Published in final edited form as:

Arthritis Rheumatol. 2017 May ; 69(5): 1035–1044. doi:10.1002/art.40038.

SLAMF7 engagement restores defective effector CD8+ T cells activity in response to foreign antigens in systemic lupus erythematosus

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Abstract

Objective—Effector CD8+ T cell function is impaired in SLE and associated with compromised ability to fight infections. SLAMF7 engagement has been shown to enhance NK cell degranulation. Thus, we characterized the expression and function of SLAMF7 on CD8+ T cells subsets isolated from peripheral blood of SLE patients and healthy subjects.

Methods—CD8+ T cell subset distribution, SLAMF7 expression and cytolytic enzyme expression (perforin, GzmA, GzmB) were monitored on cells isolated from SLE patients and healthy controls by flow cytometry. CD107a expression and IFNγ production in response to viral antigenic stimulation were assessed by flow cytometry in the presence or absence of an anti-SLAMF7 antibody. The antiviral cytotoxic activity in response to SLAMF7 engagement was determined using a flow cytometry-based assay.

Results—The distribution of CD8+ T cell subsets is altered in the peripheral blood of SLE patients with decreased effector cell subpopulation. Memory CD8+ T cells from SLE patients display decreased amounts of SLAMF7, a surface receptor that characterizes effector CD8+ T cells. Ligation of SLAMF7 increases CD8+ T cell degranulation capacity and the percentage of IFNγ-producing cell in response to antigen challenge in SLE and healthy controls. Moreover, SLAMF7 engagement promotes cytotoxic lysis of target cells in response to viral antigenic stimulation.

Conclusion—Activation of SLAMF7 through a specific mAb restores defective SLE effector CD8+ T cells function in response to viral antigens and represents a potential therapeutic option in SLE.

Keywords

SLE; T-lymphocyte cells; Autoimmune disease; Cytotoxicity

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Profound T cell abnormalities characterize T cells from patients with systemic lupus erythematosus (SLE) [1, 2]. Even though CD4+ T cells have been extensively studied, the role of CD8+ T in SLE cells is less well understood. Studies suggest a defect in cytotoxic CD8+ T cells activity in SLE and an accelerated disease in lupus-prone mice in which cytotoxic CD8+ T cells major killing pathways have been deleted [3–5]. Additionally, SLE has been associated with defective cytotoxic responses against foreign antigens, an abnormality considered to contribute to the increased rate of infections, the leading cause of mortality in SLE [6–8].

Signaling lymphocytic activation molecules family members (SLAMF) are type I transmembrane glycoprotein cell surface receptors that deliver downstream signals upon their engagement and modulate the magnitude of the immune response [9]. SLAMF7 (CS1, CRACC, CD319) is expressed on NK cells, NK T cells, CD8+ T cells, and subsets of B cells [10]. SLAMF7 acts as a self-ligand through the binding of the N-terminal Ig domain of SLAMF7 [10]. In NK cells, SLAMF7 uses the adaptor protein Ewing's sarcoma's/FLI1-activated transcript 2 (EAT-2) to promote downstream signaling, while other SLAMF molecules preferentially bind to SLAMF associated protein (SAP) [11]. SLAMF7 has been extensively studied in patients with multiple myeloma, a malignant disorder of plasma cells. A monoclonal antibody (mAb) directed against SLAMF7 has been shown to eliminate myeloma cells by activating NK cells and by stimulating antibody-dependent cellular cytotoxicity [12–14]. SLAMF molecules, and their downstream adaptor protein SAP, have been suggested to play a role in SLE pathogenesis and to represent a potential therapeutic target [15–17]. However, limited data are available concerning the role of SLAMF7 in SLE and some studies have proposed that SLAMF7 can play a role in the pathogenesis of the disease [18, 19]. The gene encoding SLAMF7 is located on chromosome 1 within 1q23 locus, a region known to be associated with an increased susceptibility for SLE [20]. SLAMF7 expression has been reported altered in NK cells, plasmacytoid dendritic cells and a subset of B cells in SLE patients [18, 19], but the role and function of SLAMF7 in SLE CD8+ T cells has not been established.

In the present study, we show that the frequency of effector CD8+ T cells is decreased in the peripheral blood of SLE patients compared to healthy controls. In addition, we demonstrate that the expression of SLAMF7 is decreased in SLE CD8+ T cells and that the presence of SLAMF7 characterizes cytotoxic CD8+ T cells. More importantly, engagement of SLAMF7 with a specific monoclonal antibody (mAb) enhances degranulation and cytotoxicity of CD8+ T cells in response to viral peptides. Despite the reduced SLAMF7 expression on SLE CD8+ effector T cells, activation of SLAMF7 via an anti-SLAMF7 mAb restores the defective SLE effector CD8+ T cells function in response to viral antigens and therefore, represents a potential therapeutic option to enhance immune response against foreign antigen.

