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SLAMF1 engagement inhibits T cell-B cell interaction and diminishes IL-6 production and plasmablast differentiation in systemic lupus erythematosus

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Abstract

Objective: SLAMF1 homophilic interactions promote immunoglobulin production and T cell-B cell (T-B) cross-talk. SLAMF1 is overexpressed on T and B cells in patients with SLE. We conducted studies to determine the role of SLAMF1 monoclonal antibody in modulating T-B cell interaction and B cell activation.

Materials: Anti-IgM pre-stimulated naïve or total B cells from healthy donors or patients with SLE were co-cultured with autologous T cells under CD3/CD28 stimulation in the presence or absence of SLAMF1 monoclonal antibody. Naïve B cells were stimulated with anti-IgM and CD40L in the presence of SLAMF1 antibody. Cytokine production by CD4+ T cells and B cells was examined by flow cytometry and/or qPCR. Plasmablast formation and T-B conjugates were assessed by flow cytometry. IgG and ANA production was determined by ELISA.

Results: SLAMF1 ligation in a human peripheral blood T-B cell culture system reduces conjugate formation, IL-6 production by B cells, IL-21 and IL-17A by T cells, Ig and autoantibody production in both healthy controls and patients with SLE. Whereas the SLAMF1 monoclonal antibody affects directly the function of isolated peripheral B cells by decreasing IL-6 and Ig production in vitro, it does not affect stimulation and cytokine production by isolated T cells stimulated in vitro.

Conclusions: SLAMF1 antibody inhibits T-B cell interaction and suppresses B cell cytokine production and differentiation and therefore it represents a therapeutic tool in the treatment of patients with SLE.

Keywords

SLE; B cells; SLAMF1; autoimmunity; IL-6

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Systemic Lupus Erythematosus (SLE) patients are characterized by alterations in B cell subset distribution in the peripheral blood (1), defects in early BCR-initiated signaling events (2) and spontaneous (auto)antibody and IL-6 production (3, 4). The pathophysiological significance of B cells in SLE is underscored by the beneficial clinical outcome of belimumab, an antibody blocking a B cell stimulating cytokine that affects survival of B cells and plasma cells (5).

Signaling lymphocytic activation molecules family members (SLAMF1–9) are type I transmembrane glycoprotein cell surface receptors that deliver downstream signals upon their engagement and modulate the magnitude of the immune response [9]. The SLAMF encoding genes are located on chromosome 1 within 1q23, a region known to be associated with increased susceptibility for SLE development (6, 7).

SLAMF1 is expressed on T cells, B cells and dendritic cells but not on monocytes or natural killer cells (8, 9). Under physiological conditions SLAMF1 acts as a self-ligand. The −262 A/G and −188 A/G polymorphisms in the promoter region of SLAMF1 are linked to higher SLAMF1 mRNA expression following stimulation of SLE peripheral blood mononuclear cells with phytohemagglutinin and may contribute to increased SLE susceptibility (10). Moreover, SLAMF1 is up-regulated on the cell surface of both T cells and B cells in the peripheral blood of patients with SLE, suggesting a potential role of SLAMF1 in SLE immunopathogenesis (11, 12).

In B cells, SLAMF1 is up-regulated following activation and it has been shown that co-engagement with membrane or soluble forms of recombinant SLAMF1 may promote B cell proliferation and differentiation into immunoglobulin secreting cells (ISC) (13). Interestingly, it has been shown that the SLAMF1 (A12) monoclonal antibody (SLAMF1 mAb) and its $F(ab')_2$ fragment slightly diminished SAC-or anti-CD40-mediated proliferation in B cells, suggesting that SLAMF1 mAb may have an inhibitory effect on B cells activation (13).

We report that the presence of SLAMF1 mAb in human peripheral blood T-B cell cultures reduces T-B cells interaction and IL-6 production by B cells. As a result, IL-21 and IL-17A production by T cells and Ig and autoantibody production by B cells is also diminished in healthy subjects and patients with SLE.

Whereas the SLAMF1 mAb affects directly the function of isolated peripheral B cells by decreasing IL-6 and Ig production in vitro, it does not affect stimulation and cytokine production by isolated T cells stimulated *in vitro*. These data argue that SLAMF1 engagement may have unrecognized beneficial effect in conditions where T-B cell interaction is crucial in disease pathogenesis.

Materials and Methods

SLE patients and healthy controls.

Patients (n=26) fulfilling the American College of Rheumatology criteria for lupus were recruited at the Rheumatology Department at Beth Israel Deaconess Medical Center (14).

Disease activity score was measured using the SLEDAI scoring system (supplementary Table S1). Age-, sex-, and ethnicity-matched healthy individuals were evaluated as controls. Informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

mAbs and reagents.

A complete list of the flow cytometry mAbs and of the reagents used for T and B cell *in vitro* stimulation is provided in Supplementary Table S2.

Cell isolation.

Peripheral blood mononuclear cells were isolated by density gradient centrifugation (Lymphocyte Separation Medium, Corning Life Sciences). Total T and B cells were isolated by negative selection (RosetteSep, Stem Cell Technologies). Naïve B cells were negatively selected from total B cells using the human naïve B cell isolation kit II (Miltenyi Biotec). The positive fractions representing memory B cells were also collected. Naïve CD4+ T cell purification was performed with Naïve CD4+ T cell Isolation Kit II (Miltenyi Biotec).

T cell stimulation.

Total or naïve CD4+ T cells were stimulated in complete RPMI (supplemented with 10% fetal bovine serum, 100mg/ml streptomycin and 100U/ml penicillin), with pre-coated antibodies (anti-CD3 1μg/ml; anti-CD28 1μg/ml, anti-SLAMF1 5μg/ml or isotype control 5μg/ml). Where indicated, cells were re-stimulated with phorbol 12-myristate 13-acetate (PMA, 25ng/ml) and ionomycin (0.5μg/ml) in the presence of Brefeldin A (GolgiPlug 1μl/ml; BD Biosciences) for 6h.

B cell stimulation.

Total, naïve or memory peripheral blood B cells were stimulated with the $F(ab)_2$ fragment of an affinity purified mouse anti-human μ heavy chain antibody [$F(ab)_2$ anti-IgM, 1μg/ml] followed by soluble CD40 ligand (CD40L, 2μg/ml), in the presence of a mouse anti-human SLAMF1 mAb (5μg/ml) or a mouse IgG1κ isotype control (5μg/ml) for the indicated time points. In some experiments, cells were cultured in the presence of a pharmacological inhibitor against SHP-2 (SHP099, purchased from Cayman Chemical).

For cytokine detection, cells were re-stimulated with PMA (25ng/ml) and ionomycin (0.5μg/ml) in the presence of Brefeldin A (GolgiPlug 1μg/ml) for the final 6h of culture. For B cell differentiation, naïve B cells were stimulated as mentioned above in the presence of IL-4 (10ng/ml, Peprotech) for 7d. IL-4 was replenished every 3d. For immunoglobulin production, naïve B cells (50×10^3/200μl, 96-U bottom, complete medium) were stimulated with $F(ab)_2$ anti-IgM (1μg/ml), CD40L (2μg/ml) and IL-4 (10ng/ml), in the presence or absence of SLAMF1 mAb (5μg/ml) or an isotype control, for 12 days.

T cell-B cell co-culture.

