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Review Article

2B4 (CD244, SLAMF4) and CS1 (CD319, SLAMF7) in systemic lupus erythematosus and cancer

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ABSTRACT

Signaling Lymphocyte Activation Molecule (SLAM) family receptors are expressed on different types of hematopoietic cells and play important role in immune regulation in health and disease. 2B4 (CD244, SLAMF4) and CS1 (CD319, CRACC, SLAMF7) were originally identified as NK cell receptors regulating NK cell cytolytic activity. 2B4 is expressed on all NK cells, a subpopulation of T cells, monocytes and basophils. Unlike other activating and inhibitory receptors, 2B4 (CD244) interaction with its ligand CD48 has been shown to mediate both activating and inhibitory functions. Defective signaling via 2B4 due to mutations in signaling adaptor SAP contributes to X-linked lymphoproliferative Disease (XLP). Expression of 2B4 and CS1 are altered in systemic lupus erythematosus (SLE). CS1 is overexpressed in multiple myeloma (MM) and anti-CS1 mab (Elotuzumab/Empliciti) has been approved by FDA as a breakthrough drug for treatment for MM patients. CAR -T cells or CAR- NK cells containing full length CS1 or the signaling domain of 2B4 with TCR-ζ have shown promising results to treat cancer and autoimmune diseases.

1. Introduction

Signaling Lymphocyte Activation Molecule (SLAM) family receptors are expressed on different types of hematopoietic cells and are known to be involved in immune regulation [1,2]. The SLAM family is comprised of nine receptors, which are CD150 (SLAM, SLAMF1), CD48 (SLAMF2), Ly-9 (CD229, SLAMF3), 2B4 (CD244, SLAMF4), CD84 (SLAMF5), NTB-A (SLAMF6), CS1 (CRACC, CD319, SLAMF7), BLAME (SLAMF8) and CD2F-10 (SLAMF9) [3,4]. The need for SLAM family receptors in immune response was highlighted when mutations in the SRC homology 2 domain protein 1A gene (*SH2D1A*), which encodes for SLAM-associated protein (SAP), was observed in patients with X-linked lymphoproliferative syndrome (XLP) [5]. XLP is a serious immunodeficiency characterized by an inability of the immune system to effectively respond to Epstein-Barr virus (EBV) [5–7]. SAP-deficient mice have revealed impaired NK cell cytotoxicity, defects in the formation of germinal centers, an absence of NK-T cells, and a decrease in memory B cells [5,8]. Furthermore, genetic studies revealed that SAP interacts with a tyrosine motif (TxYxxV/I/A) located in the cytoplasmic tail of SLAM family receptors [5,9]. All members of the SLAM family except 2B4 are homophilic. 2B4 interacts with CD48 and hemophilic interactions of other SLAM family receptors regulate various immune responses. This review summarizes the role of 2B4 (SLAMF4) and CS1 (SLAMF7) in autoimmunity and cancer.

1.1. 2B4 (SLAMF4, CD244)

We have originally identified 2B4 in mice as an activation receptor on all resting and IL-2 activated NK cells and splenic T cells involved in non-MHC-restricted killing [5,6]. Ligation of surface 2B4 by anti-2B4 monoclonal antibody enhanced the lysis of target cells by NK cells. Signaling through 2B4 via anti-2B4 monoclonal antibody activates the lytic activity of NK cells and non-MHC-restricted T cells and is able to induce IFN-γ secretion and granule exocytosis in IL-2 activated NK cells [6]. Addtionally, it has been demonstrated in vivo that murine 2B4 (m2B4) can also function as an inhibitory receptor [7]. 2B4 belongs to the CD2 subfamily which also includes CD2, CD48, CD58, CD84, CD150 (SLAM), CD319 (CS1 or CRACC), and CD229 (Ly9). Furthermore, identification of CD48 has the high affinity ligand of 2B4 implicated a broader role of 2B4 in immune regulation [8,9]. 2B4 contains an N-terminal extracellular domain, distal Ig V-like domain, an Ig-C2-like domain, a single transmembrane domain, and a cytoplasmic tail containing four tyrosine motifs [5,9]. m2B4 exists in two isoforms, 2B4—S and 2B4-L, that result due to alternative splicing. The difference between these two isoforms is found in the cytoplasmic domain; the first 61 amino acids of the cytoplasmic domain are shared among the two isoforms, but 2B4—S has an additional 32 unique amino acids and 2B4-L has an additional 89 unique amino acids [10]. Studies in which 2B4—S and 2B4-L were transfected into a rat leukemia cell line (RNK-16) suggested that 2B4—S functions as an activation receptor, whereas 2B4-L is inhibitory [10,11]. These functional differences are most likely

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attributed to the difference in tyrosine residues on the cytoplasmic domains.