MATERIAL AND METHODS

Patients and controls

SLE patients (n=79) were diagnosed according to the American College of Rheumatology classification criteria [21], and were recruited from the Division of Rheumatology at Beth

Israel Deaconess Medical Center. Age-, sex-, and ethnicity-matched healthy individuals were chosen as controls. Disease activity score was measured using the SLE Disease Activity Index (SLEDAI) scoring system (Table 1). Informed consent was obtained as approved by the Institutional Review Board after the nature and possible consequences of the studies were explained, in compliance with the Helsinki Declaration.

Cell isolation

Peripheral blood mononuclear cells (PBMC) were enriched by density gradient centrifugation (Lymphocyte Separation Medium, Corning Life Sciences). T cells were isolated by negative selection (RosetteSep, Stem Cell Technologies).

Flow cytometry

Cells were stained for dead cells (Zombie Aqua/NIR Fixable Viability Kit; Biolegend), and then labeled for surface antibodies. Cells were permeabilized (Cytofix/Cytoperm, BD Biosciences) and stained for cytokines and/or cytotoxic enzymes. Data were acquired on a LSR II SORP (BD Biosciences) and analyzed using FlowJo (version 10.1r5, FlowJo Enterprise).

Degranulation assay

Cryopreserved PBMC (2×10^6) were stimulated for 6 h in 1ml of complete RPMI (RPMI-1640 supplemented with 10% FBS, 100mg/ml streptomycin and 100U/ml penicillin) in the presence of Golgiplug (1μl/ml; BD) and Golgistop (1μl/ml; BD), CD107a (4μl/ml; Biolegend), and CEF (CMV-EBV-Flu) peptide mix (3μg/ml) or Staphyloccal enterotoxin B (SEB; 1μg/ml) as a positive control. When indicated, cells were also stimulated with anti-SLAMF7 mAb or a mouse IgG2b Isotype control antibody (5μg/ml, Biolegend), and a goat anti-mouse IgG cross-linker (EMD Millipore). At the end of the stimulation, cells were stained for live-dead cells (Zombie NIR Fixable Viability Kit, Biolegend), then stained for surface antibodies with CD3 (BD Horizon BUV395 anti-human CD3; BD), CD4 (PerCP eFluor 710 anti-human CD4; eBioscience), CD8 (PerCP anti-human CD8; Biolegend), fixed / permeabilized (Cytofix/cytoperm; BD) and stained for IFNγ (Alexa Fluor 647 anti-human IFNγ; Biolegend). Cells were then acquired on an LSRII SORP and analyzed by using FlowJo (version 10.1r5, FlowJo Enterprise).

Flow cytometry-based assay to measure CD8+ T cell cytotoxic activity in response to anti-SLAMF7 mAb treatment

Freshly isolated PBMC were cultured in the presence of CEF peptide mix (5μg/ml) and IL-2 (20IU/ml; Peprotech) for 6 days in complete RPMI. On day 3, half cell culture medium was replaced with fresh complete RPMI. On day 6, effector CD8+ T cells were isolated by negative selection using magnetic sorting (human CD8+ T cells isolation kit, Miltenyi Biotec). Cells were re-suspended at a concentration of 10^6/ml. 6 serial dilutions were performed. From each dilution, half cells were treated with anti-SLAMF7 mAb (5μg/ml; Biolegend) and a goat anti-mouse IgG cross-linker (EMD Millipore), while the other half was treated with an IgG isotype control (Biolegend). 100 μl of each CD8+ T cells preparation were seeded in duplicate in a 96-well U bottom plate.

Freshly isolated autologous CD4+ T sorted by magnetic negative selection (human CD4+ T cells isolation kit, Miltenyi Biotec) were used as target. Half CD4+ T cells were labeled with CFSE 0.2μM (CFSE high) and were pulsed with CEF peptide (5μg/ml) for 45 min at 37°C in complete RPMI. Other half of CD4+ T cells were labelled with CFSE 0.02μM (CFSE low) and were used as control to determine unspecific cell death background. CFSE high and low CD4+ T cells were mixed at 1:1 ratio, and re-suspended at a concentration of 2×10^5 cells/ml. 100μl of target CD4+ T cells were added to each dilution of CD8+ T cells. Cells mixtures were incubated for 6h at 37°C.

After incubation, cells were stained for live-dead cells (Zombie Aqua Fixable Viability Kit; Biolegend), CD4 (APC anti-human CD4 Ab; Biolegend) and CD8 (APC/Cy7 anti-human CD8 Ab; Biolegend) and analyzed by Flow Cytometry. Aqua+ defines dead cells. Percentage of target cells lysis in response to effector CD8+ T cells was expressed as [% of Aqua+ pulsed CD4+ T cells] – [% Aqua+ un-pulsed CD4+ T cells].

