcine endothelial cells

PECs with overexpression of either the transmembrane or secretory form of CD200 or CD47 were subjected to phagocytosis by $CD14^+$ human macrophages. (A) Both CD200 and CD200-Fc suppressed the phagocytic activity of human macrophages, and the suppressive effect of secretory CD200-Fc was stronger than that of transmembraneous CD200. (B) Both transmembraneous and secretory CD47 suppressed the phagocytic activity of human macrophages to a similar degree. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the secretory forms. PEC, porcine endothelial cell.

Inhibition of pro-inflammatory cytokine secretion from human macrophages by human CD200 and human CD47 in PECs

Human macrophages can mediate the xenogeneic immune response by secreting pro-inflammatory cytokines such as IL-6, TNFα, and IL-1β. Both the transmembrane and secretory forms of human CD200 suppressed the secretion of IL-6, TNFα, and IL-1β (Figure 7A, C, E). CD47 and CD47-Fc also suppressed the secretion of IL-6 and IL-1β (Figure 7B, D); however, both the transmembrane and secretory forms of human CD47 had little effect on TNFα secretion (Figure 7F).

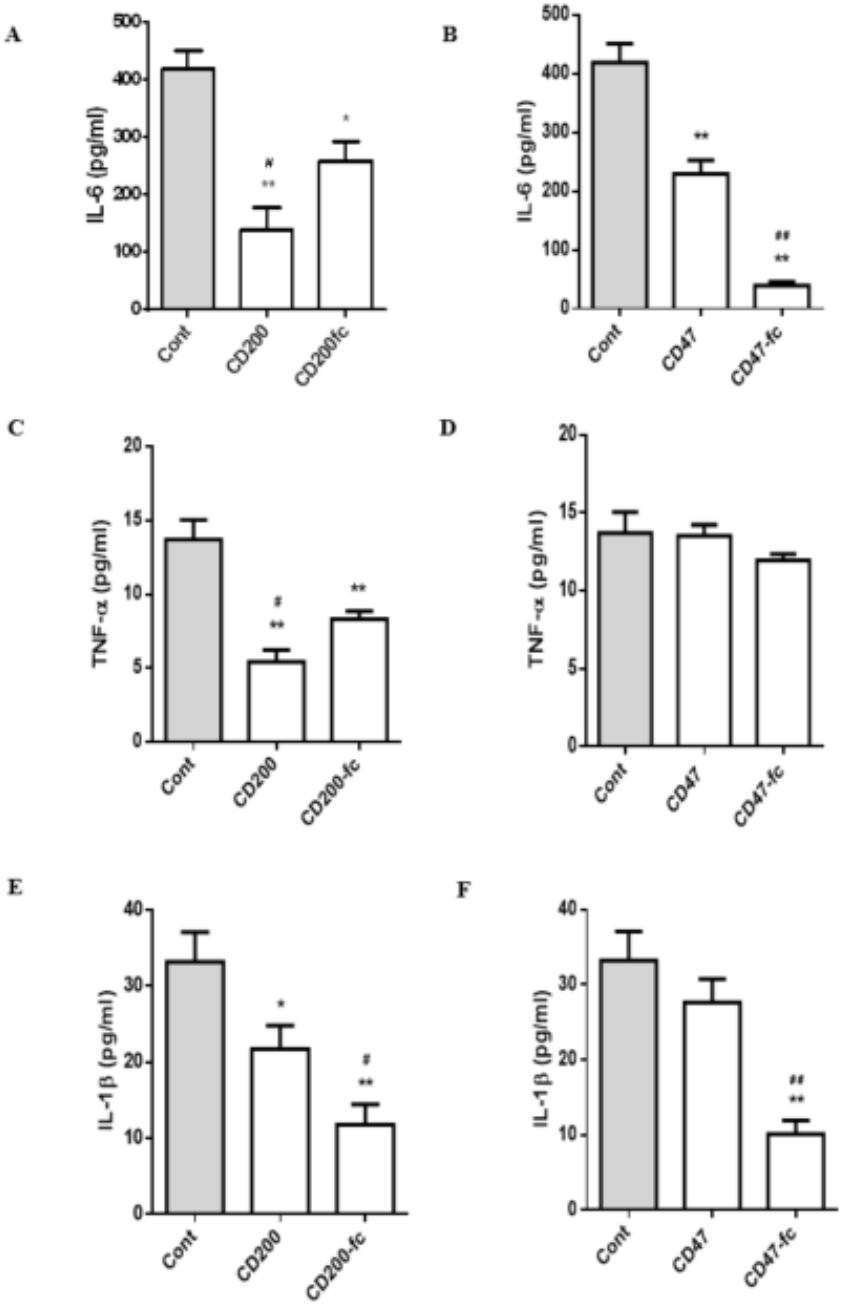
A
IL-6 (pg/ml)
500
400
300
200
100
0
Cont
CD200
CD200fc
B
IL-6 (pg/ml)
Cont
CD47
CD47-fc
C
TNF-α (pg/ml)
20
15
10
5
0
Cont
CD200
CD200-fc
D
TNF-α (pg/ml)
Cont
CD47
CD47-fc
E
IL-1β (pg/ml)
40
30
20
10
0
Cont
CD200
CD200-fc
F
IL-1β (pg/ml)
Cont
CD47
CD47-fc

Figure 7.