Human 2B4 (h2B4) is 70% similar to the long form of m2B4 and contains four tyrosine motifs in the cytoplasmic domain. h2B4 is expressed in NK- and T-cell lines and in lymphokine-activated killer (LAK) cells as well as in spleen, lymph node, and peripheral blood leukocytes [12]. Activation of 2B4 on human NK cells by anti-2B4 antibody or CD48 stimulates cytotoxicity and IFN-γ secretion. Additionally, 2B4 has also been implicated in immune regulation and disease. It plays a regulatory role in human NK cell precursors that are differentiated in vitro [7,13]. These precursors acquire the activating receptors and cytolytic potential even before they express MHC class I-specific inhibitory receptors. This suggests that NK precursors may be potentially reactive to the surrounding cells. However, 2B4 acts as an inhibitory receptor at this stage, to prevent killing of normal autologous cells at early stages of NK cell differentiation when there are no other inhibitory receptors expressed [7,13]. Cytolytic activity of human NK cells against various target cells was greatly augmented in the presence of a monoclonal antibody against 2B4, suggesting that 2B4 can transduce activation signals to human NK cells. This increase was also seen against MHC class I deficient target cells, further suggesting that activation of human NK cytolytic activity via 2B4 is independent of the expression of class I molecules on target cells [8]. In humans, two isoforms of 2B4, h2B4-A and h2B4-B are expressed on NK cells. However, unlike the murine isoforms, both human isoforms have identical cytoplasmic domains with four immunoreceptor tyrosine-based switch motifs. The differences in the human isoforms arise by differential splicing of hnRNA that result in the addition of five amino acids between the immunoglobulin V and C2 domains in the extracellular region of h2B4-B introduces additional turns in the strands at the junction of V and C2 domain, which alters the ligand binding site. Our studies demonstrated that h2B4-A fusion protein shows strong binding to NK-92MI cells with increasing concentrations of 1–10 μg/mL whereas h2B4-B fusion protein weakly binds to NK-92MI cells and higher concentrations of the fusion protein did not bring any substantial change in the binding affinity [12,14]. Upon interaction with CD48, h2B4-A increases cytolytic activity and intracellular calcium levels in NK cells whereas h2B4-B does not activate NK cells [12]. At present, the ligand for h2B4-B has not been determined. The molecular basis for the opposing function of 2B4 in human (mainly as an activating receptor) and in mice (predominantly as an inhibitory receptor) is not fully understood.

2B4 is involved in the rejection of melanoma cells.

We generated 2B4-deficient mice to study the in vivo role of 2B4 and our results suggest a complex role of 2B4 in rejecting B16 melanoma cells [7]. Compared with $CD48^-$ melanoma cells, $CD48^+$ cells are poorly rejected by wild-type mice, suggesting that the expression of CD48 on tumor cells inhibits B16 cell killing. This is supported by the enhanced resistance to $CD48^+$ melanoma cells in $2B4^{-/-}$ male mice. However, $2B4^{-/-}$ female mice have poor resistance against both $CD48^+$ and $CD48^-$ melanoma cells, revealing a gender-specific role of 2B4 that is independent of CD48 expression on tumor cells. In vitro killing assays and in vivo depletion of NK cells using NK1.1 mAb indicated that interaction of NK cells with other immune cells may play a role in the generation of gender specificity [7]. 2B4-CD48 interaction between NK cells has also been found to activate NK cells. In view of these observations, 2B4-CD48 interactions could regulate host resistance against tumor by two distinct mechanisms: interaction of NK cell 2B4 with tumor-associated CD48 and 2B4-CD48 interactions among NK cells. Previous data, by our lab and others, shows the likely effects of both such interactions. Male and female knockout LAK cells had similar cytotoxicity against B16 cells, indicating that female NK cells do not have an intrinsic defect. This supports the possibility of a role for non-NK cells, such as T cells, in this effect. In vivo, our lab saw no gender difference in the growth of B16 tumors in NK-depleted mice. This suggests that NK cells contribute to the gender difference seen in the $2B4^{-/-}$ mice. These observations indicate that interactions between the different components of immune system play an important role in the rejection of cancer cells. The gender-specific difference seen in $2B4^{-/-}$ mice could be the result of an interaction between NK and other immune cells [7]. This gender-specific effect in $2B4^{-/-}$ mice was not seen with the mouse lung cancer cell line 3LL. Thus, the gender-related effect appears to be specific to B16 melanoma cells. If this is indeed the case, it suggests that an antigen-specific T cell response could play a role in producing this effect in a 2B4-dependent manner [7]. In fact, 2B4 is involved in generating antigen specific T cell responses and place a complex role in T and NK cell responses against viral infections [15,16].