Total or naïve B cells were prestimulated with $F(ab)_2$ anti-IgM (1 μg/ml) for 48hr and then co-cultured with autologous total T cells or naïve CD4+ T cells, as indicated, in complete medium in 48-well plates pre-coated with anti-CD3 (1μg/ml) and anti-CD28 (1μg/ml) for 5

days, at 37 ºC with 5% CO_2. Soluble SLAMF1 mAb (5μg/ml) or an isotype control were added in the culture. Where indicated, we used a $F(ab)_2$ fragment generated from SLAMF1 mAb (5μg/ml) or from normal isotype control (5μg/ml) using a $F(ab)_2$ fragmentation kit (G-biosciences) according to manufacturer's instructions.

On day 5, cells were re-stimulated with PMA (25ng/ml) and ionomycin (0.5μg/ml) in the presence of Brefeldin A (1μg/ml) for 6h. Cytokine production was examined by flow cytometry. Alternatively, co-cultures were maintained for 12h and were then examined for conjugate formation or were maintained for 7d to examine Tfh-like formation and plasmablast differentiation.

Th17 cell differentiation.

Freshly isolated naïve CD4+ T cells were cultured in complete medium with pre-coated anti-CD3 (1μg/ml) and anti-CD28 (1μg/ml) in the presence of soluble SLAMF1 mAb (5μg/ml) or an isotype control (5μg/ml), in Th17 polarizing conditions as previously described (15). On day 5, cells were re-stimulated with PMA (25 ng/ml) and ionomycin (0.5 μg/ml) in the presence of Brefeldin A (1μg/ml) for 6h. Cytokine production was examined by flow cytometry. All cytokines were purchased from Peprotech.

Flow cytometry.

Cells were stained for dead cells (Zombie Aqua/UV/NIR Fixable Viability Kit; Biolegend), and then labeled for surface antibodies (see Table S2). For cytokine detection cells were permeabilized (Cytofix/Cytoperm, BD Biosciences) and stained with the indicated antibodies (see Table S2). Data were acquired on a LSR II SORP (BD Biosciences) and analyzed using FlowJo (version 10.0.8, FlowJo Enterprise).

Real-time quantitative reverse transcriptase-polymerase chain reaction (Q-RT-PCR).

Performed as previously described (16). Primer sequences are shown in Supplemental table S3

Western Immunoblot.

Performed as previously described (17)

Proliferation experiments.

Cells were labeled with Carboxyfluorescein succinimidyl ester (CFSE, 1μM) for 5min at 37ºC and were then activated with appropriate stimuli, as indicated. The CFSE dilution was examined by flow cytometry.

Enzyme-linked immunosorbent assay.

IgG (ebioscience) and ANA (NeoScientific) secretion was determined in culture supernatants by Enzyme-linked immunosorbent assay (ELISA), according to manufacturer's instructions.

Statistical analysis.

Statistical analysis was performed using the Wilcoxon matched signed-rank test. For multiple comparisons, statistical analysis was performed using one-way Anova, followed by *post hoc* analysis with Tukey's test. Analyses were performed using GraphPad Prism (version 7). Statistical significance was reported as follows: *p≤0.05, **p≤ 0.01, ***p≤ 0.001. Data are presented as mean ± SEM.

Results

SLAMF1 ligation reduces IL-21 and IL-17A production by healthy and SLE CD4+ T cells in a T-B cell co-culture system

B cells are potent antigen presenting cells, able to initiate and maintain T cell responses, both through cell-cell contact and cytokine release. SLAMF1 is upregulated on both T cells and B cells of patients with SLE (12). We examined the effect of SLAMF1 ligation using a specific anti-human SLAMF1 mAb in the context of T-B interactions *in vitro*. Total B cells were isolated from the peripheral blood of healthy donors or patients with SLE and stimulated with a $F(ab)_2$ anti-IgM for 48h to mimic the initial capture of antigen by the B cells. Stimulated B cells were then co-cultured with autologous T cells, in the presence or absence of soluble SLAMF1 mAb for 5 days.

We examined the production of IL-21, IL-17A, IFNγ, TNFα, IL-4, IL-10 and IL-2 by CD4+ T cells on day 5 following re-stimulation with PMA and ionomycin for the final 6h of the culture. In cultures where SLAMF1 mAb was added, we observed a significant decrease in the percentage of IL-21 and IL-17A-producing CD4+ T cells in both patients with SLE and healthy subjects compared to isotype (Figure 1A, B and C). Production of IFNγ, TNFα, IL-4, IL-10 and IL-2 remained unaffected (Supplementary figures 1 and 2). For certain co-culture experiments a $F(ab)_2$ fragment of anti-SLAMF1 or of isotype control were used, with similar results with respect to IL-21 and IL-17 production (Supplementary Figure 3).

When T cells were cultured in the absence of autologous B cells, we failed to detect differences in the percentage of IL-21+ CD4+ T cells following SLAMF1 co-engagement and IL-17A production under non Th17 polarization conditions was minimal (Supplementary Figure 4A and B). To further assess the potential effect of SLAMF1 co-engagement on IL-17A production and Th17 differentiation, naïve CD4+ T cells from healthy controls were incubated with SLAMF1 mAb under Th17 polarizing conditions. We did not observe any differences in the percentages of IL-17A-producing cells following Th17 differentiation in the presence of anti-SLAMF1 compared to isotype control mAb (Supplementary Figure 4C and D). Finally, we assessed the effect of SLAMF1 mAb on CD4+ T cell activation and proliferation. Upregulation of CD69 and CD25 on the cell surface of CD4+ T cells following 48h of stimulation, as well as the percentage of proliferating CFSE-low cells following 6d of culture did not differ among cells that were treated with anti-SLAMF1 compared to cells treated with isotype control mAb (Supplementary Figure 5).

The above data suggest that the decrease in the percentage of IL-17A and IL-21-producing CD4+ T cells in the co-culture system does not represent a direct effect of the SLAMF1 mAb on T cells, but rather an effect on the T-B cell interaction.

SLAMF1 ligation inhibits plasmablast differentiation in a T-B co-culture system *in vitro*.

Production of IL-21 is instrumental for driving B cell differentiation towards ISC (18, 19). Because SLAMF1 ligation resulted in reduced IL-21 production from CD4+ T cells in our T-B co-culture system, we assessed the effect of SLAMF1 mAb in the progression of naïve B cells into plasmablasts. We isolated and prestimulated for 48h healthy naïve peripheral blood B cells and then co-cultured them with autologous total T cells for 7d. Plasmablast formation (defined as IgD-CD27+CD38^{hi}) was assessed by flow cytometry. In the presence of SLAMF1 mAb, the frequency of plasmablasts was significantly reduced compared to isotype control after 7d of culture (Figure 1D). Because IL-21 production mainly characterizes follicular helper CD4+ T cells (Tfh), a distinct subset of CD4+ helper T cells that drives antigen-specific humoral immune responses within germinal centers, we examined the effect of anti-SLAMF1 ligation on CD4+T cell differentiation towards Tfh-like cells. F(ab)$_2$ anti-IgM pre-stimulated B cells were co-cultured with naïve CD4+ T cells in the presence or absence of SLAMF1 mAb for 7d. The frequency of naïve CD4+ T cells that acquired a Tfh-like phenotype, defined by high expression of inducible co-stimulator (ICOS), programmed cell death-1 (PD-1) and chemokine (C-X-C motif) receptor 5 (CXCR5) remained unaltered (data not shown) (20, 21).

SLAMF1 ligation with SLAMF1 mAb diminishes IL-6 cytokine production by B cells.