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

Total RNA was extracted by RNeasy Mini Kit (Qiagen). Reverse transcription was performed from 500ng total RNA using the High Capacity cDNA Archive Kit (Applied Biosystems). Q-RT-PCR was performed with 40 cycles at 94°C (12s) and 60°C (60s) using SYBR Green (LightCycler 480 SYBR Green I Master, Roche). The comparative Ct method was used to quantify transcripts relative to the endogenous control gene β-actin. Primer sequences were as follows: EAT-2, forward 5′- TGTGCCTCTGTGTCTCGTTTA-3′and reverse 5′- ACCACCATCCCCTGATTTGG-3′; β-actin, forward 5′- AGAGCTACGAGCTGCCTGAC-3′ and reverse 5′- AGCACTGTGTTGGCGTACAG-3′.

Statistical analysis

Statistical analysis was performed using Student’s t-test, corrected with the Holm-Sidak method. For multiple comparisons, statistical analysis was performed using one-way Anova, followed by *post hoc* analysis with Tukey's test. Statistical analyses and illustrations were performed using FlowJo (version 10.1r5, FlowJo Enterprise), and GraphPad Prism (version 6). Statistical significance was reported as follows: *p<0.05, **p<0.01, ***p<0.001.

RESULTS

Skewed distribution of CD8+ T cell subsets in peripheral blood of SLE patients

We screened the distribution of CD8+ T cell subsets in the peripheral blood of 45 SLE patients with varying disease activity and 41 healthy controls by assessing cell surface expression of CCR7 and CD45RA. This allowed us to distinguish four differentiated CD8+ T cells subsets, i.e. naïve (CCR7+CD45RA+), central memory (CM, CCR7+CD45RA−), effector memory (EM, CCR7−CD45RA−) and terminally differentiated effector memory (TDEM, CCR7−CD45RA+) (figure 1A) [22]. Frequency of EM CD8+ T cells was reduced in SLE compared to healthy controls, while cells expressing markers of naïve CD8+ T cells were enriched (figure 1B). Moreover, skewed distribution of CD8+ T cells correlated with disease activity, because patients with active disease (as defined by SLEDAI ≥4) displayed a statistically significant decrease of EM CD8+ T cells and increase of naïve CD8+ T cells

(figure 1C and E) compared to patients with inactive disease (SLEDAI <4). CM CD8+ T cells were also decreased in SLE patients but to a lesser degree (figure 1D). We observed a statistically significant linear correlation between decreased number of TDEM CD8+ T cells and SLEDAI score, which is associated with an increased frequency of CD8+ T cells expressing naïve markers (Supplementary figure 1). Of note, there was no difference in the percentage of total CD8+ T cells between SLE patients and controls (Supplementary figure 2).

SLAMF7 is decreased in SLE CD8+ T cells

Expression of SLAMF7 was examined in T cells isolated from SLE (n=16 to 27) patients and healthy controls (n=13 to 22). SLAMF7 is mostly expressed by CD8+ T cells, as well as double negative (DN) T cells (figure 2A and supplementary figure 3A), a T cell subset that expresses CD3 but has lost CD4 and CD8 expression. In contrast, expression of SLAMF7 on CD4+ T cells is very low. Expression of SLAMF7 was found reduced in CD8+ T and DN cells isolated from SLE patients compared to healthy subjects (figure 2A). Reduced SLAMF7 expression correlated with disease activity because SLE patients with active disease display less SLAMF7 expression than inactive patients (figure 2B and supplementary figure 4). Because the distribution of CD8+ T cell subsets is altered in SLE patients, we examined the expression of SLAMF7 on each CD8+ T cell differentiated subsets (naïve, CM, EM, TDEM). We observed that SLAMF7 expression increases during cell differentiation: its expression is low in naïve CD8+ T cells, whereas most EM and TDEM express SLAMF7 (figure 2C and supplementary figure 3B). Importantly, each of the CD8+ T cells differentiated subset from SLE patients displayed decreased amounts of SLAMF7 compared to controls (figure 2C). Decreased SLAMF7 expression was prominent among CD8+ T cells isolated from SLE patients with active disease compared to those with inactive disease (figure 2D, Supplementary figure 3C and supplementary figure 4). Because it has been previously reported that SLAMF7 binds to EAT-2 in NK cells to transmit downstream signaling, we assessed the expression of EAT-2 in CD8+SLAMF7− and CD8+SLAMF7+ T cells by qPCR. We were not able to detect any EAT-2 gene expression in CD8+ T cells (Supplementary figure 5).

In summary, SLAMF7 expression increases over CD8+ T cells differentiation and is mostly expressed by EM and TDEM CD8+ T cells. Moreover, SLAMF7 expression is decreased in SLE CD8+ T cells compared to healthy controls.

