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REVIEW

The immunological function of CD52 and its targeting in organ transplantation

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Abstract

Introduction CD52 (Campath-1 antigen), a glycoprotein of 12 amino acids anchored to glycosylphosphatidylinositol, is widely expressed on the cell surface of immune cells, such as mature lymphocytes, natural killer cells (NK), eosinophils, neutrophils, monocytes/macrophages, and dendritic cells (DCs). The anti-CD52 mAb, alemtuzumab, was used widely in clinics for the treatment of patients such as organ transplantation. In the present manuscript, we will briefly summarize the immunological function of CD52 and discuss the application of anti-CD52 mAb in transplantation settings.

Findings We reviewed studies published until July 2016 to explore the role of CD52 in immune cell function and its implication in organ transplantation. We showed that ligation of cell surface CD52 molecules may offer costimulatory signals for T-cell activation and proliferation. However, soluble CD52 molecules will interact with the inhibitory sialic acid-binding immunoglobulin-like lectin 10 (Siglec10) to significantly inhibit T cell proliferation and activation. Although the physiological and pathological significances of CD52 molecules are still poorly understood, the anti-CD52 mAb, alemtuzumab, was used widely for the treatment of patients with chronic lymphocytic leukemia, autoimmune diseases as well as cell and organ transplantation in clinics.

Conclusion Studies clearly showed that CD52 can modulate T-cell activation either by its intracellular signal pathways or by the interaction of soluble CD52 and Siglec-10 expressing on T cells. However, the regulatory functions of CD52 on other immune cell subpopulations in organ transplantation require to be studied in the near future.

Keywords CD52 · Grafts · Transplantation · Immune rejection

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Introduction

CD52, also known as CAMPATH-1 antigen, is a glycoprotein of 12 amino acids, anchored to glycosylphosphatidylinositol (GPI) [1]. It is a nonmodulating membrane glycoprotein and is present on the cell surface of immune cells, such as mature lymphocytes, monocytes, and dendritic cells (DCs) [2–4], and other tissues and cells, such as the male genital tract and mature sperm cells [5, 6], but not on hematopoietic progenitors, erythrocytes, or platelets

[7, 8]. The anti-CD52 mAb, alemtuzumab, was used clinically for the treatment of patients with chronic lymphocytic leukemia, relapsing–remitting multiple sclerosis, steroid-refractory acute graft versus host disease (GVHD) and organ transplantation [9–13]. Although targeting CD52 molecules was used as an approach to treat patients in clinics, the physiological and pathological significance of CD52 molecule is less understood so far. In the present manuscript, we will briefly summarize the immunological function of CD52 and discuss the application of anti-CD52 mAb in transplantation settings.

CD52 molecule and its cellular expression

Human CD52, a small glycoprotein with a molecular mass of approximately 21–28 KDa, is a peptide of 12 amino acids that is encoded by the CD52 gene located in chromosome 1. CD52 molecule is anchored to glycosylphosphatidylinositol (GPI) [2, 7]. The molecular regulation on CD52 expression is less known so far. It is reported that the treatment of all-trans-retinoic acid (ATRA) can significantly induce CD52 expression at both mRNA and protein levels in leukemic cells through promyelocytic leukemia–retinoic acid receptor α [14]. Such an ATRA-induced high level of CD52 expression might potentially serve as a novel therapeutic target in treatment of acute promyelocytic leukemia [14].

CD52 is present on the most differentiation stages of all lymphocytes and also on eosinophils, monocytes/macrophages, and DCs [5, 6, 15, 16]. Furthermore, it is found within the male genital tract and is present on the surface of mature sperm cells. Unlike T cells, B cells, NK cells, monocytes, and DCs [7], it is used to be widely believed that neutrophils do not express CD52 molecules on the cell surface [17]. Within the granulocyte population, it has been reported that eosinophils, but not neutrophils, express CD52 molecules [16]. However, it was recently reported that neutrophils indeed express CD52 at both mRNA and protein levels, although the level of surface CD52 molecule on neutrophils is lower than those on eosinophils, T cells, and B cells [18]. Human neutrophils are susceptible to alemtuzumab-mediated lysis in the presence of complement [18]. It is shown that CD52 is not expressed on hematopoietic progenitors, erythrocytes, or platelets. However, a subpopulation of $CD34^+$ cells in the bone-marrow expresses CD52 as well as other lymphoid markers, representing lymphoid-committed progenitors [19–21].