It has been reported that SLAMF1 co-engagement inhibits the production of proinflammatory cytokines, such as TNFα and IL-6, by CD40L-activated human dendritic cells (22). Moreover, production of IL-17A and IL-21 by CD4+ T cells depends on IL-6 (23). We sought to examine whether SLAMF1 co-engagement exerts a similar effect on cytokine production in human B cells. As SLAMF1 is expressed at higher levels on naïve B cells compared to memory B cells (8), we assessed cytokine expression following F(ab)$_2$ anti-IgM and/or CD40-mediated stimulation in sorted naïve and memory human peripheral blood B cells in the presence or absence of SLAMF1 mAb or an isotype control. As previously reported, a sequential activation of B cells through the BCR followed by CD40 engagement leads to high IL-6 and TNFα production (24). In the presence of soluble SLAMF1 mAb, naïve B cells that were submitted to dual stimulation exhibited significantly less IL-6 production, whereas the frequency of memory IL-6-producing B cells remained unaffected (Figure 2A and B). On the other hand, the percentage of TNFα-producing naïve and memory B cells was not significantly affected by SLAMF1 co-engagement (Supplementary Figure 6A and B). Reduced IL-6 production was also validated by qPCR. We did not record any differences in TNFα mRNA levels (Supplementary Figure 6C and D). IL-6 and TNFα production by B cells were also examined in the T-B co-culture system. As expected, we observed a significant reduction in the frequency of IL-6-producing B cells, whereas TNFα production remained unchanged following SLAMF1 ligation (Figure 2C and D). Based of the above data, more than just inhibiting T-B interaction, SLAMF1 ligation may have a direct effect on B cells.

SLAMF1 co-engagement inhibits B cell differentiation towards plasmablast and immunoglobulin secreting cells.

It is well established that IL-6 promotes B cell growth and terminal differentiation to Ig producing cells both directly and indirectly (3) (23). Because SLAMF1 co-engagement results in reduced IL-6 production by naïve B cells, we hypothesized that treatment with SLAMF1 mAb would negatively affect B cell differentiation. We differentiated naïve B cells from the peripheral blood of healthy donors towards plasmablasts with consecutive dual BCR and CD40-mediated stimulation, in the presence or absence of SLAMF1 mAb. Plasmablast formation was evaluated on day 7 as the percentage of IgD-CD27+CD38^{hi} cells. We found that in the presence of SLAMF1 mAb the percentage of IgD-CD27+CD38^{hi} plasmablasts was significantly diminished compared to isotype control (Figure 3A and B). Moreover, IgG production was also reduced when B cells were differentiated towards plasmablasts in the presence of anti-SLAMF1 compared to isotype control mAb (Figure 3C) and can be explained, in part, by reduced IL-6 production by B cells.

Reduced percentage of plasmablast-like cells and diminished IgG production following SLAMF1 co-engagement *in vitro*, was not the result of increased cell death, as the percentage of live cells (defined as Aqua negative cells) at the end of culture was similar between anti-SLAMF1 treated cells and isotype control treated cells (Figure 3D). In addition, the inhibitory effect of SLAMF1 mAb in plasmablast differentiation and Ig secretion, was not due to a generalized B cell unresponsiveness. B cells upregulated CD69 and CD86 at 12h and 72h of stimulation respectively in the presence of SLAMF1 mAb as effectively as in the presence of an isotype control (Supplementary Figure 7A and B). Moreover, B cells proliferated normally in the presence of SLAMF1 mAb (Supplementary Figure 7C and D). To better assess the effect of SLAMF1 mAb on B cells, we incubated healthy B cells with F(ab)$_2$ anti-IgM in the presence of anti-SLAMF1 or isotype control for 0, 5,10 or 30 minutes and we examined protein tyrosine phosphorylation (pTyr) by Western immunoblotting. In the presence of anti-SLAMF1, pTyr levels were decreased, suggesting a possible inhibitory effect of SLAMF1 mAb on B cells via BCR-mediated signaling regulation (Supplementary Figure 8). It has been reported that SLAMF1 mediates signaling via phosphatase SHP-2 (25). To further address the mechanism via which anti-SLAMF1 affects BCR-initiated signaling, we cultured healthy B cells with F(ab)$_2$ anti-IgM in the presence of SLAMF1 mAb and a small drug SHP-2 inhibitor (SHP099) and assessed pTyr levels by Western immunoblotting. Indeed, in the presence of SHP099 pTyR levels were restored following SLAMF1 co-engagement, indicating that BCR-mediated signaling can be directly modulated in the presence of SLAMF1 mAb in an SHP-2 dependent manner in single cell population cultures (Supplemental Fig 9).

SLAMF1 ligation regulates IL-6 production and plasmablast differentiation of SLE B cells.

SLE B cells spontaneously produce IL-6 *in vitro* that drives B cell differentiation and (auto)antibody production (3). We examined whether treatment of peripheral blood SLE B cells with anti-SLAMF1 regulated IL-6 production and differentiation of naïve SLE B cells. We stimulated naive B cells from SLE patients with F(ab)$_2$ anti-IgM followed by CD40L in the presence of SLAMF1 mAb or isotype control for 72h. We observed that the frequency of IL-6-producing B cells was significantly decreased when cells were co-engaged with anti-

SLAMF1 compared to isotype control mAb, whereas no significant differences were detected in TNFα production (Figure 4A, B and C). IL-6 production by SLE B cells was also examined in the T-B co-culture system. We observed a statistically significant reduction in the percentage of IL-6 producing SLE B cells in the presence of anti-SLAMF1 (Figure 4D) Finally, when we differentiated naïve SLE B cells towards ISC, the percentage of IgD-CD27+CD38^{hi}, as well as ANA production, were significantly reduced in the presence of anti-SLAMF1 compared to cells treated with isotype control (Figure 5).

SLAMF1 mAb prevents T cell-B cell conjugate formation *in vitro*

Considering that the presence of SLAMF molecules are important for cell-cell interactions, we hypothesized that SLAMF1 mAb could inhibit the formation of T-B conjugates (26). B cells isolated from the peripheral blood of healthy volunteers or SLE patients were co-cultured with autologous total T for 12h in the presence or absence of soluble SLAMF1 mAb. Conjugate frequencies were measured by flow cytometry. In the presence of SLAMF1 mAb, formation of CD4+CD19+ T-B conjugates, was decreased in both healthy individuals and patients with SLE (Figure 6).

Discussion

B cells in SLE are able to stimulate T cells via upregulation of co-stimulatory molecules and reciprocally, activated T cells provide substantial help to autoreactive B cells, thus driving autoantibody production (27). Inhibition of T-B cell interaction and co-stimulation to constraint activation of adaptive immunity is an appealing therapeutic approach for patients with SLE and other autoimmune diseases. Treatment with mAbs directed against co-stimulatory molecules, such as CD28, ICOS and CD40L have either already been attempted in patients with SLE with variable results in terms of efficacy and/or safety (5), or are currently under evaluation.

In this study we show that in the context of an *in vitro* T-B cell co-culture system, SLAMF1 ligation with a SLAMF1 mAb limits the frequency of IL-21 and IL-17A producing CD4+ T cells in healthy controls and, more importantly, in patients with SLE. Our data suggest that this inhibition occurs at two levels: (1) through an inhibition of T-B cell direct interaction and (2) through a modulation of B cell activation and BCR signaling, which affects the production of IL-6 by B cells.

