Mouse CD47 cDNA Plasmid Construction and Transfection

The entire coding region of the CD47 cDNA was PCR-amplified from reverse-transcribed mouse lymphocyte cDNA with primers (sense) 5′-GCGAAGTGACAGAGTTATCC-3′ and (antisense) 5′-TGGCTCACATGCCATGATGC-3′. The amplified PCR product was digested with *Eco*RI/*Not*I and cloned into the pRc/CMV vector (kindly provided by Dr. Tadashi Furusawa, National Institute of Animal Research Industry, Japan), which had been predigested with the same restriction endonucleases. Rat insulinoma cells (INS-1E) were transfected with either pRc/CMV-mouse CD47 or the empty plasmid, using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Five hours after the transfection, cells were selected with G418 (Sigma-Aldrich; 800 μg/mL) for 1 week to generate stable cell lines and analyzed for expression of mouse CD47 by FACS analyses.

Diabetic Mice Generation

Rag2$^{-/-}$ γ chain $^{-/-}$ mice were rendered diabetic through a single i.p. administration of 200 mg/kg streptozotocin (Sigma-Aldrich) at 6 days prior to injection of rat INS-1E cells. Diabetic mice with non-fasting blood glucose levels of >400 mg/dL on the day of transplantation were used as the recipients. The blood glucose levels were monitored with a blood glucose test meter (Medisafemini GR-102; Terumo, Somerset, NJ, USA). In the absence of INS-1E cell transplantation, diabetes persisted in all diabetic mice (blood glucose level: 0.350 mg/dL), and no spontaneous reversal of diabetes was observed for at least the next 3 months.

In vivo Phagocytic Assay

Target cells were stained with the fluorescent dye 5/6-CFSE (Molecular Probes, Eugene, OR, USA) according to the manufacturer's protocol. Either CFSE-labeled mCD47-INS-1E cells (10×10^6) or cont-INS-1E cells (10×10^6) were injected into the peritoneal cavity of streptozotocin-induced diabetic Rag2$^{-/-}$ γ chain $^{-/-}$ mice. After 6 h, the recipient intraperitoneal cells were harvested and the macrophages that phagocytosed the target cells could be identified by FACS analysis, based on CFSE labeling.

Statistical analysis. Significant differences between groups were determined using Student's t-test. A p-value of <0.05 was considered statistically significant.

Results

Rat CD47 does not Induce Tyrosine Phosphorylation of SIRPα in Mouse Macrophages

In the CD47-SIRP system, the interaction between SIRP, on macrophages, and CD47, on target cells, inhibits phagocytosis of the target cells by promoting phosphorylation of tyrosine in the cytoplasmic domain, and recruitment of Src homology 2 domain-containing protein tyrosine phosphatase-1, which is the major regulator of phagocytic responses [20].

To determine whether rat CD47 can interact with mouse SIRPα, we assessed tyrosine phosphorylation of SIRPα in mouse macrophages after contact with either rat or mouse RBCs. Western blotting revealed that incubation of mouse peritoneal cavity macrophages with mouse RBCs resulted in SIRPα tyrosine phosphorylation, as expected (Fig. 1). However, after incubation with rat RBCs, this tyrosine phosphorylation was not induced in mouse macrophages above the level in control macrophages, which had been incubated with medium alone, indicating that rat CD47 fails to induce SIRPα tyrosine phosphorylation in mouse macrophages.

Mouse CD47 Expression on Rat Cells Markedly Reduces the Susceptibility to Phagocytosis by Mouse Macrophages

To determine whether expression of mouse CD47 on rat cells could efficiently prevent their phagocytosis by mouse macrophages, we generated rat insulinoma cell lines that express mouse CD47 by transfecting rat cells with a mouse CD47-expressing plasmid, pRc/CMV-mouse CD47 (Fig. 2A). Mouse CD47 expression on the transfected INS-1E cells was confirmed by FACS analysis (Fig. 2B). The expression level of CD47 on the pRc/CMV-mouse CD47 vector-transfected INS-1E cells (mCD47-INS-1E) was higher than that on mouse PBMCs, whereas the control vector-transfected INS-1E (cont-INS-1E) cells tested negative for mouse CD47. We have confirmed constant expression of mouse CD47 on mCD47-INS-1E cells in diabetic Rag2$^{-/-}$ γ chain$^{-/-}$ mice without further G418-selection at least until 8 days after the inoculation. Western blotting revealed that incubation of mouse macrophages with mCD47-INS-1E resulted in significant tyrosine phosphorylation of SIRPα, indicating that mCD47-INS-1E cells functionally interact with mouse SIRPα (Fig. 2C). Except for functional mouse CD47 expression, mCD47-INS-1E cells were comparable to cont-INS-1E cells with respect to their morphology, proliferation rates, and insulin producing activity (data not shown).

The phagocytic activities of mouse macrophages toward both INS-1E cell lines were evaluated by *in vivo* assays. CFSE-labeled mCD47-INS-1E or cont-INS-1E cells were injected into the peritoneal cavity of streptozotocin-induced diabetic Rag2$^{-/-}$ γ chain $^{-/-}$ mice, which lack T, B, and NK cells. The recipient intraperitoneal cells were harvested after 6 h, and macrophages that phagocytosed the target cells were then detected as CD11b- and CFSE-double–positive cells, using FACS analysis (Fig. 3A). The proportion of CFSE$^+$ cells among all the CD11b$^+$ cells was significantly lower in cells obtained from mCD47-INS-1E recipients than from cont-INS-1E recipients. This result indicated that mouse CD47 expression on rat cells markedly reduced the susceptibility of these cells to phagocytosis by mouse macrophages (Fig. 3B).