Figure 7. CD200 and CD47 suppression of the secretion of pro-inflammatory cytokines from human macrophages in response to xenogeneic stimulation

The cytokine secretions of human macrophages stimulated by PECs were measured by ELISA. (A) Both CD200 and CD200-Fc suppressed IL-6 secretion from human macrophages. (B) Both CD47 and CD47-Fc suppressed IL-6 secretion from human macrophages. (C) Both CD200 and CD200-Fc suppressed TNF-α secretion from human macrophages. (D) However, neither CD47 nor CD47-Fc suppressed TNF-α secretion from human macrophages. (E) CD200 and CD200-Fc had a significant suppressive effect on IL-1β secretion from human macrophages. (F) CD47-Fc had a significant suppressive effect on IL-1β secretion from human macrophages. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the secretory forms. ELISA, enzyme-linked immunosorbent assay; PEC, porcine endothelial cell.

Suppression of the proliferation of human macrophages by human CD200 and human CD47 in PECs

The suppressive activity of human CD200 and human CD47 against the proliferation of human macrophages in response to xenogeneic stimulation was measured by CFSE staining. The proliferation of CFSE-labeled human macrophages was suppressed by CD200, specifically secretory CD200-Fc (Figure 8A). In addition, both the transmembrane and secretory forms of CD47 suppressed the proliferation of human macrophages (Figure 8B).

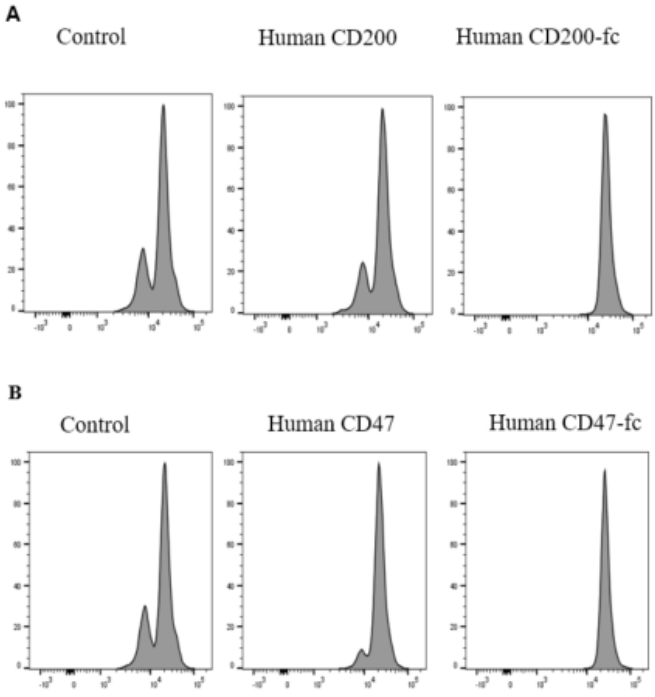

Figure 8.

Figure 8. CD200 and CD47 suppression of the proliferation of human macrophages in response to xenogeneic stimulation

The proliferation of human $CD14^+$ macrophages stimulated by PECs was assessed by CFSE staining. (A) In comparison with CD200, CD200-Fc was more efficient in suppressing macrophage proliferation. (B) The suppression of macrophage proliferation in the CD47-PEC group was greater than in the control PEC group. The proliferation of human macrophages was lowest in the presence of hCD47-Fc-PECs. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the secretory forms. CFSE, carboxyfluorescein succinimidyl ester; PEC, porcine endothelial cell.

Additive effects of human CD200 and human CD47 on the cytotoxic activity of human macrophages against PECs

LDH assay demonstrated that a combination of transmembrane CD200 and CD47 had additive effects on the cytotoxic activity of human macrophages (Figure 9A). A combination of secretory CD200-Fc and CD47-Fc also demonstrated better suppression than either CD200-Fc or CD47-Fc (Figure 9B). However, CD200 and CD47 did not demonstrate any additive effects in suppressing early or late apoptosis (Figure 9C–F).