Twelve amino acids-constituted CD52 molecules with an asparagine-linked amino-terminal oligosaccharide were tethered on the cell surface by its GPI anchor [22]. Near the N-terminus is a large and complex carbohydrate with negatively charged sialic acid residues attached to the asparagine 3 amino acid. The GPI anchor holds the CD52 molecule on the outer layer of cell membrane by linking to its C-terminus [8]. Since it is negatively charged at high levels and expressed on the cell surface of lymphocytes, it was expected that its function is anti-adhesion, allowing cells to freely move around. Because the GPI anchor is cleavable by phospholipases [1], Bandala-Sanchez et al. investigated this possibility in the activated T cells. It is true that cell-bound CD52 was released to be soluble CD52 by the action of phospholipase C on its carbohydrate moiety. Soluble CD52 molecules directly bind the inhibitory sialic acid-binding immunoglobulin-like lectin 10 (Siglec10) [23], which also mediates the immune regulation.

The roles of CD52 on immune cells

Cross-linking CD52 on B-cell line Wien 133 and T-cell line Jurkat T cells which expresses high levels of endogenous CD52 and transfected to express high levels of CD52, respectively, resulted in slower cell growth kinetics, followed by the induction of apoptosis, which appeared to be independent of the Fas/Fas L pathway [24]. In addition, it has been indicated that cross-linking of anti-CD52 mAb (alemtuzumab) on B tumor cells can occur naturally through Fc receptor interaction and lead to the activation of specific cellular pathways and induction of caspase-dependent apoptosis [24–28]. Anti-CD52 mAb significantly augmented the anti-CD3 mAb-mediated proliferative response of human naïve and memory $CD4^+$ and $CD8^+$ T cells when these two mAbs were co-immobilized or cross-linked in solution by the same secondary Ab [29]. CD52 ligation induces a profile of tyrosine phosphorylated proteins in both primary and Jurkat T cells dependently upon both CD45 and TCR molecule expressions [30]. Anti-CD52 mAb increases protein tyrosine phosphorylation in T cells after cross-linking using the secondary Ab [29]. CD52 ligation induces the increases of tyrosine phosphorylation in proteins, such as TCRζ, SLP-76, $p95^{vav}$ $p120^{cbl}$, and LAT, which become phosphorylated upon TCR stimulation [29], indicating that CD52 may utilize the molecular signaling machinery of the TCR to promote intracellular signal pathways during T-cell activation. Further studies showed that CD52 signal transduction in T cells is dependent upon CD45 and TCR expression, which was supported by the direct associations of TCR and CD52 as measured by FRET technique [29]. However, CD52 ligation fails to induce PLCγ1 activation and calcium signals in Jurkat T cells, which is in contrast to TCR ligation [30].

In contrast, studies showed that CD52 recognized by mAb 4C8 (IgG3) can act as a costimulatory molecule for the induction of regulatory $CD4^+$ T cells [31, 32]. Human $CD4^+CD25^-CD45RO^+$ T cells expanded by stimulation

