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CD47 in Xenograft Rejection and Tolerance Induction

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Abstract

Robust immune responses to xenografts remain a major obstacle to clinical translation of xenotransplantation, which could otherwise be a potential solution to the worldwide shortage of organ donors. The more vigorous xenograft rejection relative to allograft rejection is largely accounted for by the extensive genetic disparities between the donor and recipient. Xenografts activate host immunity not only by expressing immunogenic xenoantigens that provide the targets for immune recognition and rejection, but also by lacking ligands for the host immune inhibitory receptors. This review is focused on recent findings regarding the role of CD47, a ligand of an immune inhibitory receptor SIRP α , in xenograft rejection and induction of xenotolerance.

Introduction

Clinical transplantation is the most effective therapy for end-stage organ failure, but many patients in need do not receive this therapy due to the severe shortage of human organ donors. This supply-demand imbalance could be corrected by the ability to transplant organs, tissues and cells from pigs (i.e., xenografts) into humans [1, 2]. However, transplants across discordant species barriers are subject to vigorous immunologic rejection. In addition to adaptive immune responses, which play critical roles in the rejection of both allo- and xenografts, innate immunity also mediates vigorous xenograft rejection. Transplantation of organs from pigs results in hyperacute rejection in primate recipients due to the presence of anti-Gal α 1-3Gal (α 1,3Gal) natural antibodies (Abs) in their sera. If hyperacute rejection is overcome, delayed humoral rejection (i.e., acute vascular rejection) is likely to develop. Successful generation of α 1,3Gal transferase (α 1,3GalT) knockout (KO) pigs [3–7] may overcome the anti- α 1,3Gal Ab-mediated xenograft rejection, but non- α 1,3Gal-specific anti-pig Abs, produced either prior to (natural Abs) or after transplantation present an additional barrier to acceptance of transplanted organs from GalT KO pigs to humans [8, 9]. Furthermore, the cellular innate immune responses, including those mediated by NK cells [10–14] and macrophages (see discussion below) have also been shown to contribute to the rejection of porcine xenografts. This review summarizes recent insights into the role of CD47 in xenograft rejection and induction of xenotolerance.

CD47 serves as a ‘marker of self’ to prevent phagocytosis of hematopoietic cells

Macrophages are essential to both innate and adaptive immune responses. They initiate the innate immune response by recognizing pathogens via a variety of receptors with specificity

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for pattern-recognition molecules [15, 16]. Macrophages also express inhibitory receptors that control macrophage activity, including phagocytosis [17]. A number of inhibitory receptors have been reported to inhibit macrophage function, including signaling regulatory protein (SIRP) α , CD200 receptor [18, 19], paired Ig-like receptor (PIR)-B [20], immunoglobulin-like transcript (ILT) 3 [21], and CD33-related receptors [22]. SIRPs are cell surface proteins belonging to Ig superfamily and can be separated into at least three subfamilies, namely SIRP α , SIRP β and SIRP γ [23, 24]. SIRP α (also known as CD172a, SHPS-1) is expressed on macrophages and dendritic cells (DCs), and it recognizes CD47 as a 'marker of self' [24–27]. CD47 (also known as integrin-associated protein) is a member of the Ig superfamily with a single extracellular Ig domain followed by five transmembrane segments that is ubiquitously expressed on most cells and interacts functionally with integrins [27–29]. The critical role for CD47-SIRP α signaling in preventing phagocytosis was first demonstrated in CD47 KO mice. Hematopoietic cells from CD47 KO mice were rapidly cleared from the bloodstream by macrophages in syngeneic wild-type (WT) mice [30–32]. CD47-SIRP α signaling also reduced the sensitivity of antibody- and complement-opsonized cells to phagocytosis [33, 34]. Comparison of the susceptibility to phagocytosis between cells from CD47 KO (CD47^{-/-}), heterozygote (CD47^{+/-}) and WT (CD47^{+/+}) mice indicated that the level of CD47 expression was positively correlated with the protective strength. CD47 upregulation was recently found to serve as a mechanism for leukemia stem cells/progenitors to avoid phagocytosis [35, 36]. Because only CD47 KO cells were destroyed when CD47 KO and WT cells were co-transplanted into CD47^{+/+} WT mice [32], CD47 expression appeared to act in a cell autonomous manner to inhibit phagocytosis.