1.2. Expression of 2B4 on NK cells, T cells and monocytes altered in Systemic Lupus Erythematosus

Family-based association studies of SLE families and rheumatoid arthritis patients have identified single nucleotide polymorphisms (SNP) in introns of the SLAM family members CS1 and 2B4. Because mutations in the intron sequence can affect splicing events, our lab investigated whether differential expression of splice variants of 2B4 is observed in SLE patients. Healthy individuals expressed five- to eight-fold higher mRNA levels of 2B4-A than 2B4—B [17]. However, some SLE patients showed more predominance (more than 10-fold) of 2B4-A over 2B4—B than in healthy controls. Interestingly, some patients with active SLE showed comparable levels of 2B4-A and 2B4—B. These data indicate clearly that splicing of h2B4 mRNA is regulated differentially in SLE [17]. To further elucidate if 2B4 expression is altered in SLE patients, we analyzed the proportion of 2B4-expressing cells in total PBMCs, CD3+ T cells, CD56+ NK cells, and CD14+ monocytes in patients with SLE and healthy controls. The proportion of 2B4-positive cells in total PBMCs and T cells was not significantly different between healthy controls and SLE patients. However, the proportion of 2B4-expressing cells was decreased significantly in NK cells and monocytes from patients with SLE compared to healthy controls. Although all monocytes are known to express 2B4, monocytes from two patients with SLE showed almost no expression of 2B4. Interestingly, when we compared the expression of 2B4 at the single-cell level, the expression of 2B4 was downregulated significantly by all 2B4-expressing cells, including total PBMCs, T cells, NK cells, and monocytes. Research done in the laboratory of George Tsokos showed a selective loss of 2B4+ CD8+ T cells in SLE patients compared to healthy controls [18]. Their study further demonstrated that 2B4+ CD8+ T cells from SLE patients had decreased cytotoxic capacity and proliferative response to viral peptides [18]. Consistent with the 2B4 splice variant mRNA results, these data indicate clearly that the expression of 2B4 is altered in SLE [17]. A possible mechanism for this 2B4 downregulation can be suggested from previous studies, showing that 2B4-mediated stimulation of human NK cells leads to the downregulation of surface 2B4 by reduced promoter activity as well as receptor internalization [19]. This implies that 2B4–CD48 interaction might be involved actively in SLE. Furthermore, our study using 2B4-deficient mice showed that 2B4–CD48 interactions play a regulatory role in generating gender-specific immune responses. This gender-specific immune response was mediated by NK cells [10,17]. Thus, one could speculate that reduced expression of 2B4 on NK cells from SLE patients may be involved in the gender bias seen in SLE.

1.3. 2B4 mediated immunotherapy for cancer and other immune diseases

Recently, a wealth of information has emerged from several laboratories on the various functional roles of 2B4 in immune regulation. In X-linked lymphoproliferative disease (XLPD), NK cells cannot be activated via surface 2B4. The molecular adaptor protein SLAM-associated protein (SAP)/src homology 2 domain-containing adaptor molecule (SH2D1A) associates with the cytoplasmic tail of 2B4 and SLAM