with anti-CD3 and anti-CD52 (4C8) mAbs could greatly suppress T effector cells with polyclonal or allogeneic stimulation in a cell contact-dependent and cytokines (IL-10 and TGF-β)-independent manner [31, 33]. Co-injection of these induced regulatory $CD4^+$ T cells significantly suppressed lethal GVHD in severe combined immunodeficiency disorder mouse recipients caused by human peripheral blood mononuclear cells [33]. Importantly, it was recently uncovered that human- and mouse-activated $CD52^{high}CD4^+$ T cells significantly suppressed T effector cells and were distinctive from $CD4^+CD25^+Foxp3^+$ regulatory T cells [23]. The immunosuppressive ability of $CD52^{high}CD4^+$ T cells was mainly mediated by soluble CD52 released by phospholipase C. Soluble CD52 molecules directly bind to the inhibitory receptor Siglec-10 and impaired the phosphorylation of the TCR–associated kinases Lck and Zap70 and subsequently the T cell activation [23]. The inhibitory effect on T cells of soluble CD52 molecules is also dependent on the presence of glycan moiety in CD52 molecules. Therefore, CD52 plays complicated roles on T-cell activation via multiple pathways (Fig. 1). CD52 can act as costimulatory molecules to activate effectors and regulatory T cells. On the other hand, CD52 acts as a ligand for Siglec-10 to inhibit T cell activation. In addition, the elevated levels of $CD4^+CD39^+Foxp3^+$ regulatory T cells were observed in human peripheral blood mononuclear cells of relapsing–remitting multiple sclerosis (RRMS) patients with anti-CD52 treatment [34]. A unique subset of $CD39^+$ regulatory T cells is identified in gut-associated lymphoid tissues of anti-CD52 mAb-treated EAE mice, which is accompanied by the amelioration of the disease [34]. Thus, CD52 may put a crucial impact on regulatory T-cell induction. Unfortunately, the roles of CD52 on other immune cells, such as innate immune cells, are less studied. As Siglec-10 is widely expressed on many immune cell subpopulations, the effects of CD52 on other immune cells need to be determined as well.

Targeting CD52 in transplanted patients

It is reported that Campath-1H negatively modulates CD4 and CD8 expression on T-cell surface in vitro and in vivo through T-cell activation and apoptosis-independent pathways [35]. While, some studies conclude that Campath-1H itself negatively modulated cell surface CD4 and CD8 expression without activating T cells [35]. To determine whether the down-regulation of surface CD4 and CD8 expression by Campath-1 H was due to T-cell activation or other pathways processed in vivo, the authors exposed the whole blood from healthy volunteers to Campath-1 H in vitro. Flow cytometry assay revealed a dose-dependent