Because SLAMF1 acts through homophilic interaction, ligation of SLAMF1 with a specific monoclonal antibody or its F(ab)$_2$ fragment, could affect cell-cell interaction and interfere with T cell activation, proliferation and differentiation. There are several observations in literature indicating that anti-SLAMF1 specific antibodies may interfere with SLAMF1 homotypic interactions. Initial *in vitro* studies conducted in human preactivated Th0, Th1 and Th2 clones suggested that SLAMF1 ligation with the use of a SLAMF1 mAb or its F(ab)$_2$ fragment, strongly upregulated IFNγ production and was even found to redirect Th2 clones to acquire a Th1-like phenotype (28, 29). On the contrary, antigen receptor-dependent production of IFNγ, following anti-CD3 activation, was inhibited on BI-141 T cell clone expressing a constitutively activated SLAMF1-SAP pathway (30). As SLAMF1 mAb clone

A12 recognizes the external V1 domain of SLAMF1, it is probable it may be disrupting SLAMF1-SLAMF1 homotypic interactions.

In support of the above, we noticed that in the presence of SLAMF1 mAb the formation of T-B conjugates was significantly reduced in normal individuals, and more importantly, in patients with SLE.

Then, we have further characterized the effect of SLAMF1 ligation on direct B cell activation and BCR signaling. In our co-culture system (and single cell assays) we noted a significant decrease in IL-6, but not on TNFα production, by B cells in healthy controls and SLE patients, when cells were incubated with SLAMF1 mAb.

Indeed, upon stimulation B cells are known to become a rich of source of cytokines that contribute to the outcome of the immune response. B cells may promote or inhibit T cell immune responses and differentiation via cytokine production (31). B cells from patients with SLE can produce significant amounts of IL-6 even in the absence of stimulation (32).

A fundamental function of IL-6 is to promote B cell maturation into Ig secreting cells in an autocrine/paracrine way (3). However, IL-6 also indirectly affects B cell progression to antibody producing cells as it drives CD4+ T cells to secrete IL-21, a cytokine with a major role in B cell antibody class switch process, differentiation and Ig secretion (18, 33). Data from studies in murine lupus and humans with SLE have shown that IL-6 plays an important role in sustaining B cell overactivity and autoantibody production and has a direct role in mediating tissue damage (23, 34). When we differentiated naïve B cells from healthy donors and patients with SLE, either alone or with autologous T cells, plasmablast formation and IgG and ANA production was reduced in the presence of anti-SLAMF1.

Failure of B cells to differentiate into Ig secreting cells was not due to a general unresponsiveness following treatment with SLAMF1 mAb. B cell proliferation, as well as upregulation of activation markers was not affected by SLAMF1 mAb. This is in agreement with previously published data demonstrating that B cell stimulation with SLAMF1 mAb, or its $F(ab)_2$ fragment, had no effect on proliferation and activation (13), whereas soluble and membrane forms of SLAMF1 protein promotes human B cell proliferation and Ig synthesis (13). Our data also indicate that SLAMF1 ligation with a mAb may have a direct effect on BCR-mediated signaling, as it diminished overall pTyr levels following BCR stimulation via SHP2 (13, 30).

IL-6 is known to drive Th17 differentiation (35) and promote IL-21 production by activated CD4+ T cells (36, 37). SLE patients are characterized by increased IL-21 and IL-17 levels in the serum that correlate with disease activity (38–40). Moreover IL-17 producing T cells are expanded in the periphery of SLE patients and are detected in the kidneys of patients with lupus nephritis, indicating a link between IL-17 production and lupus immunopathogenesis (38). When we cultured human naïve CD4+ T cells under Th17 polarizing conditions the presence of SLAMF1 mAb did not affect IL-17 production. On the contrary, we observed a reduction on IL-17A production by CD4+ T cells upon SLAMF1 ligation, detected only when T cells were co-cultured with autologous B cells, indicating a regulatory effect of the

SLAMF1 mAb on IL-17A production, either by directly inhibiting T-B cell interaction and/or suppressing IL-6 production by B cells.

Production of IL-21 mainly characterizes follicular helper CD4+ T cells (Tfh), a subset of CD4+ helper T cells that drives antigen-specific humoral immune responses within germinal centers (20, 21). IL-21-producing CD4+ T cells and CXCR5+ICOS+PD-1+ Tfh-like cells are expanded in the peripheral blood of SLE patients and are found in kidney sections from patients with lupus nephritis (41–44). In our study, SLAMF1 ligation did not interfere with the generation of Tfh-like CD4+ T cells *in vitro*. This is in agreement with previously published data using SLAM−/− mice which demonstrated that even though Tfh differentiation in germinal centers remained intact, cytokine production by Tfh was nevertheless affected (45).

Although total B-cell depletion therapies failed to deliver the expected results in the treatment of patients with SLE in controlled clinical trials (5), the role of B cells in the immunopathogenesis of lupus should not be overlooked and more targeted treatments need to be explored. The efficacy of tocilizumab, a human monoclonal antibody directed against the α chain of the IL-6 receptor (IL-6R), has already been assessed in an open label phase I dose-escalating study in patients with moderately active SLE (46). Treatment with tocilizumab resulted in improvement of SELENA-SLEDAI scores and reduction in dsDNA antibody titers. However, the concurrent development of neutropenia and severe infections posed a significant limiting factor to continuation of treatment. Therefore, newer and safer treatments targeting the IL-6 pathway should be explored. Moreover, SLAMF1 mAb inhibits T-B cell interaction which may have a beneficial effect in the context of autoimmunity.

In summary, we show that SLAMF1 co-engagement *in vitro* regulates IL-6 cytokine production and inhibits differentiation of naïve B cells towards immunoglobulin secreting cells in both healthy individuals and patients with SLE. More importantly we show that in a T-B cell co-culture system the presence of SLAMF1 mAb reduces T-B cell interaction, thus interfering with IL-21 and IL-17A production by T cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Dorner T, Jacobi AM, Lee J, Lipsky PE. Abnormalities of B cell subsets in patients with systemic lupus erythematosus. J Immunol Methods. 2011;363(2):187–97. [PubMed: 20598709]
2. Liossis SN, Kovacs B, Dennis G, Kammer GM, Tsokos GC. B cells from patients with systemic lupus erythematosus display abnormal antigen receptor-mediated early signal transduction events. J Clin Invest. 1996;98(11):2549–57. [PubMed: 8958217]