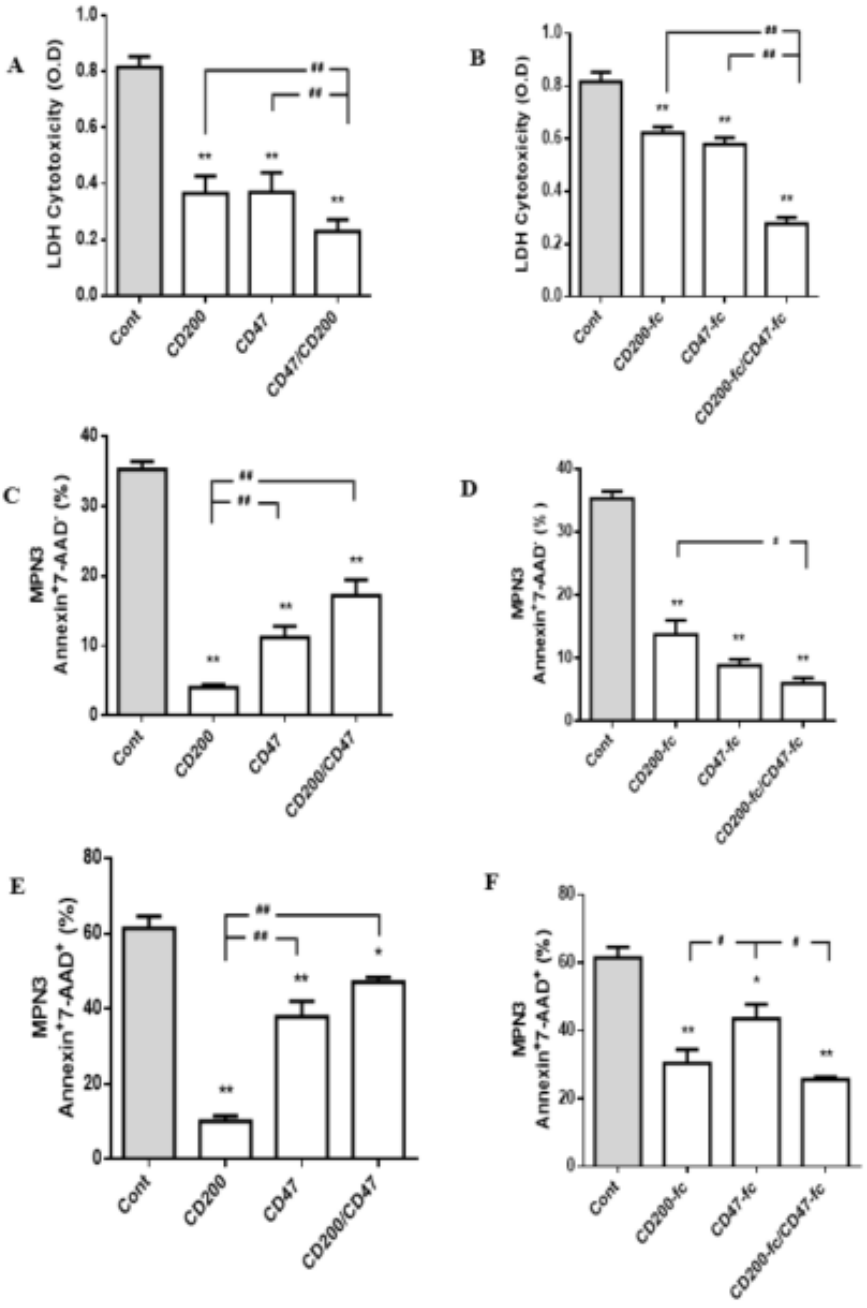
A
LDH Cytotoxicity (O.D)
Cont
CD200
CD47
CD47/CD200
B
LDH Cytotoxicity (O.D)
Cont
CD200-fc
CD47-fc
CD200-fc/CD47-fc
C
MPN3
Annexin+7-AAD- (%)
Cont
CD200
CD47
CD200/CD47
D
MPN3
Annexin+7-AAD- (%)
Cont
CD200-fc
CD47-fc
CD200-fc/CD47-fc
E
MPN3
Annexin+7-AAD+ (%)
Cont
CD200
CD47
CD200/CD47
F
MPN3
Annexin+7-AAD+ (%)
Cont
CD200-fc
CD47-fc
CD200-fc/CD47-fc

Figure 9.

Figure 9. Additive effects of human CD200 and human CD47 on the cytotoxic activity of human macrophages against porcine endothelial cells

(A) The cytotoxic activity of human macrophages against PECs was suppressed to a greater extent in the double transmembrane group with overexpression of both hCD200 and hCD47 (hCD200/hCD47-PECs) than in the hCD200-PEC or hCD47-PEC group in LDH assays. (B) The cytotoxic activity of human macrophages against PECs was also suppressed to a greater extent in the double secretory group (hCD200-Fc/hCD47-Fc-PECs) than in the hCD200-Fc-PEC or hCD47-Fc-PEC group in LDH assays. (C) However, the early apoptosis (Annexin-V^+/7-AAD^-) of PECs was reduced to a lesser extent in the hCD200/hCD47-PEC group than in the hCD200-PEC or hCD47-PEC group in flow cytometric analysis. (D) The early apoptosis (Annexin-V^+/7-AAD^-) of PECs was reduced to a greater extent by hCD200-Fc/hCD47-Fc than by hCD200-Fc or hCD47-Fc in flow cytometric analysis. (E) The late apoptosis (Annexin-V^+/7-AAD^+) of PECs was reduced to a lesser extent by hCD200/hCD47 than by hCD200 or hCD47 in flow cytometric analysis. (F) The late apoptosis (Annexin-V^+/7-AAD^+) of PECs was reduced to a greater extent by hCD200-Fc/hCD47-Fc than by hCD47-Fc in flow cytometric analysis. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the double group. LDH, lactate dehydrogenase; PEC, porcine endothelial cell.