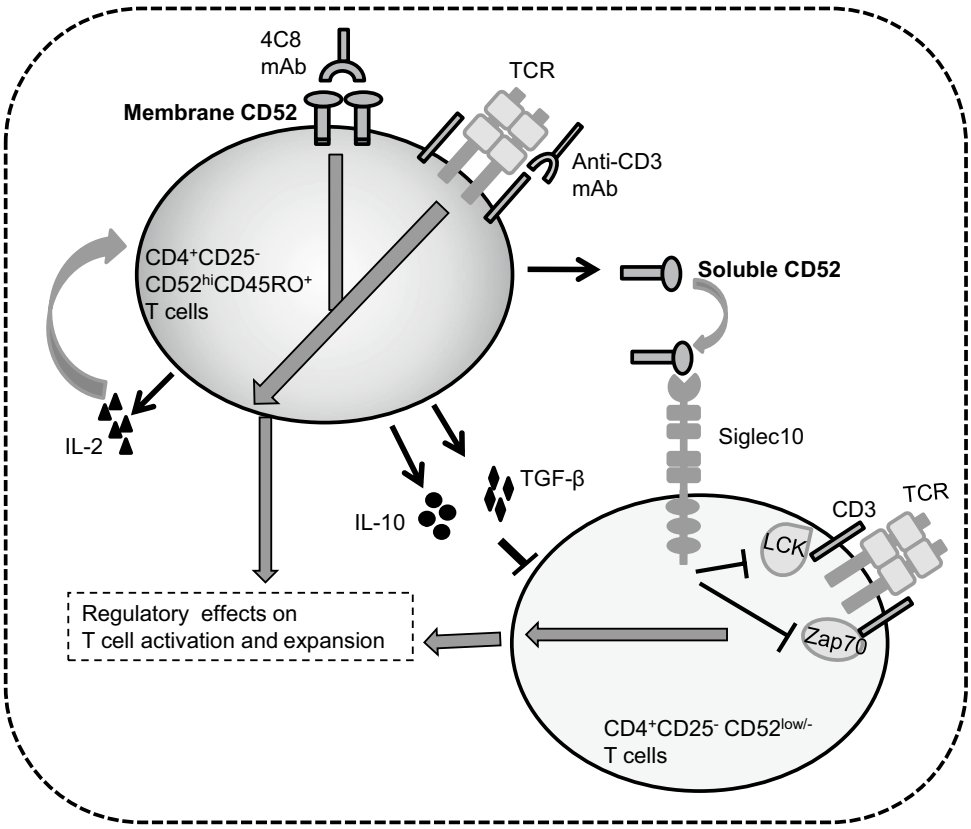

Fig. 1 Impacts on T cells via membrane CD52 and soluble CD52. Costimulation of $CD4^+$ T cells with anti-CD3 and anti-CD52 mAb (4C8 mAb) induces the regulatory T cells and full activation of $CD4^+$ T cells with high level IL-2 production. Upon re-stimulation, 4C8 mAb-costimulated cells produce high levels of IL-10 and TGF-β, suppressing bystander T cells. Furthermore, the immunosuppressive effects of $CD52^{high}$ T cells may be mediated by soluble CD52 molecules, which can bind to the inhibitory receptor Siglec-10 expressed on the cell surface of T cells and subsequently impaired phosphorylation of the T-cell receptor-associated kinases Lck and Zap70 and T-cell activation

downmodulation of cell surface CD4 and CD8 expression without T-cell activation [35]. Furthermore, Campath-1H could kill T cells in vivo through complement and non-complement-mediated mechanisms [36, 37]. Isolating different subtypes of T cells and incubating them with Campath-1 H with or without complement and/or serum, it is found that $CD4^+$ T cells were 60 and 40% cell death and $CD8^+$ T cells were 23 and 77% cell death in the peripheral blood [38]. Thus, the elimination of $CD4^+$ and $CD8^+$ T cells by Campath-1 H was mediated by complement- and noncomplement-mediated mechanisms, respectively. $CD4^+$ T cells express about twice the amount of surface CD52 than $CD8^+$ T cells, consistent with primarily complement-mediated killing $CD4^+$ T cells [38]. It is demonstrated that alemtuzumab induces rapid apoptosis of NK cells and a strong induction of inflammatory cytokines, which is exclusively mediated via the binding of the IgG1 Fc to the low affinity receptors for IgG, CD16 (FcγRIII) [39]. Furthermore, anti-mouse CD52 mAb could deplete $\gamma\delta^+$ IELs, causing the decrease in mucosal KGF expression and EC proliferation and then slowing down the epithelial turnover and tetrad the repair of the damaged epithelium [40]. In summary, alemtuzumab, a humanized immunoglobulin IgG1 mAb against CD52, may cause cell death by several mechanisms, including complement-mediated cell lysis, antibody-mediated cellular cytotoxicity, and enhancing apoptosis both caspase-dependent and caspase-independent pathways [41–44].

Alemtuzumab-treated patients exhibited a nearly complete deletion of $CD4^+$ T cells at day 7 and peak expansion of $CD4^+CD25^+CD127^-$ regulatory T cells at month 1 [45]. The increased percentages of TGF-β, IL-10, and IL-4-producing $CD4^+$ T cells reached a maximum at month 3, whereas a significant decrease in the percentages of Th1 and Th17 cells was detected at months 12 and 24 [46]. These indicate the differential reconstitution of T-cell subsets and selective delayed $CD4^+$ T-cell repopulation after alemtuzumab treatment [47].

Monocyte-derived DCs (moDCs) express abundant CD52. Langerhans cells and dermal-interstitial DCs, however, do not express CD52. It has been proposed that alemtuzumab not only induced T-cell depletion, but also removed moDCs and alleviated their presentation of host-derived antigens to donor T cells in mitigating GVHD [6, 48, 49]. It is also reported that treatment with alemtuzumab causes a strong and sustained reduction of the number of peripheral DCs and a significant shift from moDCs to plasmacytoid DC subsets (pDCs) as indicated by the decreased moDC/pDC ratio as early as 1 month post-transplantation, suggesting the functional significance of CD52 in the setting of allogeneic organ transplantation [50]. Recently, it is reported that Campath-1G causes the rapid depletion of circulating host DCs before allogeneic transplantation but does not delay donor DC reconstitution [51]. Although neutrophils expressed low levels of CD52 molecules than lymphocytes and eosinophils, incubation of alemtuzumab with neutrophils results in dose-dependent and complement-mediated lysis in the presence of complement, offering an explanation for the etiology of alemtuzumab-associated neutropenia [18, 52–54]. Enhanced cell numbers, cytotoxicity, and IFN-γ secretion of NK cells were detected when human peripheral blood mononuclear cells were stimulated with the anti-CD3 and anti-CD52 (alemtuzumab) mAbs in vitro [55]. It is a simple and safe methodology to gain large numbers of NK cells ex vivo, which is a promising candidate for immunotherapy.