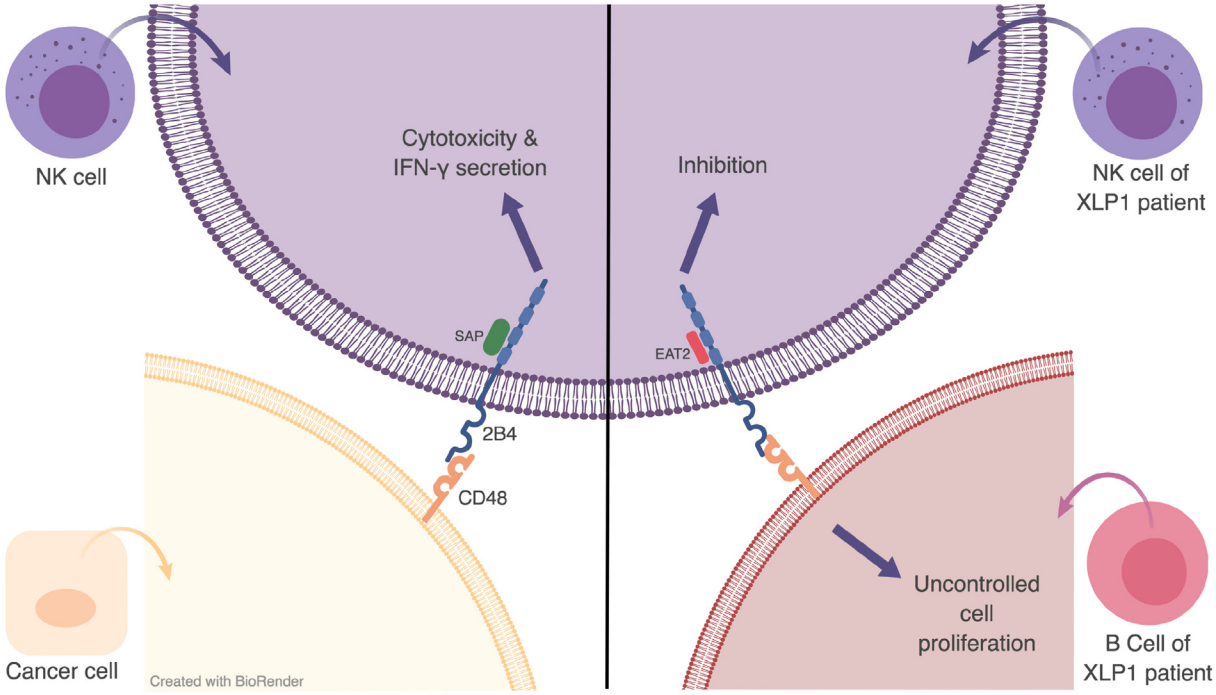

Fig. 1. Duel function of 2B4-CD48 interaction based on adaptor protein recruitment. In healthy humans, 2B4 on NK cells binds to CD48 expressed on cancer cells and leads to the recruitment of SAP (SLAM-associated protein) to the cytoplasmic domain of 2B4 and ultimately triggers a signaling cascade leading to NK cell cytotoxicity and IFN-γ secretion (left panel). Patients with XLP1, a subset of X-linked lymphoproliferative disease (XLPD), possess a mutation in the gene coding for SAP which leads to a nonfunctional binding domain which prevents binding to the cytoplasmic domain of 2B4. In the absence of SAP binding, upon 2B4-CD48 interaction the adaptor protein EAT-2 (Ewing's sarcoma-associated transcript 2) binds to the cytoplasmic domain of 2B4 and leads to the inhibition of killing EBV (Epstein-Barr virus) infected B cells. Additionally, the 2B4-CD48 interaction leads to aberrant signaling and uncontrolled cell proliferation of EBV infected B cells. As a result, the EBV infection cannot be controlled and contributes to the pathogenesis of XLPD (right panel).

[9]. XLP1, a subset of XLPD, is due to a mutation of the *SH2D1A* gene. The mutations in *SH2D1A* are diverse and can range from a complete deletion, unstable, or nonfunctional SH2 domain on SAP which prevents binding to the cytoplasmic domain of 2B4. These mutations also cause aberrant signaling via 2B4 and SLAM during T/B-cell interactions leading to uncontrolled cell proliferation of B cells during Epstein-Barr Virus (EBV) infection [4,20]. When 2B4 on these NK cells interacts with CD48 on EBV-infected cells, an inhibitory signal is sent, instead of the activating signal when functional SAP is present. As a result, the EBV infection cannot be controlled, contributing to the pathogenesis of XLPD [21]. Blocking the inhibitory signal to NK cells via 2B4 by a monoclonal antibody could be a therapeutic option in XLPD. A schematic representation of the different role of 2B4 in NK cells against cancer and autoimmune diseases is given in Fig. 1. Altvater et al., utilized a recombinant chimeric receptor that link extracellular single-chain Fv fragments specific for tumor-associated antigens to the signaling domain of 2B4 and/or TCRζ [22]. Although 2B4 signaling alone failed to induce T cell proliferation or effector function, it significantly increased activation signals when combined with the signaling domain of TCRζ. Therefore, 2B4 can be used as a costimulatory signal in T cells as an amplifier of TCR-mediated signaling [22]. In a separate study Altvater et al. utilized the same chimeric 2B4-TCRζ receptor and presented a strategy to redirect NK cell effector functions towards autologous leukemia cells by activating signaling pathways and overcoming their natural NK resistance. The increased NK response was in an antigen specific manner and was also demonstrated against neuroblastoma cells [23]

The anti-tumor activity of NK cells is increased by alloferon, an immunomodulatory peptide that stimulates natural cytotoxicity and anti-viral effects. Alloferon is able to increase NK cell natural cytotoxicity against tumor cells via the upregulation of 2B4. Additionally, alloferon increased IFN-γ and TNFα production by NK cells. When alloferon was administered to nude mice inoculated with colon cancer, tumor growth was completely inhibited [24]. These data demonstrate that 2B4 upregulation is sufficient for NK activation and tumor rejection in mice.