Suppression of the phagocytic activity of human macrophages by *co-expression of human CD200 and human CD47*

The suppressive effects of a combination of human CD200 and human CD47 on the phagocytic activity of human macrophages were compared with those of CD200 alone or CD47 alone. The co-expression of CD200 and CD47 was more effective than CD200 or CD47 in both transmembrane (Figure 10A) and secretory (Figure 10B) forms.

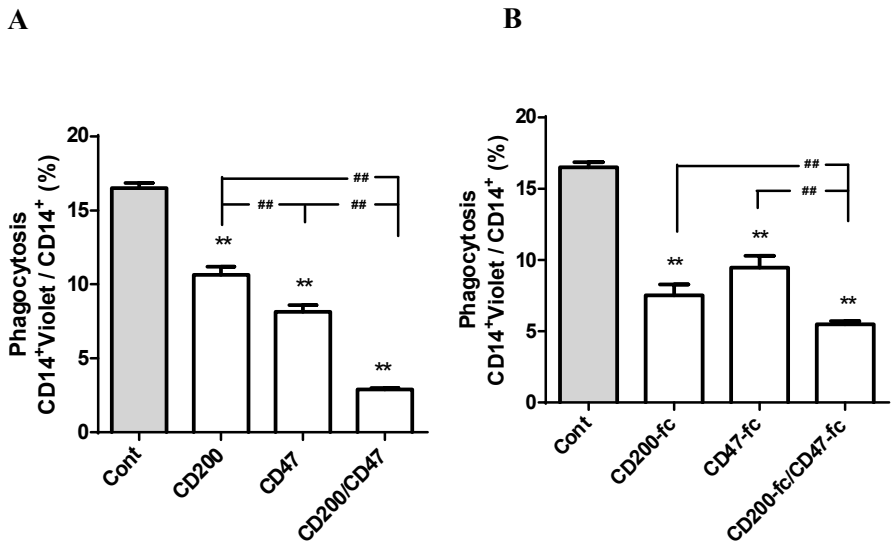

Figure 10.

Figure 10. Suppressive effects of human CD200 and human CD47 co-expression on the phagocytic activity of human macrophages

The suppressive effects of human CD200 and human CD47 co-expression (double group) on the phagocytic activity of human macrophages were compared with those of CD200 alone or CD47 alone. The double group was more effective than CD200 or CD47 in both (A) transmembrane and (B) secretory forms. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the double group.

Comparison of the cytokine suppressive potency between single- and co-expression of human CD200 and human CD47

We compared the suppressive effects of hCD200/hCD47 with those of hCD200 or hCD47 on cytokine secretion by human macrophages. The additive effects of human CD200 and CD47 were not consistent in suppressing various pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β (Figure 11A–F). CD200 and CD47 did not have an additive effect in either the transmembrane or secretory form.