To investigate whether these protections were due to CD47 overexpression or to the species-specific effect of mouse CD47, another line of rat insulinoma cells (mCD47^{low}-INS-1E) labeled with CFSE, which expressed lower levels of mouse CD47 (MFI 330.67) than the original mCD47-INS-1E cells (MFI 506.88), were injected into the peritoneal cavity of streptozotocin-induced diabetic Rag2$^{-/-}$ γ chain$^{-/-}$ mice. Intraperitoneal cells of the recipients were harvested after 6 h, and macrophages that phagocytosed the target cells were detected using FACS analysis. The proportion of CFSE$^+$ cells among all CD11b$^+$ cells was significantly lower in cells obtained from mCD47^{low}-INS-1E recipients than in those from cont-INS-1E recipients. However, no significant difference was observed in phagocytic activity between mCD47-INS-1E recipients and mCD47^{low}-INS-1E recipients. This result indicated that, in this model, the protection was not simply due to overexpression of mouse CD47 (Fig. 3B).

Diabetic Rag2$^{-/-}$ γ chain $^{-/-}$ Mice became Normoglycemic after Receiving mCD47-INS-1E

Next, mCD47-INS-1E or cont-INS-1E cells were injected into the peritoneal cavity of Rag2$^{-/-}$ γ chain $^{-/-}$ mice with streptozotocin-induced diabetes; the blood glucose levels of these

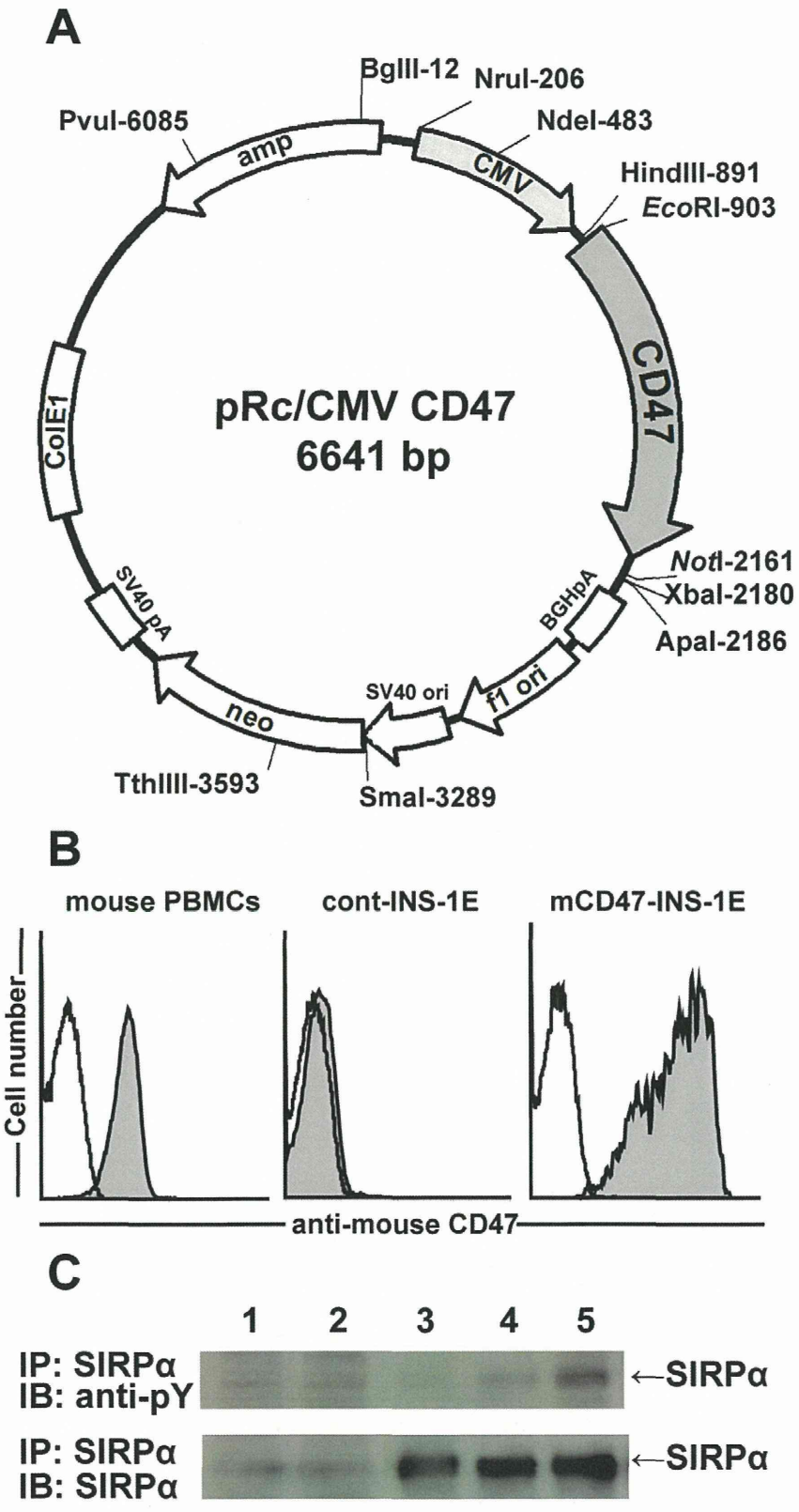

Figure 2. Generation of mouse CD47-expressing rat cell line. (A) Structure of pRc/CMV-mouse CD47. The entire coding region of the mouse CD47 cDNA was PCR-amplified. The amplified PCR product was digested and full-length mouse CD47 cDNA was inserted into the expression vector pRc/CMV. (B) Expression of mouse CD47 on a transfected rat insulinoma cell (INS-1E) was confirmed by FACS analysis. Representative histograms obtained by FACS analysis for mouse PBMCs, pRc/CMV-transfected rat INS-1E cells (cont-INS-1E), and pRc/CMV-mouse CD47-transfected rat INS-1E