SLAMF7 is expressed by effector CD8+ cytotoxic T cells

To further examine the properties of SLAMF7+ CD8+ T cells, we assessed the co-expression of SLAMF7 and cytolytic enzymes (perforin, granzyme (Gzm)A and GzmB) in CD8+ T cells isolated from peripheral blood of SLE patients (n=18) and controls (n=15). Expression of cytolytic enzyme perforin, GzmA and GzmB was found to be restricted to SLAMF7+ CD8+ T cells (figure 3A and B). As previously described [7], an altered expression of cytolytic enzymes was found in SLE patients in comparison to healthy controls: perforin and GzmB expression were reduced, while GzmA expression was slightly increased.

In sum, SLAMF7 expression defines effector cytotoxic CD8+ T cells and its expression is decreased in SLE and is associated with an altered expression of CD8+ T cells cytotoxic enzymes. Expression of cytolytic enzymes was also examined in differentiated subsets of CD8+ T cells. As expected, expression of cytolytic enzymes is higher in effector (especially EM and TDEM CD8+ T cells) than in naïve CD8+ T cells. When SLE and controls were compared, we observed the same alteration as in total CD8+ T cells: there is a trend toward a decreased expression of perforin and GzmB, while expression of GzmA is increased in SLE EM and TDEM CD8+ T subsets (Supplementary figure 6).

SLAMF7 engagement restores SLE CD8+ T cells effector function in response to viral antigens

To examine the degranulation capacity of CD8+ T cells in response to viral antigens, we stimulated PBMC isolated from SLE patients (n=8) or healthy individuals (n=8) with a mix of MHC class I restricted T cell epitopes from human CMV, EBV and influenza virus (CEF) [7, 23, 24]. PBMC were stimulated with or without anti-SLAMF7 mAb. Cells were stained for CD3, CD8, CD107a (Lysosomal-associated membrane protein 1(LAMP-1), a marker of degranulation) and IFNγ and we examined the proportion CD8+ T cells that are CD107a / IFNγ double positive, a population that has been associated with anti-viral protective immunity [25, 26]. Staphylococcal enterotoxin B (SEB) served as a positive control.

SLAMF7 engagement with a specific mAb increased CD8+ T cells response to CEF stimulation by enhancing the frequency of CD8+ CD107a / IFNγ double positive T cells (figure 4). Compared to healthy controls, SLE patients have fewer CD8+ CD107a / IFNγ double positive T cells in response to CEF (figure 4). In the presence of anti-SLAMF7 mAb, the frequency of SLE CD8+ CD107a / IFNγ double positive T cells in response to CEF stimulation was restored to levels comparable to the one observed in healthy control (figure 4).

SLAMF7 engagement enhances cytolytic activity of CD8+ T cells in response to viral antigens

Because SLAMF7 engagement favors CD8+ T cells degranulation upon viral antigenic stimulation, we asked whether SLAMF7 activation can trigger CD8+ T cell-mediated lysis of target cells. We performed a flow cytometry-based assay to measure the capacity of CEF-multi-specific CD8+ T cells to kill autologous target CD4+ T cells loaded with cognate CEF peptide (modified from reference [27]). Non-loaded autologous CD4+ T cells were used as target cell death background control. Expanded effector CD8+ T cells were co-cultured at different ratios with target cells. CEF-loaded target CD4+ T cell lysis was augmented in the presence of increasing numbers of CEF antigen multi-specific effector CD8+ T cells (figure 5A, B and C and Supplementary figure 7). When CEF-expanded CD8+ T cells were activated with anti-SLAMF7 mAb prior to co-culture with target CD4+ T cells, killing of CEF loaded autologous target CD4+ T cells was significantly increased compared to cells treated with an isotype control antibody (figure 5A, B and C). In SLE patients, treatment with anti-SLAMF7 mAb – compared to treatment with an isotype control - also enhanced effector CD8+ T cells lysis of target cells in response to CEF peptide (figure 5C). SLAMF7 mediated enhancement of target cells lysis in response to CEF peptides was similar in SLE

patients and controls (figure 5B and C). In sum, SLAMF7 engagement enhances CD8+ T cytotoxicity in response to viral antigenic peptides.

DISCUSSION

In this study, we show that CD8+ T cells differentiated subset distribution is altered in peripheral blood from SLE patients. Skewed CD8+ T cells distribution is mainly characterized by a decreased number of EM CD8+ T cells, a CD8+ subset which produces effector cytokines – such as IFNγ - and rapidly become cytotoxic upon antigenic challenge [28]. This subset is poised for a rapid response at the onset of a microbial infection [29]. CM CD8+ T cells are also decreased in the peripheral blood of active SLE patients. CM mainly home to lymph node and their main role is to proliferate and generate effector cells upon antigen stimulation [28]. The reason why CD8+ T cells distribution is altered in SLE remains unknown. One explanation could be that effector CD8+ tend to become double negative T cells (CD3+CD4−CD8− T cells) – a T cell subsets that has been linked to SLE immunopathogenesis - in the context of continuous antigenic stimulation and chronic inflammation [30].