1.4. CS1 (SLAMF7, CD319)

CS1 was first identified, cloned, and characterized as a NK cell receptor [9]. Since then, CS1 has been found to play an important role in immune cell function. CS1 is expressed on different types of cells such as NK cells, $CD8^+$ T cells, B cells, and activated dendritic cells [9,25]. NK cells contribute its role as innate lymphoid cells in immune responses against both tumor and infected cells. NK cell function includes utilizing a mechanism called antibody-dependent cell-mediated cytotoxicity (ADCC) to kill target cells, natural cytotoxicity, and cytokine secretion [26]. NK cells use its ability to recognize and eradicate tumor or infected cells through NK receptor interactions with ligands on target cells [27]. NK cell function is based on the intricate balance of activating and inhibitory signals sent to the NK cells due to ligand-receptor interactions [28]. Among the NK cell receptors, CS1 expressed on NK cells has been found to be homophilic, which means that CS1 can bind to itself on NK cells or bind to CS1 expressed on other immune cells [29]. (Fig. 2).

CS1 as a NK cell receptor can bind to CS1 expressed on target cells [29]. CS1-CS1 interaction between NK cells and leukemia cells has been shown to enhance killing of leukemia cells as a result of activation of NK cells [29]. Furthermore, the CS1-CS1 homophilic interaction is determined to be similar to homophilic interactions displayed by CD150 and CD84. These homophilic interactions from these three CD2-subset receptors results in the activation of immune cells and its functions. Some members of the CD2 receptor family are expressed in soluble form and bind to receptors on target cells [29,30]. CD48 is expressed as a

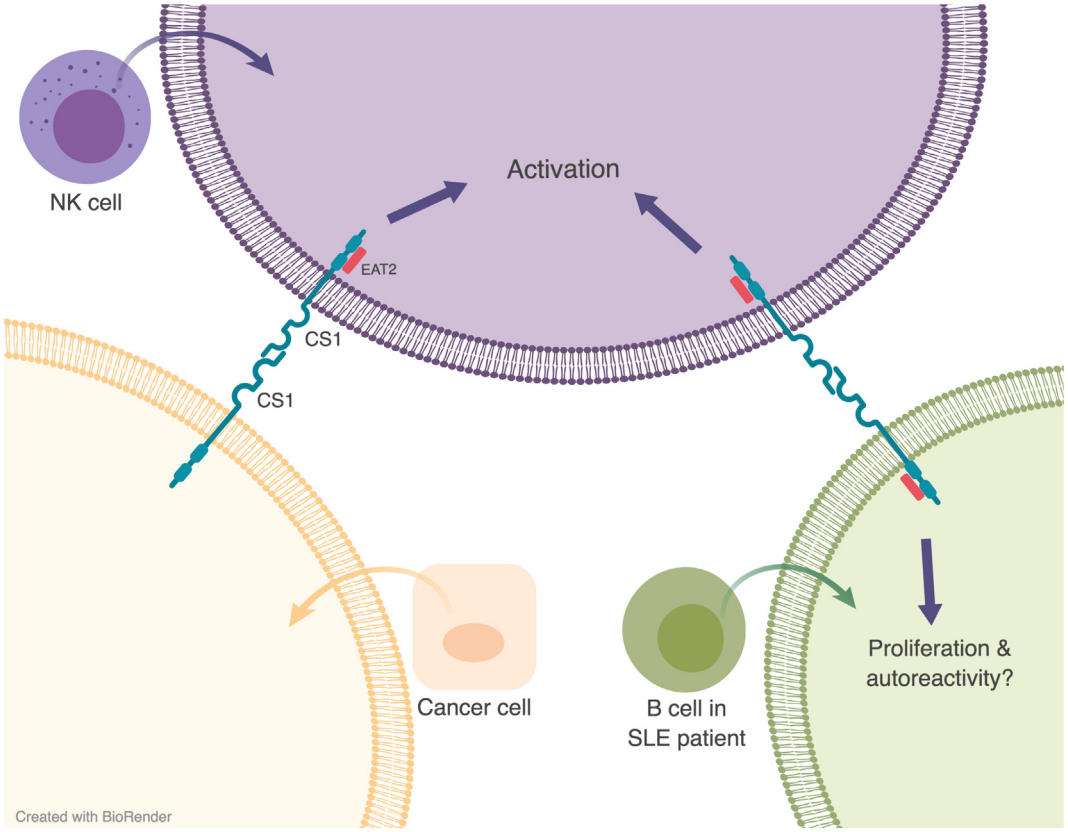

Fig. 2. CS1-CS1 interaction regulates NK cell function in cancer and autoimmunity. NK cell activation via CS1-CS1 interaction may be responsible for B cell proliferation and autoreactivity in systemic lupus erythematosus (SLE) patients. Overexpression of CS1 has been demonstrated in some cancers, such as multiple myeloma. When CS1 on NK cells binds to CS1 on cancer cells, an activation signal is sent, and NK cells increase in effector functions. It has been demonstrated that SLE patients have an increased number of CS1 positive B cells when compared to healthy controls. In SLE patients, CS1 on NK cells binds to CS1 on B cells, which may lead to the B cell proliferation and autoreactivity seen in SLE.

membrane-bound protein or in soluble form and regulate immunity and tolerance [31,32]. While human 2B4 exist in membrane bound form, rat 2B4 can exists in membrane bound as well as soluble forms [33,34]. CS1 on NK cells is expressed as a membrane-bound protein and interacts with other membrane-bound CS1 located on various target cells [29].