cells (mCD47-INS-1E) are shown. Open and filled histograms represent staining with isotype control and with anti-mouse CD47 mAb, respectively. (C) Tyrosine phosphorylation of SIRPα in mouse macrophages was induced by incubation with pRc/CMV-mouse CD47-transfected rat INS-1E cells (mCD47-INS-1E), but not with control vector-transfected rat INS-1E cells (cont-INS-1E). Differentiated mouse macrophages were incubated with mCD47-INS-1E or cont-INS-1E at 37°C for 30 min. The cells were lysed, and the lysates were mixed with mouse anti-mouse SIRPα antibodies and 50% slurry of protein G-sepharose beads by rotation at 4°C for 8 hrs. Precipitated proteins were separated by 8% 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. Mouse CD47-transfected INS-1E (mCD47-INS-1E) alone (lane 2), mouse macrophages incubated in medium alone (lane 3) or mouse macrophages incubated with cont-INS-1E (lane 4) or mCD47-INS-1E (lane 5) are shown. Immunoblotting with anti-mouse SIRPα was used as loading control. IP, immunoprecipitation; IB, immunoblotting; anti-pY, anti-phosphotyrosine.
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mice were monitored for 7 days. Diabetic Rag2$^{-/-}$ γ chain $^{-/-}$ mice became normoglycemic after receiving mCD47-INS-1E. In contrast, the mice that received cont-INS-1E failed to achieve normoglycemia (Fig. 4). Thus, the in vivo transplant model proved that genetically engineered expression of mouse CD47 in rat insulinoma cells could inhibit macrophage-mediated xenograft rejection.

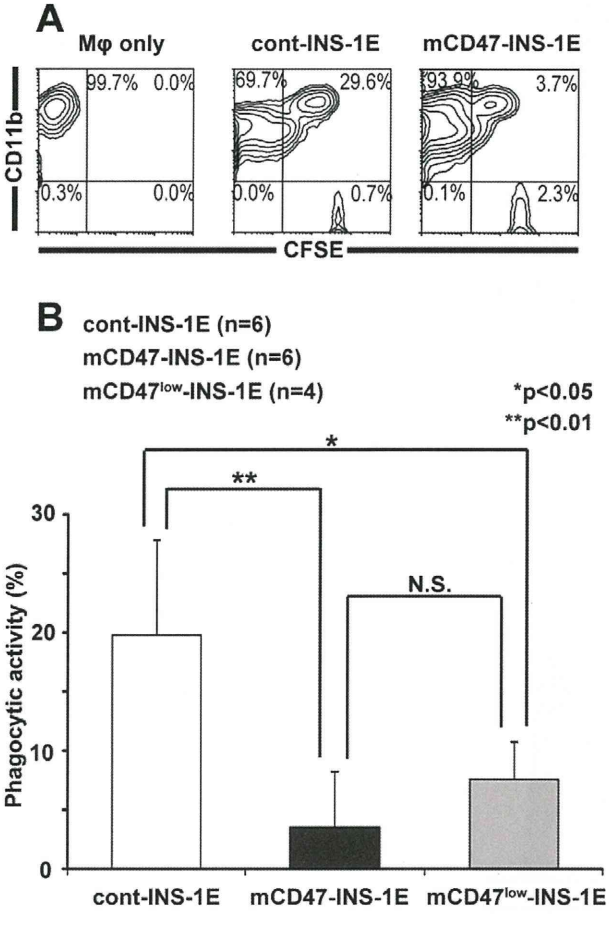

Figure 3. Mouse CD47-expressing rat INS-1E cells attenuate phagocytosis by mouse macrophages. (A) CFSE-labeled pRc/CMV-mouse CD47-transfected rat INS-1E cells (mCD47-INS-1E) and control vector-transfected rat INS-1E cells (cont-INS-1E) were injected into peritoneal cavity of streptozotocin-induced diabetic Rag2$^{-/-}$ γ chain $^{-/-}$ mice. After 6 h, the intraperitoneal cells from the recipient mice were harvested. Mouse macrophages counterstained with allophycocyanin-conjugated anti-mouse CD11b and phagocytosis of CFSE-labeled targets were measured by FACS analysis. Representative FACS profiles are shown. Regions representing non-phagocytosing macrophages are shown in the upper left quadrants, regions representing phagocytosing macrophages are shown in the upper right quadrants, and regions representing residual targets are shown in the lower right quadrants. (B) Phagocytic activity was calculated by the following formula: phagocytic activity = (percentage of engulfing macrophages/percentage of total harvested macrophages) ×100. Data are given as the means ± SD.
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CD47-SIRPα Signaling Blockade Prevents the Effects of Mouse CD47 on Macrophage-mediated Xenograft Rejection

We further explored the practical contribution of CD47-SIRPα signaling to the successful engraftment of mCD47-INS-1E xenografts in the diabetic Rag2$^{-/-}$ γ chain $^{-/-}$ mice by using the anti-mouse SIRPα Ab (P84) to block this signaling. Mouse SIRPα, on the peritoneal macrophages of Rag2−/− γ chain −/− mice, had not been decreased and had been capped after the intraperitoneal injection of P84 for at least 8 days (Fig. 5A and B). Twenty-four hours after P84 injection, either mCD47-INS-1E or cont-INS-1E cells were injected into the peritoneal cavity of the diabetic Rag2$^{-/-}$ γ chain $^{-/-}$ mice. Irrespective of which cell line recipients received, mice failed to achieve normoglycemia (Fig. 5C), indicating the essential role of CD47-SIRPα signaling in overcoming macrophage-mediated rejection of xenografts.