Reduced frequency of EM and CM CD8+ T cells in patients with SLE may contribute to the increased infection rates that characterize lupus patients [6–8]. Furthermore, effector function of CD8+ T cells (cytotoxic activity and cytokines production), which is considered a hallmark of anti-viral protective immunity, is compromised in SLE [7, 25]. Expression of the cytolytic enzymes perforin, GzmA and GzmB is dysregulated in SLE CD8+ T cells compared with CD8+ cells isolated from healthy individuals. CD8+ T cells from patients with SLE display significantly decreased levels of perforin and GzmB, whereas levels of GzmA appear to be slightly elevated. Perforin and GzmB are associated with cytotoxic activity of effector CD8+ T cells [31]. On the other hand, GzmA is much less cytotoxic than GzmB and is more likely involved in pro-inflammatory process, as it activates monocytes to secrete inflammatory mediators and cleaves IL-1β from a propeptide to its activated form [32, 33]. Elevated levels of GzmA have been described in plasma and synovial fluid of RA patients, and in the blood of patients with chronic viral infection or allergic diseases [34]. Increased levels of GzmA in SLE patients may represent a response to the underlying inflammation.

Moreover, the proportion of CD8+ T cells that are positive for both CD107a and IFNγ is reduced following stimulation of SLE PBMC with viral antigens, thus further underscoring the defective effector function of SLE CD8+ T cells. This is in agreement with our previously published data showing that degranulation of CD8+ T cells in response to anti-CD3 stimulation is decreased in SLE [7].

In both normal donors and SLE patients, the presence of SLAMF7 defines cytotoxic effector CD8+ T cells. Cytolytic enzymes perforin, GzmA and GzmB are restricted to the SLAMF7+ CD8+ T population. Compared to CD8+ T cells isolated from normal donors, expression of SLAMF7 is decreased in SLE CD8+ T cells. Reduced SLAMF7 expression was observed in each memory CD8+ differentiated subsets (CM, EM and TDEM). This difference of expression was more prominent in patients with active SLE.

The role of SLAMF7 has been extensively studied in patients with multiple myeloma, where ligation of SLAMF7 has been suggested to promote NK cytotoxicity and antibody-dependent cell-mediated cytotoxicity (ADCC) followed by a significant clinical benefit. In this study we demonstrate that engagement of SLAMF7 with a specific anti-SLAMF7 antibody is able to promote CD8+ T cells degranulation, cytokine production and cytotoxicity in response to viral peptides. Interestingly, even though SLAMF7 expression is decreased in SLE patients, its engagement enabled the restoration of degranulation, IFNγ production and cytotoxic activity to levels comparable to controls. Our data also suggest that enhancement of CD8+ T cells effector function in response to antigen may be a part of the mechanism leading to a favorable anti-tumor response in patients treated with anti-SLAMF7 mAb [35]. It would be of interest to assess whether SLAMF7 ligation can expand the CD8+ T cell response against tumor antigens in patients with multiple myeloma or other tumors. Moreover, in NK cells, SLAMF7 has been shown to recruit the SH2 adaptor protein EAT-2 to initiate the cytotoxic signaling cascade [11, 36]. In CD8+ SLAMF7+ T cells, we were not able to detect EAT-2 expression, suggesting that NK cells and CD8+ T cells use different pathways to promote cytotoxic response to SLAMF7 engagement. Whether SLAMF7-initiated responses in CD8+ T cells are mediated via SAP or via other adaptor molecules remains to be determined.

The reason why SLAMF7 expression is decreased in CD8+ T cells from SLE patients is not clear. In this study, we did not address the molecular mechanisms responsible for SLAMF7 decrease in SLE CD8+ T cells. An important question regarding this abnormality is whether SLAMF7 decrease is a primary defect related to the disease itself or an alteration that occurs in onset of chronic inflammation, which may limit SLAMF7 protein formation or accelerate protein degradation. In this context, persistent antigen stimulation or chronic exposure to pro-inflammatory cytokine may contribute to SLAMF7 downregulation. A genetic predisposition contributing to decreased SLAMF7 expression on SLE CD8+T cells cannot be excluded either, as the 1q23 SLAMF containing locus has been associated with lupus in both humans and mice [37, 38].

Overall our data suggest that SLAMF7 engagement restores to normal levels the defective effector function of CD8+ T cells in SLE. Correction of the CD8+ T cell antiviral responses in SLE patients should decrease the rate of infections and reduce morbidity and mortality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by National Institutes of Health grants PO1 AI065687, RO1 AI42269 and R37 AI49954 (to G.C.T.) and a SICPA foundation grant (to D.C.). CEF Control Peptide Pool has been kindly provided by Dan Barouch, who obtained it from the NIH AIDS Reagent Program.