Bouchon and colleagues has shown that CS1 expressed on immune cells such as NK cells, $CD8^+$ T cells, mature dendritic cells, and activated B cells allows activation of immune cell function through a SLAM-associated protein (SAP)-independent signaling pathway [25]. Receptors such as 2B4, CD150, CD84, and Ly-9 predominantly depend on the recruitment of SAP for activation of a signaling pathway that enhances function of NK, B, and T cells against pathogens [25,35]. Patients with X-linked lymphoproliferative disease has a deficiency in SAP which is attributed to immunodeficiency against EBV infections [20]. Characterization of the CS1 signaling pathway involves recruitment of two tyrosine phosphorylated proteins but does not associate with SAP [36]. Even though the cytoplasmic domain of CS1 has the immunoreceptor tyrosine-based switch motifs (ITSM), it was also shown that CS1 still triggers activation of ERK without depending on SAP, which then leads to NK cell cytotoxicity [36]. Interestingly, two splice variants of CS1 expressed on human NK cells were identified, which are CS1-S and CS1-L [36]. CS1-S does not have an ITSM, while CS1-L contains an ITSM which interacts with Ewing's sarcoma-associated transcript 2 (EAT-2), thus CS1-L was responsible for trigging activation of NK cytotoxicity [36].

Naïve B lymphocytes express CS1-L which gets upregulated upon activation and leads to proliferation of B cells and production of cytokines [37]. In CS1-stimulated B cells, production of IL-14, TNF, and flt3L was observed which served as growth factor cytokines that leads to B cell proliferation [37]. CS1 is also expressed in activated human monocytes after TLR-2,4,5, and 7/8 were induced with ligands [38]. Monocytes are known to contribute to protection against pathogens, but also damage tissue in chronic inflammation [38]. Hence, monocyte function is carefully regulated by upregulation or downregulation of cell surface proteins. It was determined that CS1 expression on activated monocytes serves its inhibitory role in suppressing production of proinflammatory cytokines TNF-α and IL-12p70 [38]. CS1 is also expressed on mouse $CD4^+$ T cells and has inhibited proliferation and cytokine production from T cells [39]. CS1 may also play an inhibitory role, but little is known to date on exactly how CS1 plays this role [39]. CS1 is also expressed on other cell types such as NK-T cells and macrophages, but like T cells, there still needs to be further studies on the role of CS1 on these specific cells.

1.5. CS1 in multiple myeloma

Multiple myeloma (MM), also known as plasma cell myeloma, is characterized by the proliferation of malignant plasma cells within the bone marrow which results in producing monoclonal paraprotein in both serum and end organs [40]. Complications of multiple myeloma include bone pain, anemia, hypercalcaemia, and renal impairment as a result of the production of abnormal antibodies or paraprotein from these malignant plasma cells [40]. Conventional treatments such as chemotherapy are used; however, considering the complexity and remission of this disease in MM patients, there is a need to determine alternative options for treating MM.

There is promise in utilizing CS1 as a potential immunotherapeutic

target on MM cells [41]. Tai and colleagues conducted a study demonstrating that CS1 is highly expressed on patient MM cells [42]. The same group has also shown that circulating soluble CS1 is expressed in sera of MM patients at low levels, but not in sera of healthy donors [42]. CS1 mRNA and protein levels were highly expressed in CD138-purified tumor cells from 97% of MM patients sampled in this study [42]. An anti-CS1 humanized monoclonal antibody HuLuc63 was developed to determine the effects of blocking CS1 on MM growth. HuLuc63 binds to CS1 which inhibits MM cells from binding to bone marrow stromal cells and induce ADCC against MM cells [42]. ADCC (antibody-dependent cell-mediated cytotoxicity) is defined as NK cells recognizing the Fc region of antibodies bound to antigens on target cells and inducing lysis against the target cell. Even in patients with MM cells resistant to conventional and novel therapies such as bortezomib or 17-AAG, which targets heat shock protein 90, anti-CS1 HuLuc63 monoclonal antibody still induces ADCC against these MM cells [42]. Pretreatment of peripheral blood mononuclear cells with lenalidomide, a FDA-approved drug for treatment of MM, enhances HuLuc63 monoclonal antibody function of ADCC against MM [42].