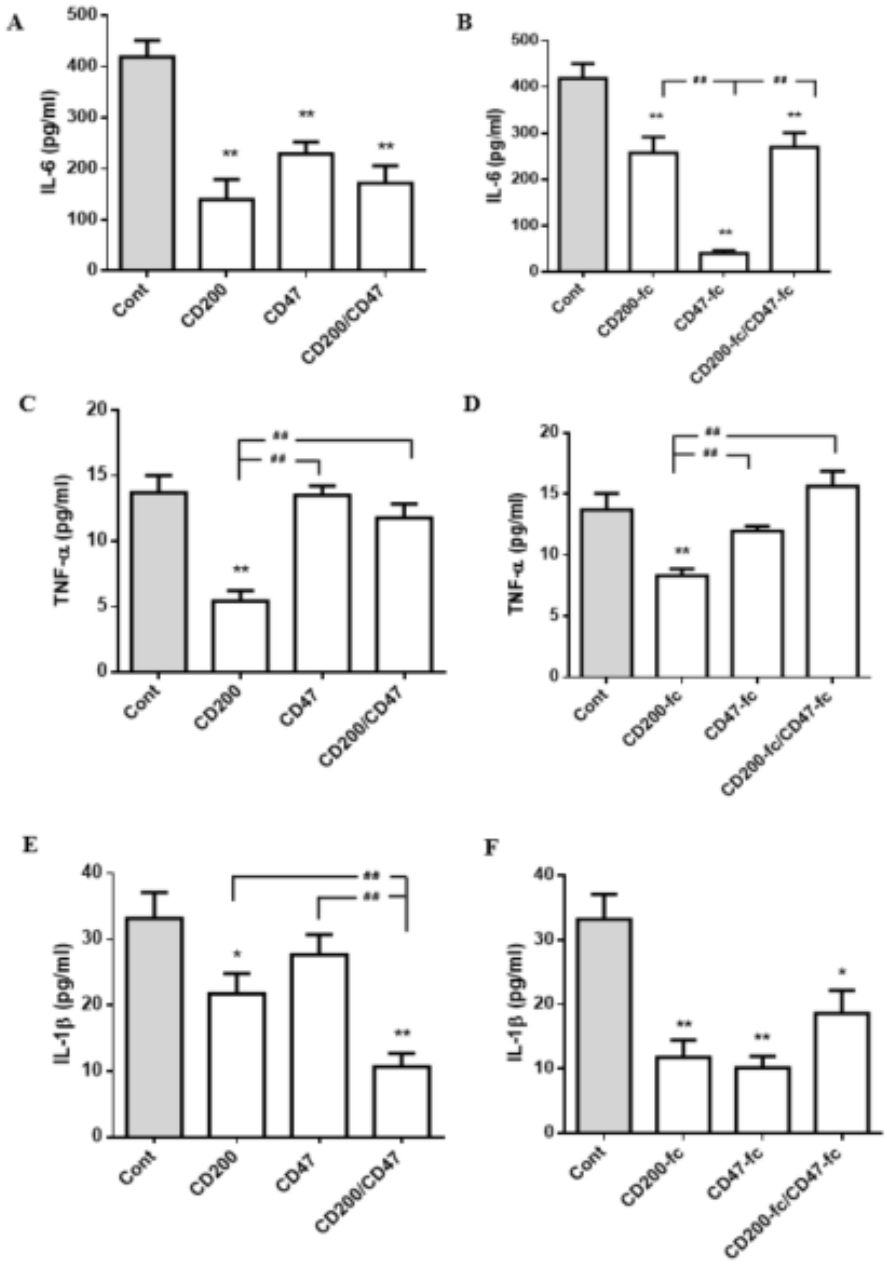
A
IL-6 (pg/ml)
500
400
300
200
100
0
**
**
**
Cont
CD200
CD47
CD200/CD47
B
IL-6 (pg/ml)
500
400
300
200
100
0
**
**
**
**
Cont
CD200-fc
CD47-fc
CD200-fc/CD47-fc
C
TNF-α (pg/ml)
20
15
10
5
0
**
##
##
Cont
CD200
CD47
CD200/CD47
D
TNF-α (pg/ml)
20
15
10
5
0
**
##
##
Cont
CD200-fc
CD47-fc
CD200-fc/CD47-fc
E
IL-1β (pg/ml)
40
30
20
10
0
*
**
##
##
Cont
CD200
CD47
CD200/CD47
F
IL-1β (pg/ml)
40
30
20
10
0
**
**
*
Cont
CD200-fc
CD47-fc
CD200-fc/CD47-fc

Figure 11.

Figure 11. Comparison of the cytokine suppressive potency between single- and co-expression of human CD200 and human CD47

The suppressive effects of human CD200/CD47 on cytokine secretion by human macrophages were compared with those of CD200 or CD47 using ELISA. The additive effects of human CD200 and CD47 were not consistent in suppressing various pro-inflammatory cytokines such as (A–B) IL-6, (C–D) TNF-α, and (E–F) IL-1β. CD200 and CD47 did not have an additive effect in either the transmembrane or secretory form except for the effect of CD200/CD47 on IL-1β (E). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the double group. ELISA, enzyme-linked immunosorbent assay; PEC, porcine endothelial cell.

Suppression of the proliferation of human macrophages by co-expression of human CD200 and human CD47

Both transmembrane human CD200 and human CD47 suppressed the proliferation of human macrophages in response to xenogeneic stimulation (Figure 12A). The co-expression of transmembraneous human CD200 and human CD47 was more effective than the single expression of transmembraneous human CD200 or human CD47 (Figure 12A). In parallel, macrophage proliferation was suppressed to a greater extent by the co-expression of secretory human CD200-Fc and human CD47-Fc than by secretory human CD200-Fc alone or human CD47-Fc alone (Figure 12B).

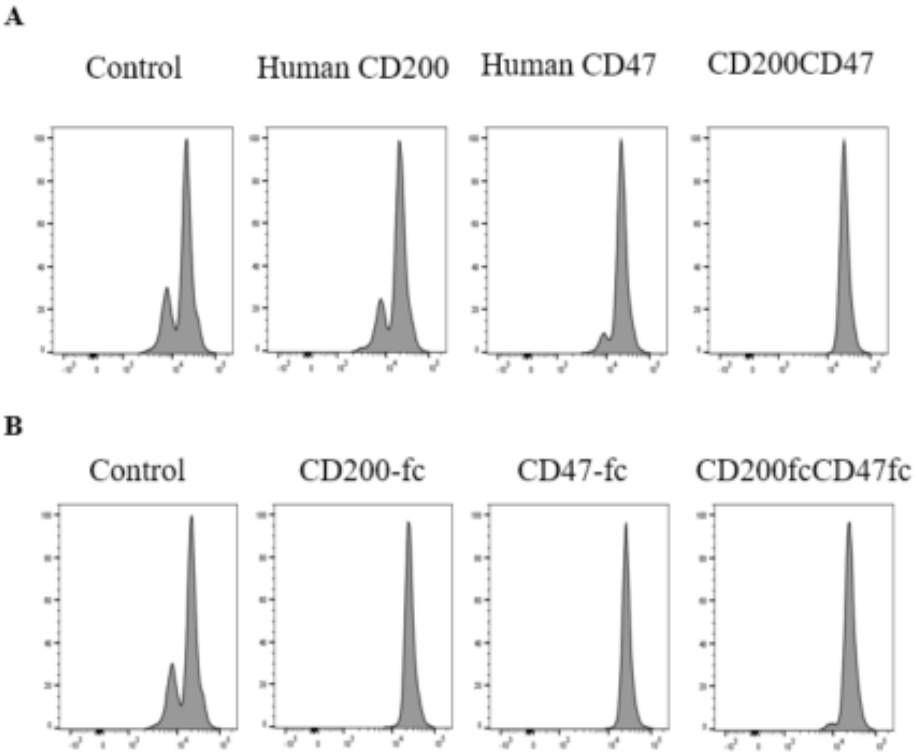
A
Control
Human CD200
Human CD47
CD200CD47
B
Control
CD200-fc
CD47-fc
CD200fcCD47fc