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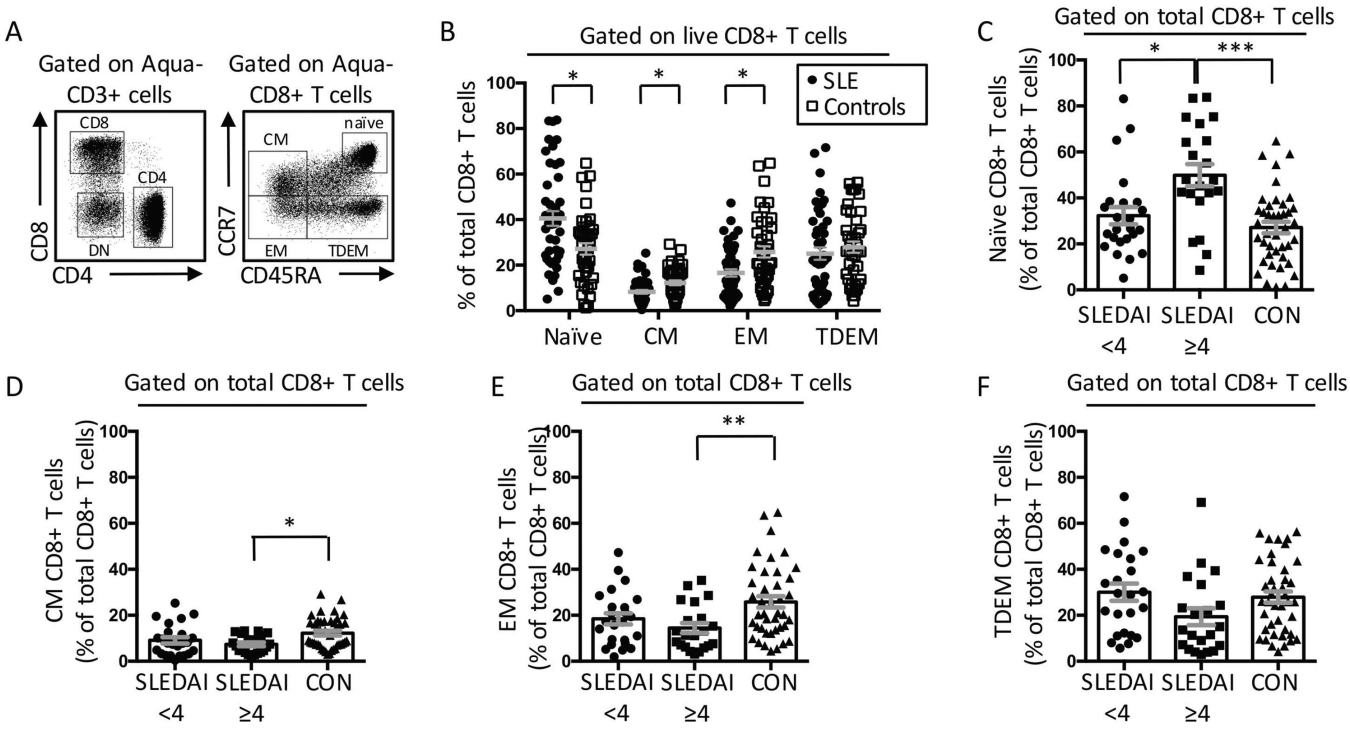

Figure 1. Skewed distribution of CD8+ T cell differentiated subsets in peripheral blood from SLE patients

(A) PBMC isolated from SLE patients were stained for CD8+ T cells differentiated subsets by examining the expression of CCR7 and CD45RA. (B) Distribution of CD8+ T cells differentiated subsets in SLE patients compared to healthy controls. Frequency of (C) naïve CD8+ T cells (D) CM, (E) EM and (F) TDEM CD8+ T cells in three cohorts: inactive SLE (SLEDAI<4), active SLE (SLEDAI≥4) and healthy controls (CON). Naive (CCR7+CD45RA+), CM: Central Memory (CCR7+CD45RA−), EM: Effector Memory (CCR7−CD45RA−), TDEM: Terminally Differentiated Effector Memory (CCR7−CD45RA+). DN: double negative (CD3+CD4−CD8−) (mean ± SEM; SLE n=45, controls n=41).