CD47-SIRPα Signaling Blockade does not Induce Phagocytosis of Congenic Cells

We further examined whether injection of P84 into the peritoneal cavity of diabetic Rag2$^{-/-}$ γ chain$^{-/-}$ mice results

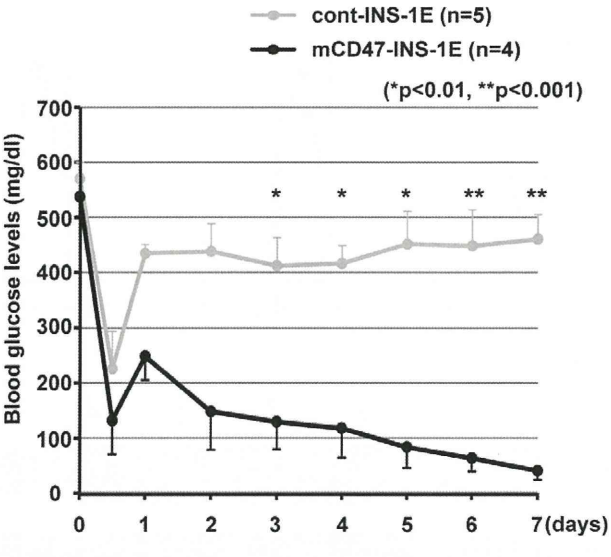

Figure 4. Diabetic Rag2$^{-/-}$ γ chain $^{-/-}$ mice became normoglycemic after receiving mCD47-INS-1E. Either mCD47-INS-1E or cont-INS-1E cells were injected into the peritoneal cavity of Rag2$^{-/-}$ γ chain $^{-/-}$ mice with streptozotocin-induced diabetes. Blood glucose levels were monitored for 7 days. Data are presented as the means ± SD.
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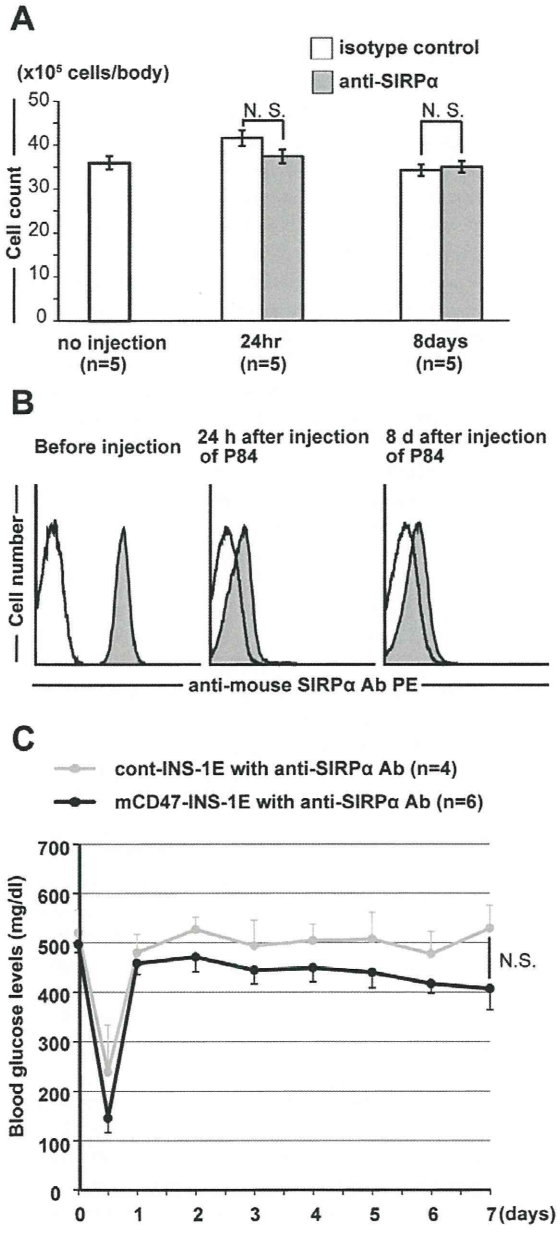

Figure 5. Inhibition of CD47-SIRPα signaling prevents the effect of genetic induction of recipient CD47 in xenografts. (A) Either P84 or control antibody was injected into the peritoneal cavity of diabetic Rag2$^{-/-}$ γ chain$^{-/-}$ mice. After injection, intraperitoneal cells from recipient mice were harvested and SIRPα+ peritoneal cells were counted. (B) Anti-mouse SIRPα mAb (P84) was injected into the peritoneal cavity of Rag2−/− γ chain −/− mice. Expression of mouse SIRPα on mouse peritoneal macrophages was confirmed by FACS analysis. Open and filled histograms represent staining with isotype control and with anti-mouse SIRPα mAb, respectively. (C) Twenty-four hours after the injection of anti-mouse SIRPα mAb (P84), either mCD47-INS-1E or cont-INS-1E cells were injected into the peritoneal cavity of the diabetic Rag2$^{-/-}$ γ chain $^{-/-}$ mice. Blood glucose levels were monitored for 7 days. Data are given as the means ± SD. N.S.: not significant.
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in phagocytosis by mouse cells. Twenty-four hours after injection of either P84 or control antibody, CFSE-labeled congenic T cells were injected into the peritoneal cavity of mice. After 6 h, intraperitoneal cells of the recipients were harvested and mouse macrophages that phagocytosed congenic T cells were then detected as CD11b- and CFSE-double–positive cells using FACS analysis. As shown in Fig. 6, no significant difference was observed in phagocytic activity between the 2 groups. This result indicated that CD47-SIRPα signaling blockade does not induce phagocytosis of congenic cells.