3. Kitani A, Hara M, Hirose T, Harigai M, Suzuki K, Kawakami M, et al. Autostimulatory effects of IL-6 on excessive B cell differentiation in patients with systemic lupus erythematosus: analysis of IL-6 production and IL-6R expression. Clin Exp Immunol. 1992;88(1):75–83. [PubMed: 1563109]
4. Nagafuchi H, Suzuki N, Mizushima Y, Sakane T. Constitutive expression of IL-6 receptors and their role in the excessive B cell function in patients with systemic lupus erythematosus. J Immunol. 1993;151(11):6525–34. [PubMed: 8245483]
5. Kyttaris VC. Novel Treatments in Lupus. Curr Rheumatol Rep 2017;19(3):10. [PubMed: 28265845]
6. Wakeland EK, Liu K, Graham RR, Behrens TW. Delineating the genetic basis of systemic lupus erythematosus. Immunity. 2001;15(3):397–408. [PubMed: 11567630]
7. Tsao BP. The genetics of human systemic lupus erythematosus. Trends Immunol. 2003;24(11):595–602. [PubMed: 14596884]
8. Romero X, Benitez D, March S, Vilella R, Miralpeix M, Engel P. Differential expression of SAP and EAT-2-binding leukocyte cell-surface molecules CD84, CD150 (SLAM), CD229 (Ly9) and CD244 (2B4). Tissue Antigens. 2004;64(2):132–44. [PubMed: 15245368]
9. Castro AG, Hauser TM, Cocks BG, Abrams J, Zurawski S, Churakova T, et al. Molecular and functional characterization of mouse signaling lymphocytic activation molecule (SLAM): differential expression and responsiveness in Th1 and Th2 cells. J Immunol. 1999;163(11):5860–70. [PubMed: 10570270]
10. You Y, Wang Z, Deng GH, Liu Y, Hao F. Detection and functional evaluation of −262A/T and −188A/G polymorphisms of SLAM gene in patients with systemic lupus erythematosus. J Rheumatol. 2010;37(11):2268–72. [PubMed: 20810499]
11. Linan-Rico L, Hernandez-Castro B, Doniz-Padilla L, Portillo-Salazar H, Baranda L, Cruz-Munoz ME, et al. Analysis of expression and function of the co-stimulatory receptor SLAMF1 in immune cells from patients with systemic lupus erythematosus (SLE). Lupus. 2015;24(11):1184–90. [PubMed: 25920347]
12. Karampetsou MP, Comte D, Kis-Toth K, Kyttaris VC, Tsokos GC. Expression patterns of signaling lymphocytic activation molecule family members in peripheral blood mononuclear cell subsets in patients with systemic lupus erythematosus. PLoS One. 2017;12(10):e0186073. [PubMed: 29020082]
13. Punnonen J, Cocks BG, Carballido JM, Bennett B, Peterson D, Aversa G, et al. Soluble and membrane-bound forms of signaling lymphocytic activation molecule (SLAM) induce proliferation and Ig synthesis by activated human B lymphocytes. J Exp Med. 1997;185(6):993–1004. [PubMed: 9091591]
14. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1982;25(11):1271–7. [PubMed: 7138600]
15. Comte D, Karampetsou MP, Kis-Toth K, Yoshida N, Bradley SJ, Mizui M, et al. Engagement of SLAMF3 enhances CD4+ T-cell sensitivity to IL-2 and favors regulatory T-cell polarization in systemic lupus erythematosus. Proc Natl Acad Sci U S A. 2016;113(33):9321–6. [PubMed: 27482100]
16. Comte D, Karampetsou MP, Yoshida N, Kis-Toth K, Kyttaris VC, Tsokos GC. SLAMF7 engagement restores defective effector CD8+ T cells activity in response to foreign antigens in systemic lupus erythematosus. Arthritis Rheumatol. 2017.
17. Karampetsou MP, Comte D, Kis-Toth K, Terhorst C, Kyttaris VC, Tsokos GC. Decreased SAP Expression in T Cells from Patients with Systemic Lupus Erythematosus Contributes to Early Signaling Abnormalities and Reduced IL-2 Production. J Immunol. 2016;196(12):4915–24. [PubMed: 27183584]
18. Ettinger R, Sims GP, Fairhurst AM, Robbins R, da Silva YS, Spolski R, et al. IL-21 induces differentiation of human naive and memory B cells into antibody-secreting plasma cells. J Immunol. 2005;175(12):7867–79. [PubMed: 16339522]
19. Ding BB, Bi E, Chen H, Yu JJ, Ye BH. IL-21 and CD40L synergistically promote plasma cell differentiation through upregulation of Blimp-1 in human B cells. J Immunol. 2013;190(4):1827–36. [PubMed: 23325890]

20. Gensous N, Schmitt N, Richez C, Ueno H, Blanco P. T follicular helper cells, interleukin-21 and systemic lupus erythematosus. Rheumatology (Oxford). 2016.
21. Blanco P, Ueno H, Schmitt N. T follicular helper (Tfh) cells in lupus: Activation and involvement in SLE pathogenesis. Eur J Immunol. 2016;46(2):281–90. [PubMed: 26614103]
22. Rethi B, Gogolak P, Szatmari I, Veres A, Erdos E, Nagy L, et al. SLAM/SLAM interactions inhibit CD40-induced production of inflammatory cytokines in monocyte-derived dendritic cells. Blood. 2006;107(7):2821–9. [PubMed: 16317102]
23. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. Nat Immunol. 2015;16(5): 448–57. [PubMed: 25898198]
24. Duddy ME, Alter A, Bar-Or A. Distinct profiles of human B cell effector cytokines: a role in immune regulation? J Immunol. 2004;172(6):3422–7. [PubMed: 15004141]
25. Wang N, Halibozek PJ, Yigit B, Zhao H, O'Keeffe MS, Sage P, et al. Negative Regulation of Humoral Immunity Due to Interplay between the SLAMF1, SLAMF5, and SLAMF6 Receptors. Front Immunol 2015;6:158. [PubMed: 25926831]
26. Cannons JL, Tangye SG, Schwartzberg PL. SLAM family receptors and SAP adaptors in immunity. Annu Rev Immunol. 2011;29:665–705. [PubMed: 21219180]
27. Moulton VR, Tsokos GC. T cell signaling abnormalities contribute to aberrant immune cell function and autoimmunity. J Clin Invest. 2015;125(6):2220–7. [PubMed: 25961450]
28. Cocks BG, Chang CC, Carballido JM, Yssel H, de Vries JE, Aversa G. A novel receptor involved in T-cell activation. Nature. 1995;376(6537):260–3. [PubMed: 7617038]
29. Aversa G, Chang CC, Carballido JM, Cocks BG, de Vries JE. Engagement of the signaling lymphocytic activation molecule (SLAM) on activated T cells results in IL-2-independent, cyclosporin A-sensitive T cell proliferation and IFN-gamma production. J Immunol. 1997;158(9): 4036–44. [PubMed: 9126961]
30. Latour S, Gish G, Helgason CD, Humphries RK, Pawson T, Veillette A. Regulation of SLAM-mediated signal transduction by SAP, the X-linked lymphoproliferative gene product. Nat Immunol. 2001;2(8):681–90. [PubMed: 11477403]
31. Bao Y, Cao X. The immune potential and immunopathology of cytokine-producing B cell subsets: a comprehensive review. J Autoimmun. 2014;55:10–23. [PubMed: 24794622]
32. Kitani A, Hara M, Hirose T, Norioka K, Harigai M, Hirose W, et al. Heterogeneity of B cell responsiveness to interleukin 4, interleukin 6 and low molecular weight B cell growth factor in discrete stages of B cell activation in patients with systemic lupus erythematosus. Clin Exp Immunol. 1989;77(1):31–6. [PubMed: 2788539]
33. Ozaki K, Spolski R, Ettinger R, Kim HP, Wang G, Qi CF, et al. Regulation of B cell differentiation and plasma cell generation by IL-21, a novel inducer of Blimp-1 and Bcl-6. J Immunol. 2004;173(9):5361–71. [PubMed: 15494482]
34. Tackey E, Lipsky PE, Illei GG. Rationale for interleukin-6 blockade in systemic lupus erythematosus. Lupus. 2004;13(5):339–43. [PubMed: 15230289]
35. Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F. Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. Nat Immunol. 2007;8(9):942–9. [PubMed: 17676045]
36. Dienz O, Eaton SM, Bond JP, Neveu W, Moquin D, Noubade R, et al. The induction of antibody production by IL-6 is indirectly mediated by IL-21 produced by CD4+ T cells. J Exp Med. 2009;206(1):69–78. [PubMed: 19139170]
37. Diehl SA, Schmidlin H, Nagasawa M, Blom B, Spits H. IL-6 triggers IL-21 production by human CD4+ T cells to drive STAT3-dependent plasma cell differentiation in B cells. Immunol Cell Biol. 2012;90(8):802–11. [PubMed: 22491065]
38. Crispin JC, Tsokos GC. IL-17 in systemic lupus erythematosus. J Biomed Biotechnol. 2010;2010:943254. [PubMed: 20379379]
39. Wang L, Zhao P, Ma L, Shan Y, Jiang Z, Wang J, et al. Increased interleukin 21 and follicular helper T-like cells and reduced interleukin 10+ B cells in patients with new-onset systemic lupus erythematosus. J Rheumatol. 2014;41(9):1781–92. [PubMed: 25028374]