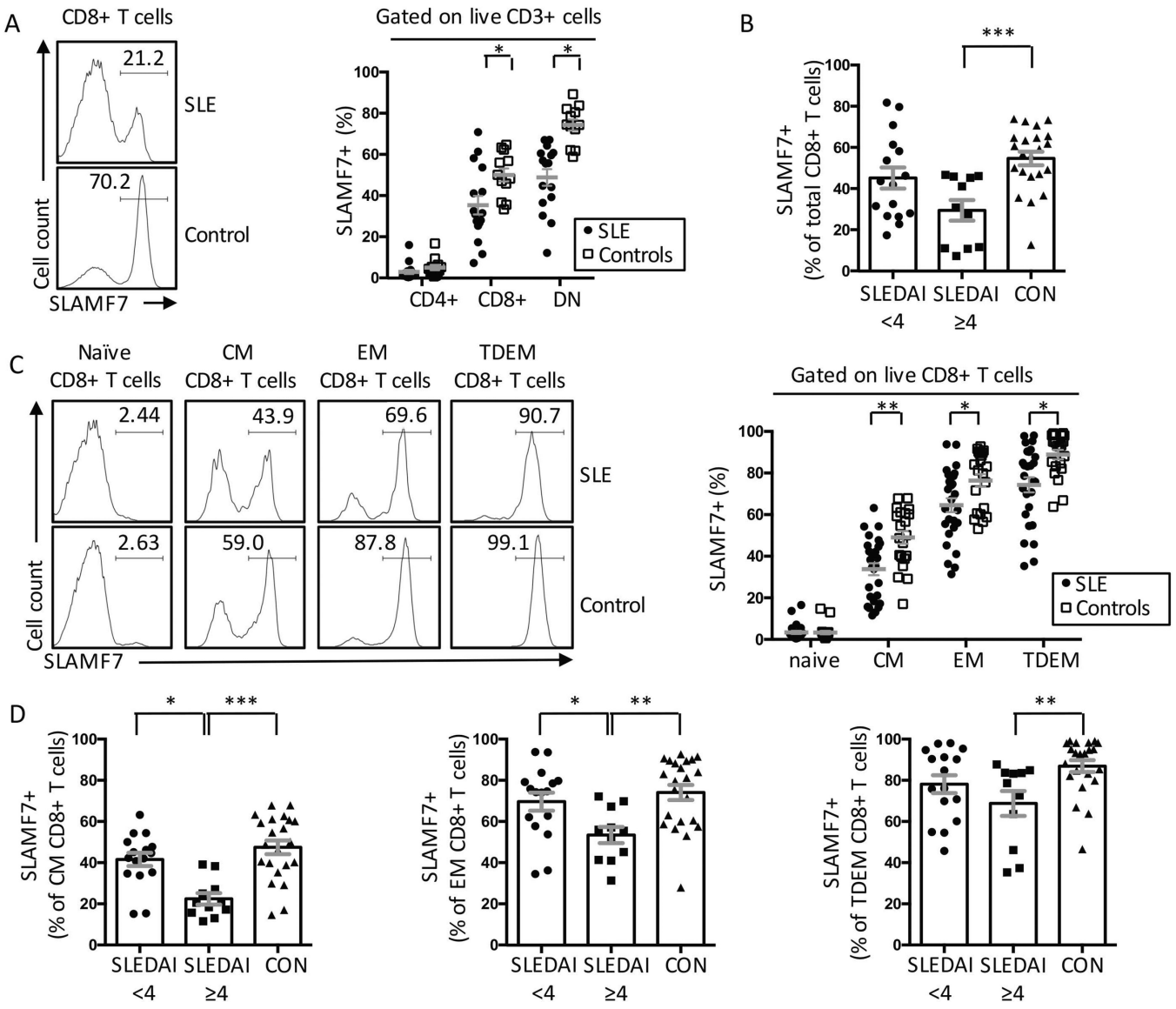

Figure 2. SLAMF7 expression is reduced on CD8+ T cells isolated from SLE patients compared to healthy controls

SLAMF7 expression was assessed by flow cytometry on T cells isolated from peripheral blood. (A) Frequency (%) of SLAMF7 expression on CD4+, CD8+ and double negative (DN) T cells isolated from SLE patients and controls. Representative plots, cumulative data and (B) correlation with disease activity: inactive SLE (SLEDAI<4), active SLE (SLEDAI≥4) and healthy controls (CON). (C) Frequency of SLAMF7 expression on central memory (CM), effector memory (EM) and terminally differentiated effector memory (TDEM) CD8+ T cells. (D) Assessment of SLAMF7 expression by CM, EM and TDEM CD8+ T differentiated subset in three cohorts: inactive SLE (SLEDAI<4), active SLE (SLEDAI≥4) and healthy controls (CON) (mean ± SEM; SLE n=16 to 27, controls n=13 to 22).