CD47 incompatibility causes xenograft rejection by macrophages

Studies in various models have shown that macrophages contribute to xenograft rejection, and their responses to xenoantigens precede the activation of T cells [37]. While macrophages have been implicated in solid organ xenograft rejection [38], these cells appeared to play more important roles in the rejection of cellular xenografts. Macrophages cause almost immediate rejection of xenogeneic bone marrow cells, even in the absence of adaptive immunity [39, 40], which poses a formidable obstacle to the application of mixed chimerism for induction of xenotransplantation tolerance. Human macrophages have been shown to phagocytose porcine RBCs in an antibody- and complement-independent manner [41]. Macrophages have also been found to mediate the rejection of porcine islet xenografts in both rodents [42–46] and primates [47]. Although host macrophages can be activated by cytokines produced by xenoreactive T cells, these powerful effects of xenogeneic macrophages could also be accounted for by the combined ability of certain xenogeneic receptors to activate macrophages [48, 49], while important inhibitory interactions are not effective [50, 51].

The rapid rejection of CD47-deficient hematopoietic cells by macrophages in syngeneic WT mice demonstrated a critical role for CD47 as a self-protection (or 'don't eat me' marker). This finding also suggested that the rapid rejection of xenogeneic cells by recipient macrophages could have resulted from the inability of donor CD47 to interact with SIRP α on the recipient macrophages. This question was first addressed in a pig-to-mouse xenogeneic combination [50], in which macrophages were found to mediate robust rejection of porcine hematopoietic cells [39]. It is known that SIRP α contains intracellular immune receptor tyrosine-based inhibitory motifs (ITIMs), and that SIRP α activation after binding to CD47 results in tyrosine phosphorylation of ITIMs, leading to recruitment and activation of protein tyrosine phosphatases [25]. It was found that porcine CD47 could not induce SIRP α tyrosine phosphorylation in mouse macrophages, and that blocking SIRP α with anti-mouse SIRP α mAb (P84) did not affect the engulfment of porcine cells by mouse macrophages [50]. These findings demonstrated the lack of cross-reaction between porcine CD47 and

mouse SIRP α . Importantly, transgenic expression of mouse CD47 on porcine cells was found to elicit strong protection against phagocytosis by mouse macrophages both in vitro and in vivo [50].

It has been reported that recombinant human SIRP α proteins bound to porcine CD47 [52]. However, the protein binding assay did not necessarily reveal whether porcine CD47 could deliver inhibitory signaling via SIRP α to human macrophages. In fact, the demonstrated inability of porcine RBCs to stimulate SIRP α tyrosine phosphorylation in human macrophages indicated a lack of functional interaction between porcine CD47 and human SIRP α [51]. In agreement with this possibility, porcine cells transfected with human CD47 showed markedly reduced sensitivity to in vitro phagocytosis by human macrophages [51]. A recent study demonstrated that, due to polymorphisms in the NOD SIRP α allele, NOD mouse SIRP α is capable of cross-reacting with human CD47, and such a cross-reactivity enabled human hematopoietic cells to survive phagocytosis in the mouse model [53]. We have recently observed that human CD47 expression can significantly promote porcine cell survival in NOD/SCID mice and in a baboon recipient (unpublished data).

The findings discussed above demonstrate that the interspecies incompatibility of CD47 is an important mechanism that triggers phagocytosis of xenogeneic cells. Given that SIRP α is also expressed on granulocytes [24], the lack of cross species inhibitory interaction between CD47 and SIRP α is likely to also augment granulocyte xenoresponses [54, 55]. Thus, transgenic pigs expressing human CD47 could prove beneficial as donors for clinical xenotransplantation.