CD52 is highly expressed on lymphocytes, and the primary usage of alemtuzumab was as an immunosuppressive agent for organ transplantation, although there is limited experience using it to treat steroid-resistant rejection [13, 44, 56–58]. While the elimination half-life of alemtuzumab is approximately 12 days [59], its clinical effects are far more persistent, and prolonged lymphocyte depletion is observed [60–63]. Using Campath-1G to deplete host lymphocytes in HLA-identical sibling bone-marrow transplants for acute myelogenous leukemia patients showed the beneficial reduction in GVHD without an increased risk of relapse [64]. The advantages of this approach are the simultaneous depletion of donor B cells to reduce the risk of EBV-associated lymphoproliferative disease and the depletion of recipient T cells to prevent graft rejection [65]. Alemtuzumab is introduced in solid organ transplantation as an induction agent and has provided a low incidence of acute rejection episodes and potential tolerogenic properties. In the following part, we will discuss the application of CD52 in clinical transplantation of kidney, heart, and stem cells.

Renal transplantation

Up to now, the most effective experience with the use of alemtuzumab in solid organ transplantation has been in renal transplantation. Initially, an IgM rat-derived antibody (Campath-1M) was used in clinical renal transplant trials and was found to have a profound effect on peripheral lymphocyte reduction, which may contribute to slowing down the acute rejection [66, 67]. Studies with the Campath-1G demonstrated a more profound and long-lasting depletion of lymphocytes [68]. In addition, Campath-1H not only deplete T cells, but also expand $CD4^+CD25^+Foxp3^+$ regulatory T cells and caused a strong reduction of peripheral DCs in allogeneic organ transplantation, sustaining the graft survival [69–72]. Six-month clinical study on kidney transplanted patients treated with alemtuzumab and tacrolimus in standard doses showed that the percentage of $CD4^+CD25^+FoxP3^+$ regulatory T cells was

19.9 ± 14.9% among patients treated with alemtuzumab and 4.1 ± 3.8% among recipients treated without alemtuzumab and 3.1 ± 1.1% among healthy controls, indicating that alemtuzumab significantly increases the percentages of $CD4^+CD25^+FoxP3^+$ regulatory T cells compared with those on renal transplant recipients of similar demography who were not treated with alemtuzumab or the healthy controls [72]. Although some studies showed that alemtuzumab therapy enables tacrolimus monotherpay with a low rate of rejection, other clinical trials found that alemtuzumab-treated patients were more liable to late rejection episodes after 12 months [73, 74]. The risk of antibody-mediated rejection was increased in pediatric renal transplant patients when Campath-1H was used without calcineurin inhibitor [75]. High doses of calcineurin inhibitor likely causes chronic nephrotoxicity, and the combination of lower calcineurin inhibitor and alemtuzumab may be one of alternative therapies for organ transplantation [76]. Recent study has found that sequential targeting of CD52 and TNF-α allows early minimization therapy in kidney transplantation [77]. Although alemtuzumab is identified as an important induction agent in organ transplantation, the optimal combination of immunosuppressive therapy and safe drug minimization remains to be further explored.

Heart transplantation

Chronic rejection of cardiac allografts remains unresolved problem in the field of transplantation and is the major reason for the late graft failure [78, 79]. The etiology of chronic rejection is often described as multi-factorial, and the donor specific antibody (DSA) is considered to have a causal effect on chronic rejection development, which is still poorly understood owing to lack of proper animal models and tools [80]. Some studies, using human CD52 transgenic mice treated with alemtuzumab, showed that mismatched cardiac allograft rejection did not happen [80]. The development of chronic rejection correlated with donor specific antibody and alloreactive B cells was shown to increase in accordance with DSA detection [81, 82]. Thus, blocking CD52 could affect the patterns of de novo alloreactive B cells and antibody formation in chronic cardiac allograft rejection [80]. Repopulation of memory T cells in allograft recipients after lymphodepletion is a major barrier to transplant tolerance induction [83–88]. When cynomolgus macaque monkeys with heart allografts were treated with alemtuzumab, naïve and memory $CD4^+$ T cells were susceptible to alemuzumab-mediated cytotoxicity, whereas naïve $CD8^+$ T cells were more resistant than memory $CD8^+$ T cells [89]. However, no significant differences in CD52 expression between lymphocyte subsets in peripheral blood and lymph node were observed [90]. Naïve $CD4^+$ and $CD8^+$ T cells expressed lower levels of caspase 3 than memory T cells [89]. Thus, after alemtuzumab infusion, residual naïve T cells in secondary lymphoid tissue may predispose to rapid recovery of memory T cell in allograft recipients.