Figure 12.

Figure 12. Suppressive effects of human CD200 and human CD47 co-expression on the proliferation of human macrophages

The proliferation of human macrophages stimulated by PECs was measured by CFSE staining. (A) Both transmembraneous human CD200 and human CD47 suppressed the proliferation of human macrophages in response to xenogeneic stimulation, and the co-expression of C human D200 and human CD47 was more effective than the single expression of human CD200 or human CD47. (B) In parallel, macrophage proliferation was suppressed to a greater extent by a combination of secretory human CD200-Fc and human CD47-Fc than by human CD200-Fc alone or human CD47-Fc alone. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the double group. CFSE, carboxyfluorescein succinimidyl ester; PEC, porcine endothelial cell.

Discussion

The present study demonstrated that overexpression of both human CD200 and human CD47 in PECs suppressed the xenogeneic immune response of human macrophages. Furthermore, the co-expression of human CD200 and human CD47 had additive effects in suppressing macrophage activation in response to xenogeneic stimulation. Both the transmembrane and secretory forms of human CD200 and human CD47 in PECs suppressed the cytotoxicity, phagocytosis, secretion of pro-inflammatory cytokines, and proliferation of human macrophages in response to xenogeneic stimulation.

The limited compatibility (73%) of amino acid sequences between human CD47 and pig CD47 might contribute to the uncontrolled xenogeneic activation of human macrophages during pig-to-human xenogeneic immune response; indeed, porcine CD47 is unable to induce the tyrosine phosphorylation of human SIRPα. (15) Overexpression of human CD47 in porcine cells can suppress the phagocytic activity of human macrophages against porcine cells in the xenogeneic immune response. (15) The compatibility of amino acid sequences between human CD200 and porcine CD200 is 82.7%. The present study demonstrated that over-expression of both human CD200 and human CD47 in PECs had suppressive effects on phagocytosis by human macrophages. The CD200-CD200R interaction is known to play a central role in the suppression of cytotoxic activity and cytokine secretion;

similar to CD200, CD47 also had suppressive activity against cytotoxicity and cytokine secretion.

CD47 transgenic pigs have been generated for xenotransplantation and demonstrated to improve both engraftment in a model of pig-to-human hematopoietic cell transplantation and xenograft survival in a model of pig-to-mouse skin transplantation using hematopoietic porcine cells with human CD47 overexpression. (16, 17) Considering the beneficial role of CD200 in suppressing macrophage activation in response to xenogeneic stimulation, CD200 transgenic pigs could suppress xenograft rejection by inhibiting the pro-inflammatory activity of macrophages and thus are suitable for xenotransplantation. However, overexpression of human CD47 in PECs or other nonhematopoietic porcine cells has not been reported to improve xenograft survival. According to the present study, the suppressive activity of the co-expression of human CD200 and human CD47 was more effective against human macrophage activation than that of the single expression of CD200 alone or CD47 alone. Therefore, we suggest that double-transgenic pigs with overexpression of both human CD200 and human CD47 would lead to better xenograft outcomes in nonhematopoietic porcine cell or organ transplantation.

The present study was limited in that only *in vitro* systems were used to investigate the role of human CD200 in suppressing macrophage activation in response to xenogeneic stimulation. Further *in vivo* studies with a

xenotransplantation model are needed to confirm our findings. Nevertheless, the present study is the first study to demonstrate the significant effects of human CD200 on macrophage activation in response to xenogeneic stimulation. In addition, the co-expression of human CD200 and human CD47 was more effective than CD200 alone or CD47 alone in suppressing human macrophage activation in the xenogeneic immune response.

In summary, both human CD200 and human CD47 suppressed the activation of human macrophages in response to xenogeneic stimulation. Moreover, the suppressive activity of the co-expression of human CD200 and human CD47 was more effective against macrophage activation than that of the expression of either CD200 or CD47. Therefore, the generation of double-transgenic pigs with human CD200 and human CD47 could be a promising approach for improving xenograft survival.