Mixed chimerism fails to inhibit macrophage-mediated rejection of CD47-deficient cells

Mixed hematopoietic chimerism is considered one of the most promising approaches to inducing transplantation tolerance. Animal studies have provided abundant evidence that induction of mixed hematopoietic chimerism is capable of inducing T, B and NK cell tolerance to allografts [56, 57]. This approach has also been successfully used to induce renal allograft tolerance in the clinic [58]. In xenogeneic transplantation, mixed chimerism has been shown to induce robust T and B cell tolerance in closely-related rodent models [59–62]. Proof-of-principle for mixed chimerism-induced T cell tolerance across a highly disparate xenogeneic barrier has also been obtained in a pig-to-mouse transplant model, in which transgenic (Tg) mice expressing three porcine cytokine transgenes (IL-3, GM-CSF and SCF) [63] were used [64]. Murine T cells developing in porcine hematopoietic chimeras were specifically unresponsive to porcine donors, as demonstrated by the acceptance of donor skin grafts and the lack of anti-donor MLR or anti-donor IgG production. A similar approach was adapted to assess the ability of mixed chimerism to induce human T cell tolerance to porcine xenoantigens in humanized NOD/SCID-Tg mice [65]. Humanized mice with a functional human immune system were created by grafting human fetal thymus/liver tissue and administering CD34⁺ cells from the same donor intravenously to NOD/SCID-Tg mice [65–67]. Induction of porcine hematopoietic chimerism in humanized NOD/SCID-Tg mice specifically eliminated immune responses towards the porcine donor antigens [65].

Unfortunately, the ability of mixed chimerism to inhibit macrophage responses to donor grafts has been less investigated. Unlike WT macrophages that recognize CD47 as a ‘marker of self’ and phagocytose CD47-deficient hematopoietic cells, macrophages from CD47 KO mice do not engulf CD47 KO cells [30], indicating that macrophages can be rendered ‘tolerant’ or unresponsive to CD47-deficient cells. Since the level of SIRP α expression on CD47 KO macrophages is virtually the same as that of WT macrophages, this tolerance to CD47 KO cells is unlikely to be induced at the level of SIRP α expression [30]. In support of

this possibility, we recently observed that SIRP α tyrosine phosphorylation in CD47 KO macrophages was able to be induced upon exposure to CD47^{+/+} RBCs (unpublished data). It is possible that the lack of CD47 expression on macrophages or in the environment may regulate macrophage function, leading to tolerance in CD47-deficient mice. Elucidation of the mechanisms for CD47 KO macrophage tolerance to CD47 KO cells would contribute to our understanding of the ability of mixed chimerism to induce macrophage tolerance to xenogeneic cells that express recipient SIRP α incompatible CD47. A recent study addressed this issue by assessing the phagocytic activity against CD47 KO cells in various types of CD47 bone marrow chimeras, in which CD47 is expressed on hematopoietic cells, non-hematopoietic cells, or on cells from both compartments [32]. Macrophages in the chimeric mice with non-hematopoietic cells expressing CD47 phagocytosed CD47 KO cells, whereas those in chimeras in which CD47 expression was limited to hematopoietic cells were tolerant of CD47 KO cells. In addition to endogenous CD47 KO cells, exogenously injected splenocytes from untreated CD47 KO mice were able to survive in the chimeras with no CD47 expression on non-hematopoietic cells. This result indicated that the alterations in macrophages, rather than target cells, were responsible for the observed macrophage tolerance.

Taken together, these findings demonstrate that the lack of CD47 expression on non-hematopoietic cells is required for inducing macrophage tolerance to CD47-deficient cells in bone marrow chimeras, and that CD47 KO mouse bone marrow transplantation cannot induce macrophage tolerance to CD47 deficient cells in WT mice. Thus, induction of mixed hematopoietic chimerism is unlikely to prevent the rejection of donor cells by macrophages in a xenogeneic combination, if the donor CD47 does not interact with the recipient SIRP α . In support of this possibility, an earlier study showed that mouse macrophages developing *de novo* in porcine hematopoietic chimeric mice remained phagocytic against porcine hematopoietic cells [39]. These observations suggested that the use of bone marrow cells from human CD47-transgenic pigs may promote the establishment of chimerism and tolerance in clinical xenotransplantation.