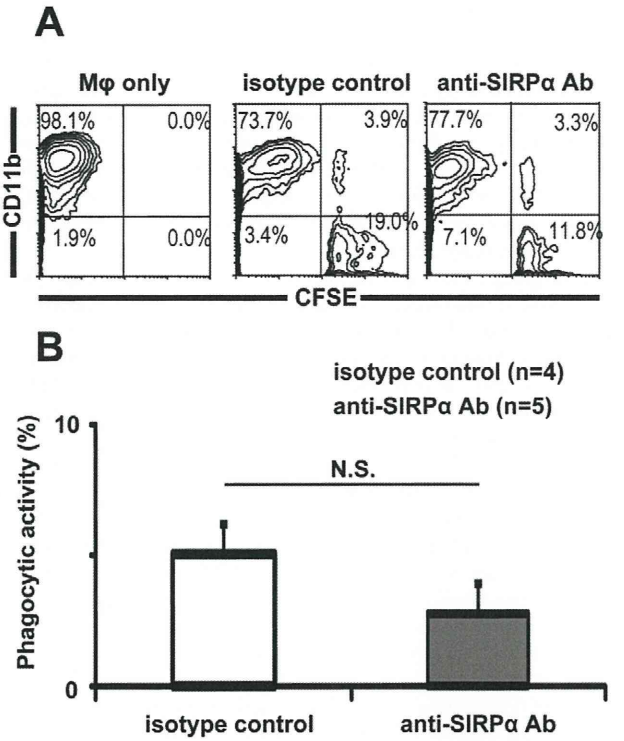

Figure 6. CD47-SIRPα signaling blockade does not induce phagocytosis of congenic cells. (A) Twenty-four hours after injection of either P84 or control antibody, CFSE-labeled congenic T cells were injected into the peritoneal cavity of mice. After 6 h, the intraperitoneal cells from recipient mice were harvested. Mouse macrophages counterstained with allophycocyanin-conjugated anti-mouse CD11b and phagocytosis of CFSE-labeled congenic T cells were determined by FACS analysis. Representative FACS profiles are shown. Regions representing non-phagocytosing macrophages are shown in the upper left quadrants, regions representing phagocytosing macrophages are shown in the upper right quadrants, and regions representing residual congenic T cells are shown in the lower right quadrants. (B) Phagocytic activity was calculated by using the following formula: phagocytic activity = (percentage of engulfing macrophages/percentage of total harvested macrophages) × 100. Data are presented as means ± SD.
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Discussion

In the present study, genetic induction of the expression of mouse CD47 on rat insulin-producing cells could deliver inhibitory signaling to SIPRα on mouse peritoneal macrophages, preventing rejection of the rat cells during observation periods. It has previously 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 on mouse, cow, or rat RBCs binds the recombinant extracellular dommain of human

SIRPα1 [21]. It has been also demonstrated that pig CD47 does not interact with mouse SIRPα [22]. Consistently, the phagocytic synapse at cell contacts has been proven to involve a basal level of actin-driven phagocytosis that is made more efficient by phospho-activated myosin in the absence of species-specific CD47 signaling [23]. Recently, we have verified that pig CD47 also does not interact with human SIRPα, and, importantly, that genetic manipulation of porcine cells for expression of human CD47 markedly reduces the susceptibility of these cells to phagocytosis by human macrophages *in vitro* [10].

Other groups have also shown that functional species-specific CD47/SIRPα interaction is required for generating improved models of mouse/human chimeras: mouse CD47-expression in transplanted human hematopoietic cells is required for optimal human T- and natural killer-cell homeostasis in mice [24]. Furthermore, the introduction of mouse CD47 into primary human hepatocytes confers a positive selective advantage upon engraftment into the mouse liver *in vivo* [25]. Currently available data from in vivo experiments assessing xenograft survival indicates that CD47 provides a potential molecular target for inhibiting macrophage-mediated rejection of xenogeneic cells. Hence, this suggests the necessity of establishing human CD47-transgenic pigs as clinically applicable donors of xenografts.

It is well known that innate immune responses mediated by monocytes/macrophages can drive and shape the process of adaptive immunity. Phagocytic activities of macrophages form a first line of defense against invading infectious microbes, and these macrophages can present antigens derived from such phagocytosed 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 host macrophages toward xenogeneic cells by genetically inducing host-type CD47 expression may also attenuate subsequent T cell immune responses against xenoantigens, while maintaining normal responses against other pathogens.

It has also been reported that a similar CD47-SIRP system negatively regulates the functions of both T cells and APCs in humans [14]. In contrast, it has been demonstrated that the interaction between CD47 on APCs and SIRPγ (also known as SIRPβ2) on T cells promotes the proliferation of antigen-specific T cells and co-stimulates T cell activation [26]. These observations raise a question as to whether interspecies incompatibility of CD47 affects CD4+ T cell-mediated responses to xenoantigens positively or negatively. In our previous study, recombinant human CD47-Fc fusion protein (which contains the extracellular domain of human CD47 fused to the Fc portion of human immunoglobulin) significantly reduced the indirect response of human CD4+ T cells to porcine antigens, but did not affect the direct response of these cells in in vitro pig-to-human mixed lymphocyte reaction assays [27]. Inhibition of the phagocytic activity of human APCs toward porcine cells by interaction between the human CD47-Fc fusion protein and the corresponding ligand, probably SIRPα, might might attenuate subsequent CD4+ T cell immune responses against porcine antigens. Taking into consideration that SIRPγ binds CD47 with a lower affinity (KD: about 23 μM) than SIRPα (KD: about 2 μM) [28], the interaction between human CD47-Fc and SIRPγ on human CD4+ T cells conferring direct xenospecificity may might not affect CD4+ T cell immune responses. Unlike this in vitro system, however, if human CD47 molecules are highly expressed on porcine APCs by genetic manipulations, there will be a risk that CD4+ T cell-mediated responses to xenoantigens are promoted. These possibilities should be addressed in further studies employing an immunocompetent animal model enabling long-term observation. In that particular case, the model utilizing rat inslinoma in mice would not be suitable, since the mouse recipients of mCD47-INS-1E cells in this study eventually died from hypoglycemia due to growth of the inocula. Further studies are needed for long-term observation employing a rat CD47-transfected normal mouse islet model.