Stem cell transplantation

Allogeneic hematopoietic cell transplantation (HCT) is the conventional treatment and a potentially curative therapeutic option for patients with the fatal hematologic malignancies, such as leukemia and lymphoma [91, 92]. The high level of CD52 expression on T cells and histiocytes makes alemtuzumab a rationale alternative for disturbing the uncontrolled immune response of hemophagocytic lymphohistiocytosis (HLH) [93]. Theoretically, $CD52^+$ T cells and histiocytes could induce HLH, facilitating the use of alemuzumab as targeted therapy to enable allogeneic SCT to be successfully performed [93, 94]. It has also been reported that lowering the alemtuzumab doses in reduced intensity conditioning allogeneic HCT is associated with a favorable early intense natural killer cell recovery [95]. The alemtuzumab dose reduction provides sufficient GVHD prophylaxis and supports improved NK cell regeneration early after allogeneic HCT in peripheral blood, which may have a positive effect on overall survival [95, 96]. Patients who received anti-CD52-treated HSCs had class-switched memory B lymphocytes, normal IgG levels, and normal responses to tetanus and *Haemophilus influenzae* type B vaccination [97]. More anti-CD52-treated patients had donor B lymphocytes [97], implying that B lymphocyte function might be better after anti-CD52-treated HSC and showing that CD52 signaling may be important for B-cell function in HSC transplantation [98]. Furthermore, some studies found that the addition of alemtuzumab resulted in a significant increase in total nucleated cells, absolute $CD34^+$ cells, myeloid and megakaryocytic progenitors, multi-lineage, and myeloid CFU, compared to cytokines alone [99]. The study suggests that the use of alemtuzumab for ex vivo expansion of cord blood HSCs may be advantageous and increases the viability of cord blood units for transplantation. This result implies that CD52 signaling plays an important role in regulating HSC proliferative capability. Although alemtuzumab may represent a rational therapeutic option for patients with fatal hematologic malignancies, its use should be restricted to second-line therapy for patients until its efficacy has been ascertained in clinical trials. GVHD is one of the major transplant-related complications in allogeneic HCT, which can be avoid by eliminating or suppressing donor-derived T cells [100]. It has also been demonstrated that administration of higher alemtuzumab effectively reduced the incidence of severe acute GVHD through the depletion of donor T cells even in mismatched unrelated transplantation [101–103]. However,

incidence of infection and relapse was high due to the delayed recovery of cellular immune function. Some studies suggested that lower alemtuzumab dose or combined with other immunosuppressive drugs, such as rapamycin or FKB506, could avoid the incidence of infections [60, 62, 104]. Therefore, optimizing the dose of alemtuzumab and the administration timing will be necessary to balance the benefit of acute GVHD suppression and the risk of viral infection. However, larger prospective studies are required to assess the alemuzumab efficacy and pursuing the importance of CD52 signaling in transplantation immunology.

Conclusions

CD52 is an important immune regulator on T-cell activation. CD52 can modulate T-cell activation either by its intracellular signal pathways or by the interaction of soluble CD52 and Siglec-10 expressing on T cells. The regulatory functions of CD52 on other immune cell subpopulations require to be studied in the near future. A humanized anti-CD52 mAb has been used in clinical solid organ transplantation as an induction agent. The effects of this treatment on transplant immune tolerance need to be investigated. Furthermore, the regulatory roles of CD52 in immune cell subpopulations in allogeneic transplantation need to be further studied in the future.

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