CD47 expression on donor cells is required for tolerance induction by donor-specific transfusion (DST)

DST has the potential to prolong allograft survival or induce tolerance, particularly when used in combination with costimulatory blockade. Multiple mechanisms, including deletion and anergy of donor-reactive T cells [68–71] and generation of regulatory T cells (Tregs) [72–74], have been suggested to mediate the immunosuppressive effect of DST. Tolerogenic DCs are known to be important for the generation of donor antigen-specific Tregs in mice treated with DST plus anti-CD154 antibodies [75]. Furthermore, the indirect antigen-presentation pathway is essential to the DST effect, as DST does not suppress alloresponses in recipients that lack antigen-presenting cells (APCs) [76].

DST has been inefficient in prolonging donor graft survival or inducing tolerance in discordant xenogeneic transplantation settings [1]. SIRP α was found to serve as an inhibitory receptor in DCs, and its signaling upon ligation of CD47 suppressed DC endocytosis [32] and activation [77]. The role of CD47 in preventing phagocytosis and in regulating DC function suggested that CD47 expression on DST donor cells may play an important role in DST-mediated immunosuppression. In fact, this was confirmed in a mouse MHC class I-disparate, C57BL/6-to-bm1, model [78]. Although DST using WT donors was able to markedly prolong donor skin survival, mice receiving DST from CD47-deficient donors showed no inhibition or even acceleration of donor skin graft rejection, compared to non-DST controls. CD47 KO DST was likely inefficient in mediating peripheral depletion of donor-reactive T cells due to rapid rejection of DST donor cells by macrophages.

However, the role of CD47 in DST is more complex than just preventing phagocytosis of donor cells, as DST using CD47^{+/-} cells that are capable of surviving phagocytosis in WT mice [33] was also less efficient than WT DST in prolonging donor skin survival. Furthermore, addition of CD47 KO cells into the WT DST inoculum significantly reduced the immunosuppressive effect of WT DST. The rapid activation of recipient CD11c⁺ DCs in mice receiving CD47 KO, but not WT, DST indicated that CD47 presence on donor cells was required to repress recipient DC activation, which has been found important in the induction of Tregs [79] and T cell tolerance [80] by DST. More recent studies have suggested that CD47 expression on donor cells is also required for induction of tolerance to fully MHC-mismatched cardiac allografts in mice after treatment with DST and anti-CD154 [81].

The studies discussed above provide clear evidence that the expression of CD47 on donor cells is essential to DST-induced tolerance or immunosuppression in allogeneic transplantation. The findings also offer a possible explanation for the poor immunosuppressive effect of DST in discordant xenogeneic transplantation settings. Although further studies in xenogeneic settings are needed to reach a conclusion, it is likely that DST will be less efficient in suppressing xenoresponses in a transplant setting when the donor CD47 does not interact with the recipient SIRPα.

Concluding remarks

CD47 is a ubiquitously expressed cell-surface molecule that serves as an essential ‘marker of self’ for macrophages and DCs, and its signaling through SIRPα prevents inappropriate self-phagocytosis. The lack of cross-species inhibitory interaction in the CD47-SIRPα pathway largely accounts for the vigorous rejection of xenogeneic cells by macrophages. However, the role for CD47 in preventing phagocytosis has only been demonstrated in hematopoietic cells. More recent data has suggested that the concept of CD47 as a ‘marker of self’ for macrophages does not apply to thymic epithelial cells [82]. Thus, further studies are needed to determine whether CD47 incompatibility plays a role in the rejection of non-hematopoietic xenografts, especially those highly susceptible to rejection by macrophages, such as xenogeneic pancreatic islets. The rapid activation of recipient CD11c⁺ DCs in mice after CD47 KO DST suggests a possible involvement of CD47 incompatibility in adaptive xenoimmune responses, which merits further investigation. Finally, considering the critical role for CD47 in preventing phagocytosis of xenogeneic hematopoietic cells and in regulating DC function, transgenic pigs expressing human CD47 are likely to be required for xenotolerance induction via hematopoietic cell transplantation, including mixed chimerism and DST.

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