It has been reported that the ability of glucocorticoids to promote macrophage phagocytosis of CD47-deficient targets could, in part, be mediated by an upregulation of expression of LDL receptor-related protein-1 (LRP1/CD91/α2-macroglobulin receptor) macrophages [29]. Since glucocorticoids are indispensable in immunosuppressive therapy after xenogeneic or even allogeneic cellular/organ transplantation, glucocorticoid-treated macrophages may enhance phagocytosis of xenogeneic cells. Therefore, genetic manipulation of xenogeneic cells for host-type CD47 expression would be particularly useful to reduce the likelihood of phagocytosis by macrophages.

It has been recently demonstrated that CD47 is a molecule commonly expressed on neoplastic cells. Its function to block phagocytosis is known, and blockade of this function leads to tumor cell phagocytosis and elimination [30,31]. Consistently, in a separate experiment, we also observed that insulinoma cell function in a syngeneic model depends on SIRPα-mediated inhibition of macrophages through engagement with its ligand CD47, i.e., treatment with anti-SIRPα Abs enhanced macrophage-mediated elimination of mouse insulinoma cells in diabetic $Rag2^{-/-}$ γ $chain^{-/-}$ mice (data not shown). It remains to be elucidated whether the observations made in neoplastic cells in this study apply to normal xenografts. However, together with our previous in vitro finding that genetic induction of human CD47 on porcine non-neoplastic lymphoblastoid cells radically reduced the susceptibility of those cells to phagocytosis by human macrophages, our results of the present study may lead to the development of approaches for attenuating macrophage-mediated xenograft rejection by genetic manipulation of porcine cells for human CD47 expression.

In conclusion, we have here demonstrated that interspecies incompatibility of CD47 significantly contributes to *in vivo* rejection of xenogeneic cells by macrophages. Our results imply that genetic induction of recipient CD47 on xenogeneic donor cells could provide inhibitory signals to recipient macrophages via SIPRα; this constitutes a novel approach to prevent macrophage-mediated xenograft rejection.

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Author Contributions

Conceived and designed the experiments: KI HO. Performed the experiments: YT HM. Analyzed the data: YT KI. Contributed reagents/materials/analysis tools: YT HT. Wrote the paper: KI HO.

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肝免疫と肝臓外科
— Liver Immunity and Surgery —

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はじめに

肝臓は腸管由来の微生物，エンドトキシン，腫瘍細胞など外来抗原を含有した門脈血が流入する臓器で，類洞内皮細胞(LSEC)，natural killer (NK)細胞，NKT 細胞，Kupffer 細胞などさまざまの免疫担当細胞が内在する。これらの細胞群は生体防御機能を司る一方で，過剰な免疫機構を制御する寛容機構も有する。肝臓内の複雑な免疫調節機構を掌握することは，肝臓外科領域の周術管理において生体防御能を損なわず癌再発や肝障害を予防/軽減する戦略を立てるうえで非常に有益な情報となる。本原稿では，肝局在免疫担当細胞のうち LSEC，NK 細胞と NKT 細胞の機能特性に関するわれわれの研究成果を紹介し，臨床治療戦略への応用について考察する。

肝 LSEC の免疫寛容誘導への関わり

1．肝臓の免疫寛容誘導機構について

肝移植では，ほかの臓器移植に比べ拒絶反応を引き起こす頻度は低い[1]。古くから肝臓は免疫寛容獲得に関わる臓器として知られるが，なぜ移植肝が拒絶されにくいのか説得力のある検証はいまだなされていない。

移植肝内で，循環リンパ球が最も高頻度かつ密接に接触するのは，解剖学構築からして LSEC である。そこでわれわれは，マウスの肝臓構築細胞の非実質細胞群から LSEC を選択的に分離し，免疫原性を解析した結果，寛容誘導特性を有することを確認した[2)3)]。

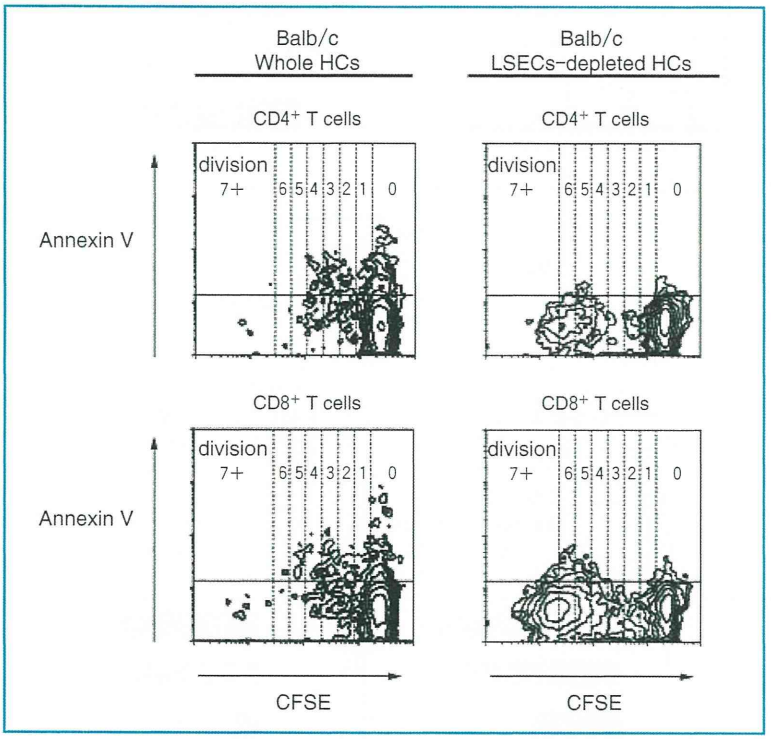

図1　肝LSECはアロ反応性T細胞にアポトーシスを誘導する

Balb/cマウスの肝臓構築細胞をstimulatorに，B6の脾リンパ球をresponderに用い，MLRによってアロ反応性のCD4$^+$およびCD8$^+$T細胞の増殖指数と存在比率を解析した。リンパ球はcarboxyfluorescein diacetate succinimidyl ester（CFSE）色素で細胞質染色した（CFSE-MLR assay）。肝構築細胞のすべてをstimulatorとしてCFSE-MLRをした場合，すなわちLSECの存在下で混合培養した異系T細胞はわずかながら分裂を認めたが，その分裂T細胞は全てアネキシンV陽性で，分裂初期にアポトーシスに陥ることがわかった。LSECを反応系から除去すると激しいT細胞の分裂/増殖を認めた。肝内ではLSECのみにCD105の表出を認め，抗CD105抗体を用い単離した。

HCs：hepatic constituent cells, LSECs：liver sinusoidal endothelial cells.