Reference

1. Garcia MR, Ledgerwood L, Yang Y, Xu J, Lal G, Burrell B, et al. Monocytic suppressive cells mediate cardiovascular transplantation tolerance in mice. J Clin Invest. 2010;120(7):2486-96.

2. Riquelme P, Tomiuk S, Kammler A, Fandrich F, Schlitt HJ, Geissler EK, et al. IFN-gamma-induced iNOS expression in mouse regulatory macrophages prolongs allograft survival in fully immunocompetent recipients. Mol Ther. 2013;21(2):409-22.

3. Fox A, Koulmanda M, Mandel TE, van Rooijen N, Harrison LC. Evidence that macrophages are required for T-cell infiltration and rejection of fetal pig pancreas xenografts in nonobese diabetic mice. Transplantation. 1998;66(11):1407-16.

4. Cadili A, Kneteman N. The role of macrophages in xenograft rejection. Transplant Proc. 2008;40(10):3289-93.

5. Fox A, Mountford J, Braakhuis A, Harrison LC. Innate and adaptive immune responses to nonvascular xenografts: evidence that macrophages are direct effectors of xenograft rejection. J Immunol. 2001;166(3):2133-40.

6. Abe M, Cheng J, Qi J, Glaser RM, Thall AD, Sykes M, et al. Elimination of porcine hemopoietic cells by macrophages in mice. J Immunol. 2002;168(2):621-8.

7. Yang YG, Sykes M. Xenotransplantation: current status and a perspective on the future. Nat Rev Immunol. 2007;7(7):519-31.

8. Gorczynski RM. Transplant tolerance modifying antibody to CD200 receptor, but not CD200, alters cytokine production profile from stimulated macrophages. Eur J Immunol. 2001;31(8):2331-7.

9. El-Ouaghlidi A, Jahr H, Pfeiffer G, Hering BJ, Brandhorst D, Brandhorst H, et al. Cytokine mRNA expression in peripheral blood cells of immunosuppressed human islet transplant recipients. J Mol Med (Berl). 1999;77(1):115-7.

10. Pietila M, Lehtonen S, Tuovinen E, Lahteenmaki K, Laitinen S, Leskela HV, et al. CD200 positive human mesenchymal stem cells suppress TNF-alpha secretion from CD200 receptor positive macrophage-like cells. PLoS One. 2012;7(2):e31671.

11. Wang H, Madariaga ML, Wang S, Van Rooijen N, Oldenborg PA, Yang YG. Lack of CD47 on nonhematopoietic cells induces split macrophage tolerance to CD47null cells. Proc Natl Acad Sci U S A. 2007;104(34):13744-9.

12. Oldenborg PA, Zheleznyak A, Fang YF, Lagenaur CF, Gresham HD, Lindberg FP. Role of CD47 as a marker of self on red blood cells. Science. 2000;288(5473):2051-4.

13. Oldenborg PA, Gresham HD, Lindberg FP. CD47-signal regulatory protein alpha (SIRPalpha) regulates Fcgamma and complement receptor-mediated

phagocytosis. J Exp Med. 2001;193(7):855-62.

14. Wang H, VerHalen J, Madariaga ML, Xiang S, Wang S, Lan P, et al. Attenuation of phagocytosis of xenogeneic cells by manipulating CD47. Blood. 2007;109(2):836-42.

15. Ide K, Wang H, Tahara H, Liu J, Wang X, Asahara T, et al. Role for CD47-SIRPalpha signaling in xenograft rejection by macrophages. Proc Natl Acad Sci U S A. 2007;104(12):5062-6.

16. Tena AA, Sachs DH, Mallard C, Yang YG, Tasaki M, Farkash E, et al. Prolonged Survival of Pig Skin on Baboons After Administration of Pig Cells Expressing Human CD47. Transplantation. 2016.

17. Tena A, Kurtz J, Leonard DA, Dobrinsky JR, Terlouw SL, Mtango N, et al. Transgenic expression of human CD47 markedly increases engraftment in a murine model of pig-to-human hematopoietic cell transplantation. Am J Transplant. 2014;14(12):2713-22.

18. Karra L, Berent-Maoz B, Ben-Zimra M, Levi-Schaffer F. Are we ready to downregulate mast cells? Curr Opin Immunol. 2009;21(6):708-14.

19. Mihrshahi R, Barclay AN, Brown MH. Essential roles for Dok2 and RasGAP in CD200 receptor-mediated regulation of human myeloid cells. J Immunol. 2009;183(8):4879-86.

20. Rijkers ES, de Ruiter T, Baridi A, Veninga H, Hoek RM, Meyaard L. The inhibitory CD200R is differentially expressed on human and mouse T and B

lymphocytes. Mol Immunol. 2008;45(4):1126-35.

21. Soliman H, Mediavilla-Varela M, Antonia S. Indoleamine 2,3-dioxygenase: is it an immune suppressor? Cancer J. 2010;16(4):354-9.