40. Lan Y, Luo B, Wang JL, Jiang YW, Wei YS. The association of interleukin-21 polymorphisms with interleukin-21 serum levels and risk of systemic lupus erythematosus. Gene. 2014;538(1):94–8. [PubMed: 24434811]

41. Chang A, Henderson SG, Brandt D, Liu N, Guttikonda R, Hsieh C, et al. In situ B cell-mediated immune responses and tubulointerstitial inflammation in human lupus nephritis. J Immunol. 2011;186(3):1849–60. [PubMed: 21187439]

42. Zhang X, Lindwall E, Gauthier C, Lyman J, Spencer N, Alarakhia A, et al. Circulating CXCR5+CD4+helper T cells in systemic lupus erythematosus patients share phenotypic properties with germinal center follicular helper T cells and promote antibody production. Lupus. 2015;24(9):909–17. [PubMed: 25654980]

43. Terrier B, Costedoat-Chalumeau N, Garrido M, Geri G, Rosenzwajg M, Musset L, et al. Interleukin 21 correlates with T cell and B cell subset alterations in systemic lupus erythematosus. J Rheumatol. 2012;39(9):1819–28. [PubMed: 22859347]

44. Choi JY, Ho JH, Pasoto SG, Bunin V, Kim ST, Carrasco S, et al. Circulating follicular helper-like T cells in systemic lupus erythematosus: association with disease activity. Arthritis Rheumatol. 2015;67(4):988–99. [PubMed: 25581113]

45. Yusuf I, Kageyama R, Monticelli L, Johnston RJ, Ditoro D, Hansen K, et al. Germinal center T follicular helper cell IL-4 production is dependent on signaling lymphocytic activation molecule receptor (CD150). J Immunol. 2010;185(1):190–202. [PubMed: 20525889]

46. Illei GG, Shirota Y, Yarboro CH, Daruwalla J, Tackey E, Takada K, et al. Tocilizumab in systemic lupus erythematosus: data on safety, preliminary efficacy, and impact on circulating plasma cells from an open-label phase I dosage-escalation study. Arthritis Rheum. 2010;62(2):542–52. [PubMed: 20112381]

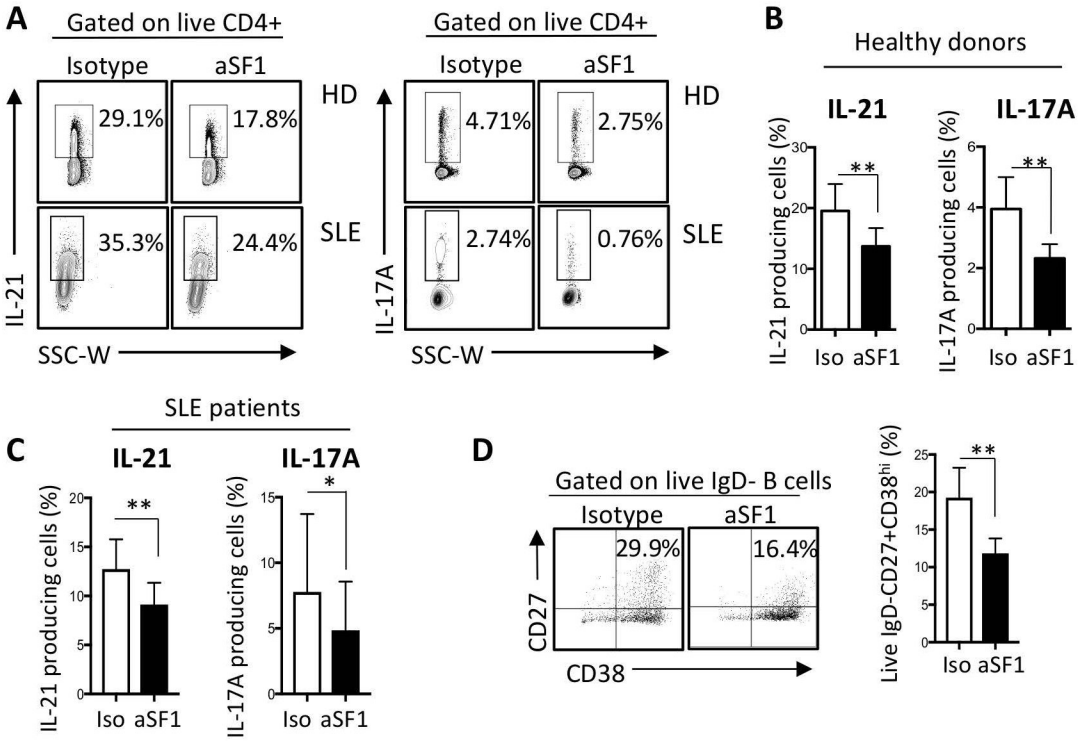

Figure 1. SLAMF1 mAb decreases IL-21 and IL-17A cytokine production from healthy and SLE CD4+ T cells and impairs plasmablast formation in a T-B cell co-culture system in vitro.

Peripheral blood B cells from healthy donors (n=8–10) or patients with SLE (n=9) were pre-stimulated with a $F(ab)_2$ anti-IgM for 48hr and were plated with autologous total T cells at 1:1 ratio under anti-CD3/CD28 stimulation for 5 days in the presence of soluble SLAMF1 mAb or an isotype control. On day 5, cells were re-stimulated with PMA and ionomycin for 6 h. Cytokine production by CD4+ T cells was evaluated by intracellular flow cytometry. (A) Representative experiments for IL-21 and IL-17A production in healthy individuals (upper panels) and SLE patients (bottom panels). Cumulative results are shown in (B) for healthy donors and (C) for SLE patients. (D) Pre-stimulated naïve B cells (n=12) were co-cultured with total T cells under anti-CD3/CD28 stimulation for 7 days. Representative flow plot (left panel) and cumulative results (right panel) of the frequency of plasmablast (defined as CD19+IgD-CD27+CD38hi). Results are expressed as percentage (%) (mean ± SEM). *P < 0.05, **P < 0.01, ***P < 0.001. Iso=isotype control; aSF1= SLAMF1 mAb; HD=Healthy Donor; SLE=patient with SLE