（文献2より引用）

2．LSECと接触したアロ反応性T細胞は寛容化する/マウス肝構成細胞の同種異系免疫原性の解析

マウスを含むいくつかの動物種において，主要組織適合性抗原（MHC）が異なる同種異系肝移植を施行した際に，移植後の免疫抑制剤を使用しなくても拒絶反応が起こらず生着する現象は以前より観察されている[4)5)]。そこでわれわれは，マウスの肝臓をコラゲナーゼ灌流法により構築細胞に分離し，同種異系リンパ球の混合培養試験（MLR）を行い，免疫原性を解析した。すなわち，Balb/c（H-2d）の肝臓構築細胞をstimulatorに，C57BL/6（B6）（H-2b）の脾リンパ球をresponderに用いMLRによって，アロ反応性のCD4$^+$およびCD8$^+$T細胞の増殖指数と存在比率を解析した[2)6)]。肝構築細胞のすべてをstimulatorとしてMLRをした場合，同種異系の組み合わせでもT細胞の分裂を認めなかった。ところが，LSECを反応系から除去すると激しいT細胞の分裂/増殖を認め，LSECがT細胞性アロ応答を抑制していることが判明した[2)]。また，LSECの存在下で混合培養した異系T細胞はわずかながら分裂を認めたが，その分裂T細胞はすべてアネキシンV陽性で，分裂初期にアポトーシスに陥ることがわ

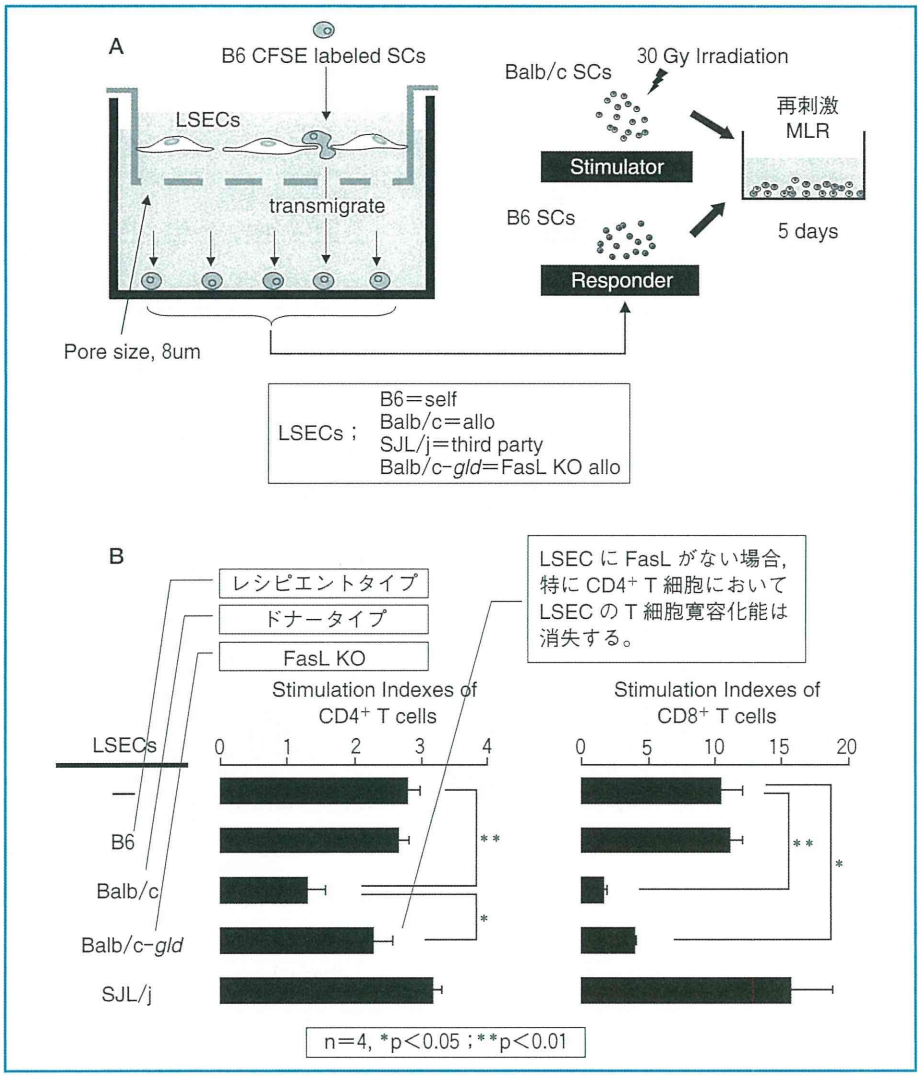

図 2　類洞内皮 transmigration assay

A：フィブロネクチンでコートした pore membrane に B6，Balb/c，Balb/c-*gld*（FasL-deficient）あるいは SJL/j マウス由来の LSEC を接着培養し，肝類洞内皮の解剖構築を模倣した *in vitro* 解析系を確立した。CFSE 色素でラベルした B6 マウスの T 細胞を重層培養しトランスマイグレートさせた後，放射線照射した Balb/c マウスの脾細胞と混合培養し MLR assay を行った。

B：Balb/c の LSEC 層を接触通過した T 細胞は，Balb/c 脾細胞抗原の刺激に対して不応答化した。

LSECs：liver sinusoidal endothelial cells, SCs：splenocytes.