22. Gorczynski RM, Cattral MS, Chen Z, Hu J, Lei J, Min WP, et al. An immunoadhesin incorporating the molecule OX-2 is a potent immunosuppressant that prolongs allo- and xenograft survival. J Immunol. 1999;163(3):1654-60.

23. Chen DX, He H, Gorczynski RM. Synthetic peptides from the N-terminal regions of CD200 and CD200R1 modulate immunosuppressive and anti-inflammatory effects of CD200-CD200R1 interaction. Int Immunol. 2005;17(3):289-96.

24. Yu K, Gorczynski RM. Persistence of gene expression profile in CD200 transgenic skin allografts is associated with graft survival on retransplantation to normal recipients. Transplantation. 2012;94(1):36-42.

25. Chung DJ, Rossi M, Romano E, Ghith J, Yuan J, Munn DH, et al. Indoleamine 2,3-dioxygenase-expressing mature human monocyte-derived dendritic cells expand potent autologous regulatory T cells. Blood. 2009;114(3):555-63.

26. Sharma MD, Hou DY, Liu Y, Koni PA, Metz R, Chandler P, et al. Indoleamine 2,3-dioxygenase controls conversion of Foxp3+ Tregs to TH17-like cells in tumor-draining lymph nodes. Blood. 2009;113(24):6102-11.

27. Elyaman W, Bradshaw EM, Uyttenhove C, Dardalhon V, Awasthi A,

Imitola J, et al. IL-9 induces differentiation of TH17 cells and enhances function of FoxP3+ natural regulatory T cells. Proc Natl Acad Sci U S A. 2009;106(31):12885-90.

28. Gorczynski R, Chen Z, Khatri I, Yu K. Long-Term Tolerance and Skin Allograft Survival in CD200tg Mice After Autologous Marrow Transplantation. Transplantation. 2014;98(12):1271-8.

29. Gorczynski RM, Chen Z, He W, Khatri I, Sun Y, Yu K, et al. Expression of a CD200 transgene is necessary for induction but not maintenance of tolerance to cardiac and skin allografts. J Immunol. 2009;183(3):1560-8.

30. Boudakov I, Liu J, Fan N, Gulay P, Wong K, Gorczynski RM. Mice lacking CD200R1 show absence of suppression of lipopolysaccharide-induced tumor necrosis factor-alpha and mixed leukocyte culture responses by CD200. Transplantation. 2007;84(2):251-7.

31. Gorczynski RM, Hu J, Chen Z, Kai Y, Lei J. A CD200FC immunoadhesin prolongs rat islet xenograft survival in mice. Transplantation. 2002;73(12):1948-53.

국문초록

서론: CD200 단백질과 CD47 단백질은 대식세포의 활성화를 억제할 수 있는데, CD200은 대식세포의 염증성 사이토카인의 분비를 억제하고, CD47은 대식세포의 식세포 작용을 억제한다. 이종 면역반응에서는, 인간 CD47를 돼지 세포에 과발현했을 때 인간 대식세포의 돼지세포에 대한 식세포 작용을 억제함으로써 이종이식 거부반응을 억제할 수 있다는 보고가 있었다. 이에, 인간 CD200과 인간 CD47의 인간 대식세포의 이종면역반응 억제능을 비교하고, 나아가 CD200과 CD47을 동시에 과발현시켰을 대의 효능을 평가하고자 하였다.

방법: 인간 CD200과 CD47를 발현하는 렌티바이러스를 제조하여 돼지 혈관내피세포에 도입한 후, 돼지 혈관내피세포가 인간 CD200 혹은 CD47을 안정적으로 세포막 표면에 발현하거나 분비할 수 있는 세포주를 구축하였다. 인간 CD200 또는 CD47 과발현 돼지 내피세포주와 인간 단핵구를 함께 배양함으로써 인간 단핵구의 염증성 사이토카인 분비, 돼지 세포에 대한 독성작용, 증식 반응과 식세포 작용에 대한 인간 CD200과 CD47을 단독 또는 병용했을 때의 억제능을 평가하였다.

결과: 인간 CD200 또는 CD47이 과발현된 돼지 혈관내피세포와 인간 단핵구의 이종 면역 반응에서, 인간 CD200과 CD47은 인간 단핵구의 돼지 세포에 대한 세포괴사 및 세포자연사 등의 세포독성작용을 억제하였고, 단핵구의 식세포 작용을 조절하였다. 또한 이종면역반응에서 인간 단핵구의 IL-1, IL-6와 같은 염증성 사이토카인의 분비를 억제하였고, 돼지세포의 이종면역자극에 대한 인간 단핵구의 증식을 억제하였다. 이런 면역반응의 조절능은 인간 CD200과 CD47이 함께 돼지 내피세포에 과발현되었을 때 더 큰 효과를 나타내었다.

결론: 인간 CD200과 CD47은 이종 면역반응에서 인간 단핵구의 활성화를 억제하였고, 함께 발현되었을 때는 더 큰 억제능을 보여, 향후 이종이식 거부반응을 억제하는데 유용하게 활용될 수 있을 것이다.

주요어: 대식세포, 이종이식, CD200, CD47, 돼지

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