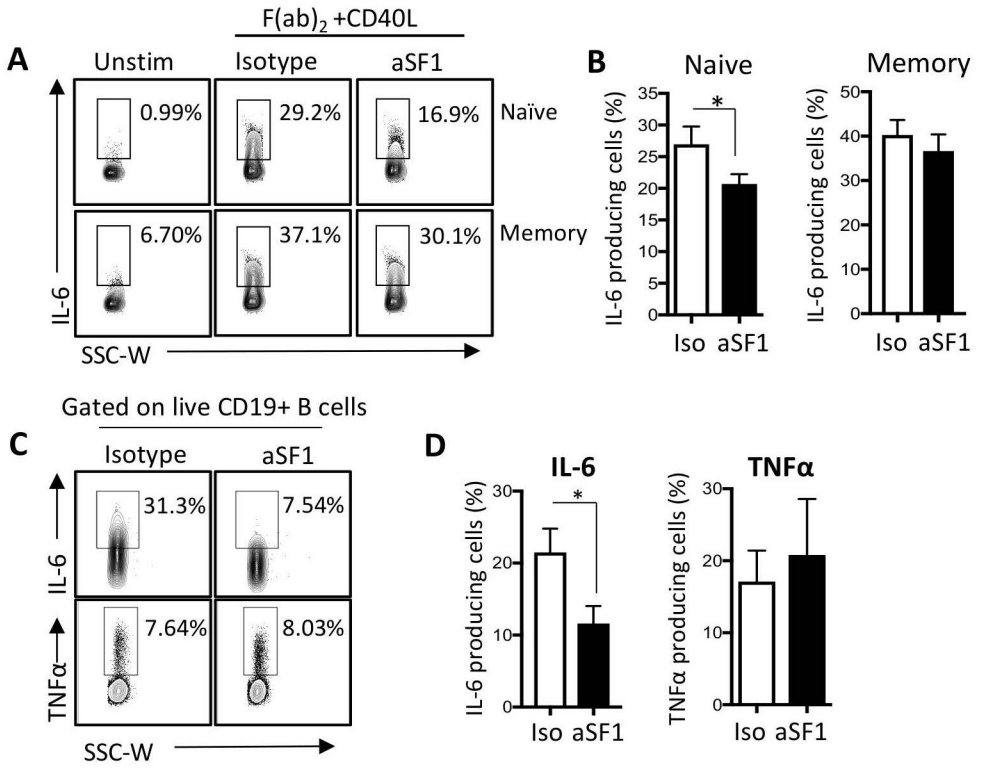

Figure 2. Engagement of SLAMF1 with SLAMF1 mAb reduces IL-6 production by healthy naïve B cells.

Naïve and memory B cells were isolated from the peripheral blood of healthy donors (n=7) and were stimulated with $F(ab)_2$ anti-IgM and CD40L for 72h in the presence of anti-SLAMF1. Cells were then re-stimulated with PMA and ionomycin for 6h. (A) representative experiment and (B) cumulative results for IL-6 production (mean % ± SEM). Total B cells were pre-stimulated with $F(ab)_2$ anti-IgM for 48hr and were plated with autologous total T cells at 1:1 ratio under anti-CD3/CD28 stimulation for 5 days in the presence of soluble SLAMF1 or an isotype control. On day 5, cells were re-stimulated with PMA and ionomycin for 6 h. (C) Representative experiment and (D) cumulative results for IL-6 and TNFα expression (mean % ± SEM). *P < 0.05, **P < 0.01, ***P < 0.001. Iso=isotype control; aSF1= SLAMF1 mAb

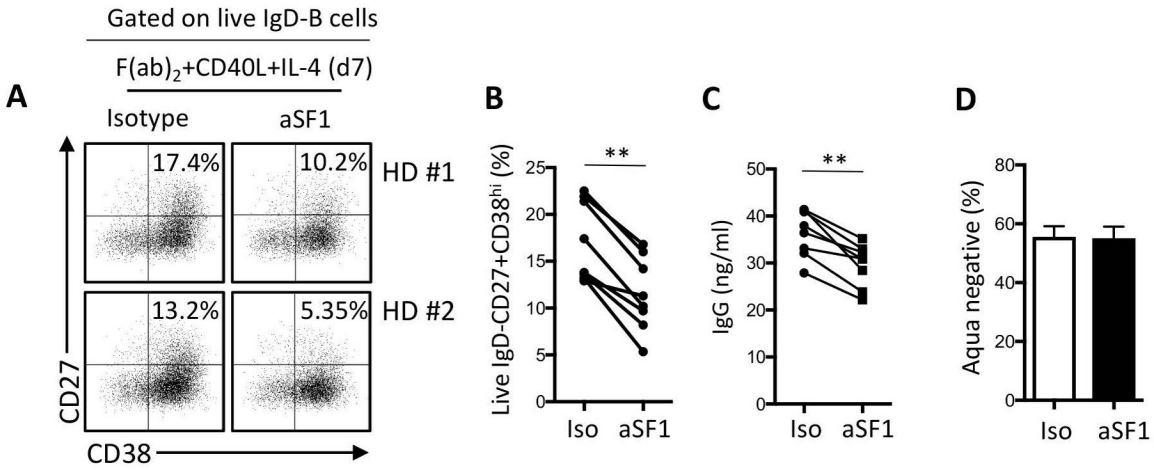

Figure 3. SLAMF1 ligation inhibits naïve B cell differentiation towards plasmablasts and prevents IgG secretion.

Naïve B cells were isolated from the peripheral blood of healthy donors (n=8). Cells were stimulated with F(ab')$_2$ anti-IgM and CD40L in the presence or absence of SLAMF1 mAb or an isotype control Ab. IL-4 (10ng/ml) was added on day 1 and was replenished every 3d. Plasmablast formation (IgD-CD27+CD38+) was assessed with flow cytometry on day 7. Two representative experiments are shown in (A) and cumulative results are shown in (B) and are expressed as percentage (%) (mean ± SEM). (C) IgG production was assessed in culture supernatants on day 12 by ELISA. (D) B cell survival in culture was evaluated on day 7 as the percentage of Aqua negative cells (mean ± SEM). *P < 0.05, **P < 0.01, ***P < 0.001. HD=Healthy Donor; Iso=Isotype control; aSF1= SLAMF1 mAb