Considering the promising results of utilizing anti-CS1 monoclonal antibodies for treatment against MM, a humanized monoclonal antibody called Elotuzumab, or Empliciti, was developed and used in clinical trials of patients with MM [43]. Elotuzumab is a monoclonal antibody that targets CS1 on MM cells and enhances NK cytotoxicity and lysis against MM cells by ADCC mechanism [43]. It has been shown that Elotuzumab also promotes CS1-CS1 interaction between $CS1^{+}$ MM cells and NK cells which may complement ADCC-induced lysis of MM cells. Utilizing these mechanisms of action, Elotuzumab in combination with bortezomib was used in phase 1 and 2 clinical trials for patients with relapsed and/or refractory multiple myeloma [44]. Treatment with Elotuzumab in combination with bortezomib showed positive results in terms of patients seeing a partial or better response of MM cells being targeted by the new treatment [44]. Interestingly, patients treated with Elotuzumab alone did not exhibit progress in MM cells being killed in contrast to the Elotuzumab in combination with bortezomib [44,45]. There is promise in treatment of MM by using anti-CS1 monoclonal antibodies, but there needs to be further studies on the mechanism of action of this drug and enhancing efficacy in combination with other conventional drugs. Higher expression of CS1 on MM cells serves as an effective target in this and future immunotherapeutic treatments for these affected patients.

1.6. CS1 in systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the deposit of immune complexes (or antigen-antibody complexes) that accumulate in tissue and induce inflammation of the affected tissue [32]. Patients with SLE have a dysfunctional immune system that attacks one's normal tissue leading to symptoms such as skin rashes, joint pain, fever, and mouth ulcers [32]. Immune complexes enhance inflammation at affected tissue by activating the complement pathway, activating immune cells, and inducing production of proinflammatory cytokines [32,33]. Currently, there is no cure for SLE, but common treatments range from immunosuppressants to nonsteroidal anti-inflammatory drugs [32]. Hence, looking at the expression of certain proteins on immune cells may reveal the mechanism of how inflammation was induced.

CS1 is already known to be a self-ligand that activates NK cell function and induces proliferation of B lymphocytes by increasing autocrine cytokine production [29]. Family-based association studies conducted in the UK and Canada have determined that within families who have patients with SLE there are mutations in the *CS1* gene which enhances the risk of being diagnosed with SLE [46]. Our study revealed that alterations in the expression of *CS1* and *2B4* genes may play a role in immune dysfunction in SLE [17]. An important feature of CS1 is that these receptors can interact with CS1 on another immune cell as self-ligands, most notably dendritic cells and NK cells [17]. It has been reported that there are different subsets of B cells circulating in patients with SLE such as memory B cells, naïve B cells, plasma B cells, and plasmablasts [47]. Kim and colleagues demonstrated that the different subsets of CD19-positive B cells in peripheral blood mononuclear cells (PBMCs) of SLE patients can further be classified based on level of CS1 expression [17]. Based on the combination of CD19 expression and CS1 expression, they were able to determine which subset of B cells (that is naïve B, memory B, plasma, etc.) correlate with the level of CS1 expression, which would ultimately provide an insight on the relationship between the pathogenesis of SLE with CS1 expression.

It has been shown by other studies that SLE disease activity has been correlated with a high frequency of plasma cells and autoreactive memory B cells [17]. The frequency of CD19-high B cells, which are autoreactive memory B cells, was correlated with SLE development while another set of studies also reported that SLE activity also correlates with high expression of CD27 and low expression of CD19 which indicates involvement of plasma cells [48,49]. Kim and colleagues have demonstrated that SLE patients have an increased number of CS1-positive B cells. Higher level of CS1 expression favors proliferation of B cells and expression of CS1 on B cells is induced by CD40-mediated B cell activation.

Another study conducted by Hagberg and colleagues aimed to determine surface proteins involved in the interaction between plasmacytoid dendritic cells (pDCs) and NK cells when activated by RNA-immune complexes in SLE [50]. This study had observed a strong upregulation of CS1 and CD229 on both pDCs and $CD56^{dim}$ NK cells but not on T and B cells by RNA-immune complexes in culture. Furthermore, cytokines IL-12 and IL-18 increased expression of CS1 on $CD56^{bright}$ NK cells. In patients with SLE, there is a decreased expression of CS1 on circulating pDCs, but when PBMCs in SLE patients gets stimulated by RNA-immune complexes the expression of both CS1 and CD229 increases on pDCs [50]. As the authors of this study explained, the low expression of CS1 on circulating pDCs and upregulated expression on RNA-immune complex-stimulated PBMCs indicates that the stimulated PBMCs in SLE patients migrates to tissue sites of inflammation. At this time, there are limited treatment options for patients with SLE. Understanding the mechanism and role of CS1 in SLE pathogenesis will allow us to counteract inflammation and disease progression in these patients.