（文献 2 より引用）

かった（図 1）。

マウス LSEC のフェノタイプを解析すると，MHC class Ⅱ，共刺激分子（CD40，CB80，CD86），細胞死誘導分子（Fas ligand ： FasL）を発現していた。単層培養膜（pore membrane）に B6，Balb/c，Balb/c-*gld*（FasL-deficient）あるいは SJL/j マウス由来の LSEC を接着培養し，肝類洞内皮の解剖構築を模倣した *in vitro* 解析系を確立した（図 2-A）。B6 マウスの T 細胞を重層培養しトランスマイグレートさせた後（transmigration assay），放射線照射した Balb/c マウスの脾細胞と混合培養し MLR assay を行った。Balb/c の LSEC 層を接触通過し

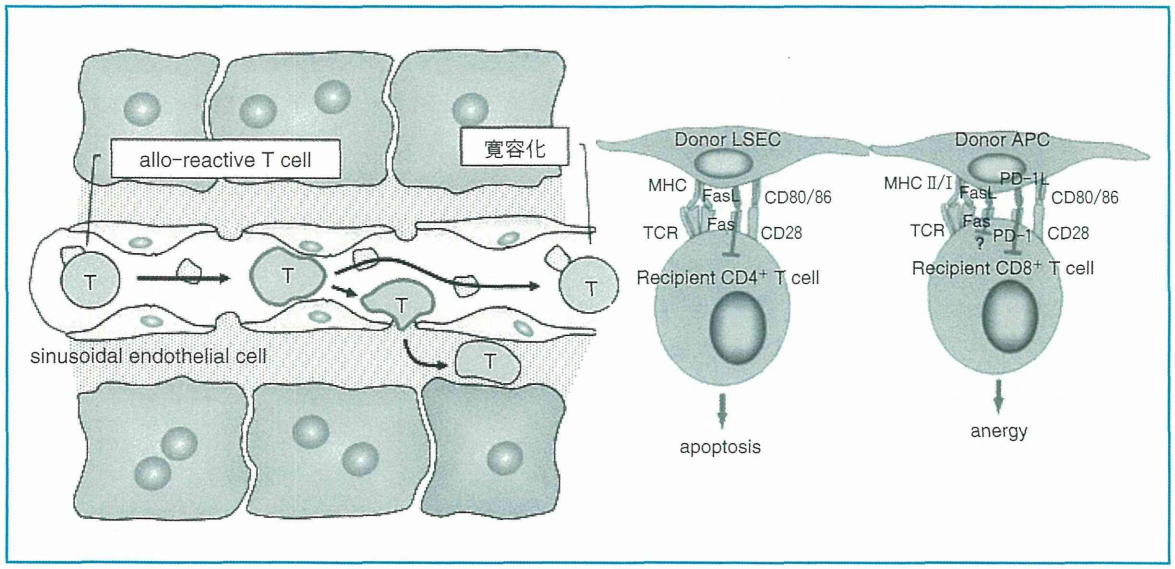

図3　マウス肝 LSEC は直接認識経路で応答する CD4$^+$ T 細胞を Fas-FasL pathway を介して寛容化する。

たT細胞は，Balb/c 脾細胞抗原の刺激に対して不応答化したが，B6 および SJL/j の LSEC 層を接触通過したT細胞は，Balb/c 脾細胞抗原の刺激に対して正常の応答を示した。また，Balb/c-*gld* の LSEC 層を接触通過したT細胞は，Balb/c 脾細胞抗原の刺激に対して部分的な応答を示した(図 2-B)[2]。LSEC 上に表出する FasL が特に CD4$^+$ T 細胞の寛容誘導へ重要な役割を果たすことが証明された。Balb/c-*gld* の LSEC 層を接触通過した CD8$^+$ T 細胞は，Balb/c 脾細胞抗原の刺激に対して有意な応答抑制を示し，CD8$^+$ T 細胞の寛容誘導には FasL 経路以外の機序も関与する可能性が考えられた(図 3)。

3．LSEC による間接認識経路を介したアロ抗原認識 T 細胞寛容化

肝臓あるいは肝細胞移植後の拒絶機構には，レシピエントのT細胞が移植肝臓内のドナー由来抗原提示細胞(APC)から MHC を直接認識する経路とレシピエント自身の APC から移植肝臓内由来のドナー抗原を間接認識する経路がある。移植抗原に対する免疫寛容の誘導には，それぞれの経路で抗原提示されるT細胞を制御しなければならない。上述機序は，マウス肝 LSEC によって抗原提示された異系T細胞が寛容誘導されることを証明したものである(直接認識経路)。これに加えて，アロ抗原を貪食したマウス肝 LSEC によって抗原提示された同系T細胞にも寛容が誘導されることを確認した(間接認識経路)[3)7)]。この知見は，門脈内アロ抗原/細胞移入後に観察されるドナー特異的寛容にも，肝 LSEC が重要な役割を果たしている可能性を示唆する。

4．LSEC が間接認識経路でアロ応答 T 細胞を寛容化する過程で NKT 細胞が重要な関わりをもつ

ドナー脾細胞の門脈投与は，臓器移植における理想である特異的免疫寛容の誘導法の1つとして研究されてきた。しかしその機序の詳細はいまだ不明で，臨床応用には至っていない。われわれは，Balb マウスに MHC class II-deficient *C2ta*^{tm1Ccum} (C2D)-B6 マウスの脾細胞を門脈内投与後，C2D-B6 の心臓を移植すると永久生着することを確認した(C2D 移植片は間接認識経路によって免