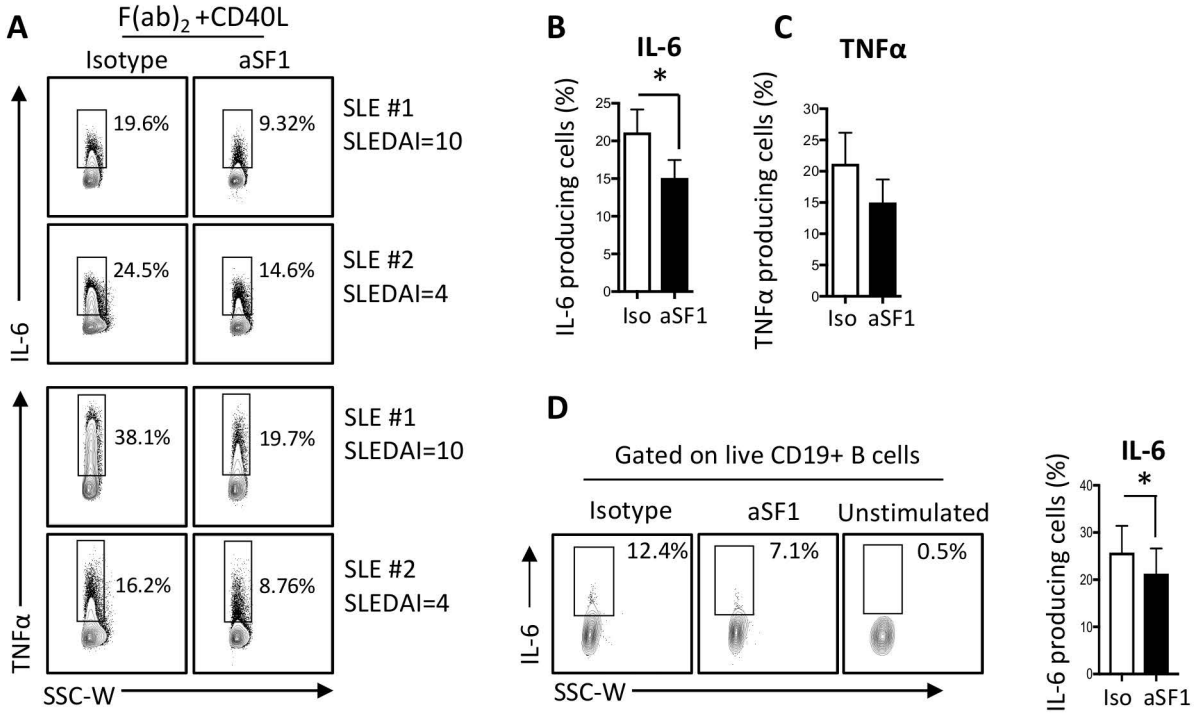

Figure 4. Treatment of SLE B cells with SLAMF1 mAb *in vitro* decreases IL-6 production.

B cells were isolated from the peripheral blood of SLE patients (n=6) and were stimulated with $F(ab)_2$ anti-IgM and CD40L for 72h in the presence of SLAMF1 mAb or isotype control. Cells were then re-stimulated with PMA and ionomycin for 6h. IL-6 and TNFα production was evaluated by intracellular flow cytometry. Representative flow plots from two SLE patients are shown in (A) and cumulative results are shown in (B). (D) Peripheral blood B cells from SLE patients (n=6) were pre-stimulated with a $F(ab)_2$ anti-IgM for 48hr and were plated with autologous total T cells at 1:1 ratio under anti-CD3/CD28 stimulation for 5 days in the presence of soluble SLAMF1 mAb or an isotype control. On day 5, cells were re-stimulated with PMA and ionomycin for 6 h. IL-6 production by B cells was evaluated by intracellular flow cytometry. Representative flow panel (left panel) and cumulative results (right panel). Results are expressed as percentage (%) (mean ± SEM). *P < 0.05, **P < 0.01, ***P < 0.001. SLE= patient with SLE; Iso= isotype control; aSF1= SLAMF1 mAb

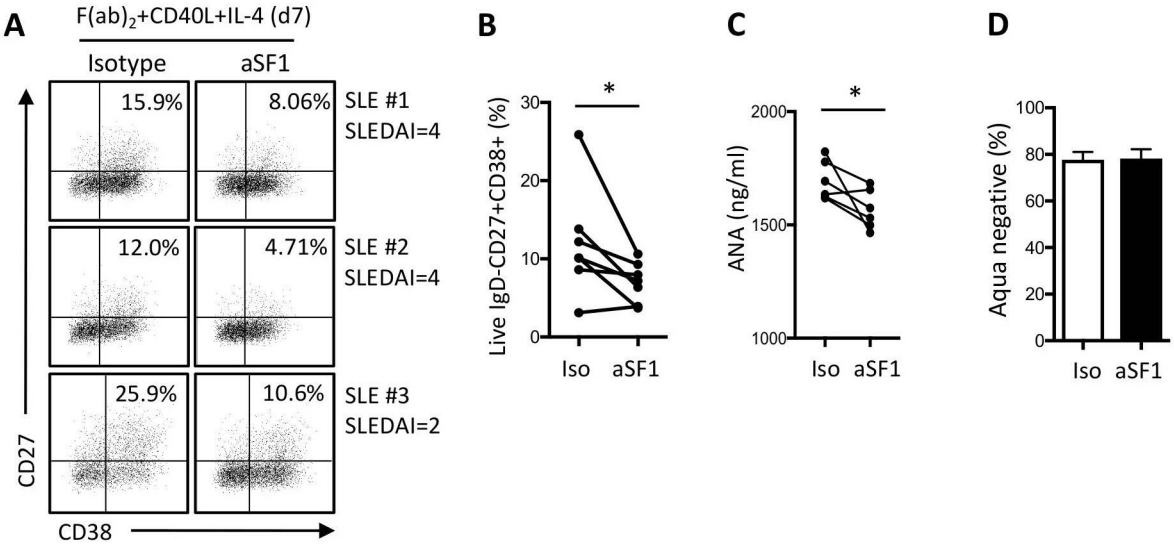

Figure 5. SLAMF1 ligation prevents naïve B cell differentiation towards plasmablasts and inhibits ANA production.

Naïve B cells were isolated from the peripheral blood of patients with SLE (n=6). Cells were stimulated with $F(ab)_2$ anti-IgM and CD40L in the presence or absence of SLAMF1 mAb or an isotype control. IL-4 (10ng/ml) was added on day 1 and was replenished every 3 days. (A) Representative flow plots of plasmablast formation (IgD-CD27+CD38+) from naïve B cells of three patients with SLE. Cumulative results are shown in (B) and are expressed as percentage (%) (mean ± SEM). (C) Naïve SLE B cells were maintained in culture for 12 days and ANA production was evaluated in culture supernatants by ELISA. (D) B cell survival in culture was evaluated on day 7 as the percentage (%) of Aqua negative cells (mean ± SEM). *P < 0.05, **P < 0.01, ***P < 0.001. SLE= patient with SLE; Iso= isotype control; aSF1= SLAMF1 mAb

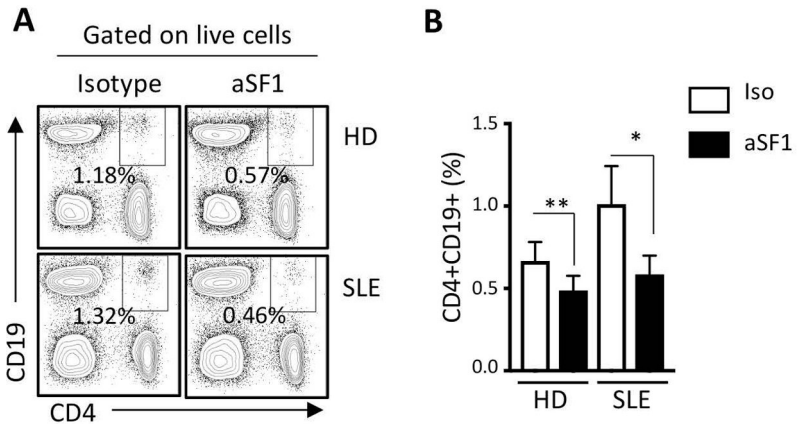

Figure 6. SLAMF1 mAb prevents T-B cell conjugate formation *in vitro*.

Total B cells isolated from the peripheral blood of healthy individuals (n=8) and patients with SLE (n=5) were pre-stimulated with $F(ab')_2$ anti-IgM for 48h and were then co-cultured for 12 h with autologous total T cells in 96 U-bottom well plates (pre-coated with anti-CD3/CD28) in the presence of SLAMF1 mAb or an isotype control. Cells were stained with anti-CD4 and anti-CD19 and conjugate formation (CD4+CD19+) was assessed by flow cytometry. (A) Representative experiments from a healthy donor (upper plots) and a patient with SLE (bottom plots). Cumulative results are shown in (B) and are expressed as percentage (%) of CD4+CD19+ (mean ± SEM). *P < 0.05, **P < 0.01, ***P < 0.001. HD=Healthy Donor; SLE= patient with SLE; Iso= Isotype control; aSF1= SLAMF1 mAb