1.7. CS1-mediated therapy

There has been increasing interest into developing chimeric antigen receptor-T cells (CAR-T) and chimeric antigen receptor-engineered natural killer cells (CAR-NK) that can target specific tumor-associated antigens [51]. It has been shown that CAR-T cells was successful in clinical settings for treatment of acute lymphoblastic leukemia [52]. For multiple myeloma, CS1 was identified as a possible ideal target for use of CAR-T or CAR-NK cells, because CS1 is overexpressed in multiple myeloma cells. It has been demonstrated that anti-CS1 humanized monoclonal antibody Elotuzumab in combination with bortezomib showed promise in phase 1 and phase 2 of clinical trials [30]. Expanding studies from monoclonal antibodies to CAR-T and CAR-NK cells will allow potential additional options for patients with MM, SLE, and other diseases.

Chu and colleagues reported that they engineered human NK cells to express chimeric antigen receptors targeting CS1 and tested for antitumor activity against MM cells in vitro and in vivo in MM-xenograft mouse models [51]. Interestingly, this study used allogeneic NK cells as a source for development of CAR-NK cells which may help inhibit graft-versus host disease and increase NK cytotoxicity due to having non-matching killer immunoglobulin-like receptors (KIRs) [51,53]. Using allogenic NK-based immunotherapy from non-HLA-related healthy donors has become a promising option for cancer therapy due to mismatching KIRs on donor NK cells and different major histocompatibility

complexes on recipient cells [54]. Hence, cytotoxicity and activation of NK cells against specific tumor-associated antigens through chimeric antigen receptors are increased while these allogenic NK cells are being spared by the recipient's immune system [54]. For MM, the need for developing CAR-NK cells over CAR-T cells is favored because only 4% of MM patients express B-lymphocyte antigen CD19 on MM cells [55]. CS1 is considered an attractive target for MM due to its high expression before and after relapse, so targeting CS1 may also be effective even if a patient's relapse of MM occurs [41]. In testing CAR-NK cells targeting CS1 on MM cells, it has been demonstrated by Chu et al. that these CAR-NK cells have an increase in production of interferon-γ (IFN-γ) in vitro and CAR-NK were able to kill human IM9 cells (MM cell line) in NOD SCID gamma mice [51]. In addition, this study went further on its development of CAR-NK cells by incorporating a CD28-CD3 ζ costimulatory domain which resulted in an enhanced anti-tumor activity against MM cells.

Development of CAR-T cells targeting CS1 was also used to eradicate MM [56]. CAR-T cells targeting CS1 has been shown to secrete higher levels of both IFN-γ and IL-2, express elevated levels of CD69 (an early activation marker on T and NK cells), and enhanced degranulation and cytotoxicity. CAR-T cells targeting CS1 has been shown to have higher cytotoxicity against MM and much lower cytotoxicity against NK and T cells similar to the anti-CS1 monoclonal antibody Elotuzumab results in phase 1 and 2 of clinical trials [51]. There are limitations to using CAR-T cells such as concerns that allogenic T cells may cause graft-versus-host disease when transferred into patients and tumor cells may develop a mechanism to evade CAR-T cells by downregulation of targeted tumor-associated antigens. The development of both CAR-T and CAR-NK cells provide advantages in treating MM but does come with its challenges of enhancing killing of tumor cells while maintaining safety of patients.

2. Conclusions

Members of the signaling lymphocyte activation molecule family (SLAMF) play crucial role in the development and immune responses against infections and cancer. 2B4 (SLAMF4) and CS1 (SLAMF7) were discovered as NK cell receptors regulating the cytolytic function of NK cells. However, expression and functional characterization of 2B4 and CS1 and their ligands on other immune cells demonstrated a broader role for these receptors in the immune system in health and disease. Signaling through 2B4 is essential for the development of NK cell tolerance. 2B4-CD48 interaction regulates the generation of antigen specific T cell responses. CS1 is overexpressed in multiple myeloma and a humanized anti-CS1 mab (Elotuzumab/Empliciti) is highly effective in controlling the disease. Defective signaling via 2B4 due to mutations in signaling adaptor molecule SAP contributes to X-linked lymphoproliferative Disease (XLP). Expression of 2B4 and CS1 are altered in systemic lupus erythematosus. CAR-T cells and CAR-NK cells containing CS1 or 2B4 ectodomain are useful immunotherapeutic tools against cancer. While these novel therapies show promise, there needs to be further studies on enhancing the efficacy and safety of these potential breakthrough treatments targeting CS1 against MM, SLE, and other diseases. A better understanding of the roles of 2B4 and CS1 will enable us to utilize/target these receptors in immunotherapy for various cancers and autoimmune diseases.

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