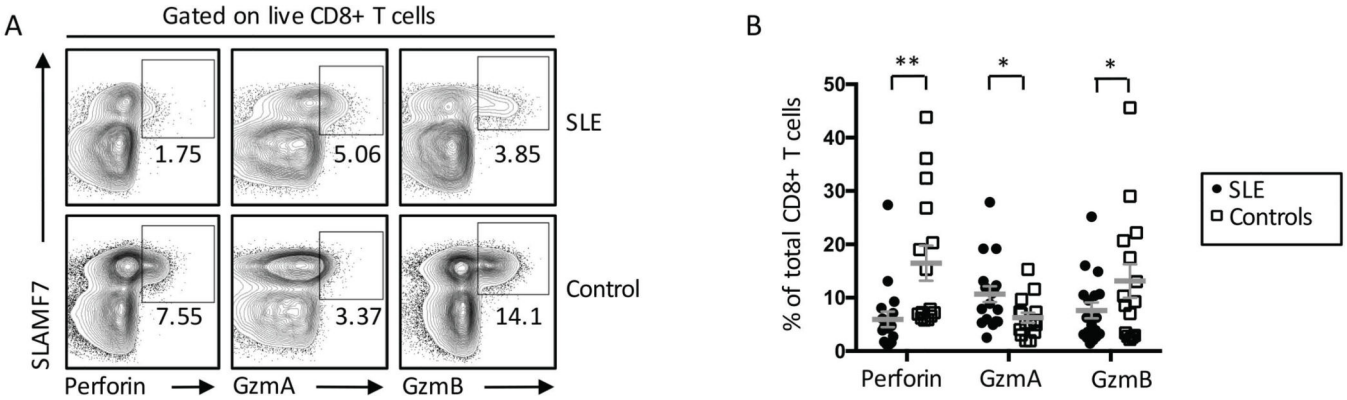

Figure 3. Expression of perforin, GzmA and GzmB is restricted to the SLAMF7+ CD8+ T cells population

Frequencies of CD8+ T cells expressing perforin, GzmA and GzmB was assessed by flow cytometry. (A) Representative flow cytometric profile of SLAMF7 vs perforin, GzmA and GzmB. Numbers indicate percentages. (B) Cumulative data from from SLE patients and healthy controls (mean ± SEM; SLE n=18, controls n=15).

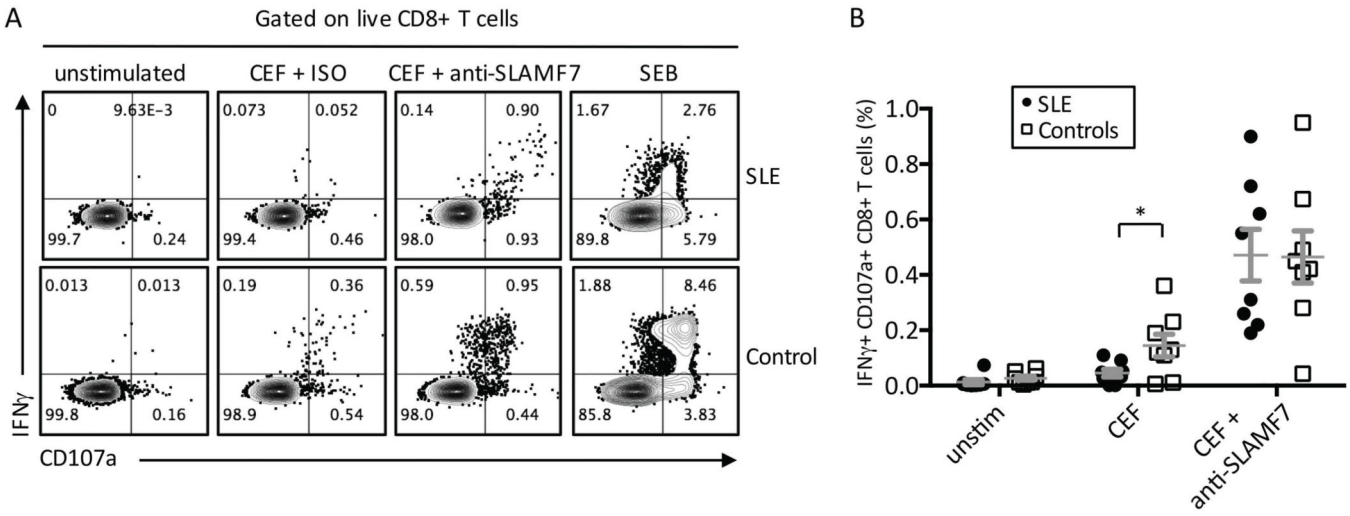

Figure 4. SLAMF7 engagement restores effector function of SLE CD8+ T cells in response to antigenic stimulation

PBMC from SLE and controls were stimulated with CEF (CMV-EBV-Flu peptide mix) for 6 hours in presence of anti-SLAMF7 mAb or an isotype control (ISO). CD107a expression and IFNg production were assessed by flow cytometry at the end of the stimulation. SEB (Staphyloccal Enterotoxin B) was used as positive control. Numbers indicate percentages. (A) Representative flow plots and (B) cumulative data (mean ± SEM; SLE n=8, controls n=8).

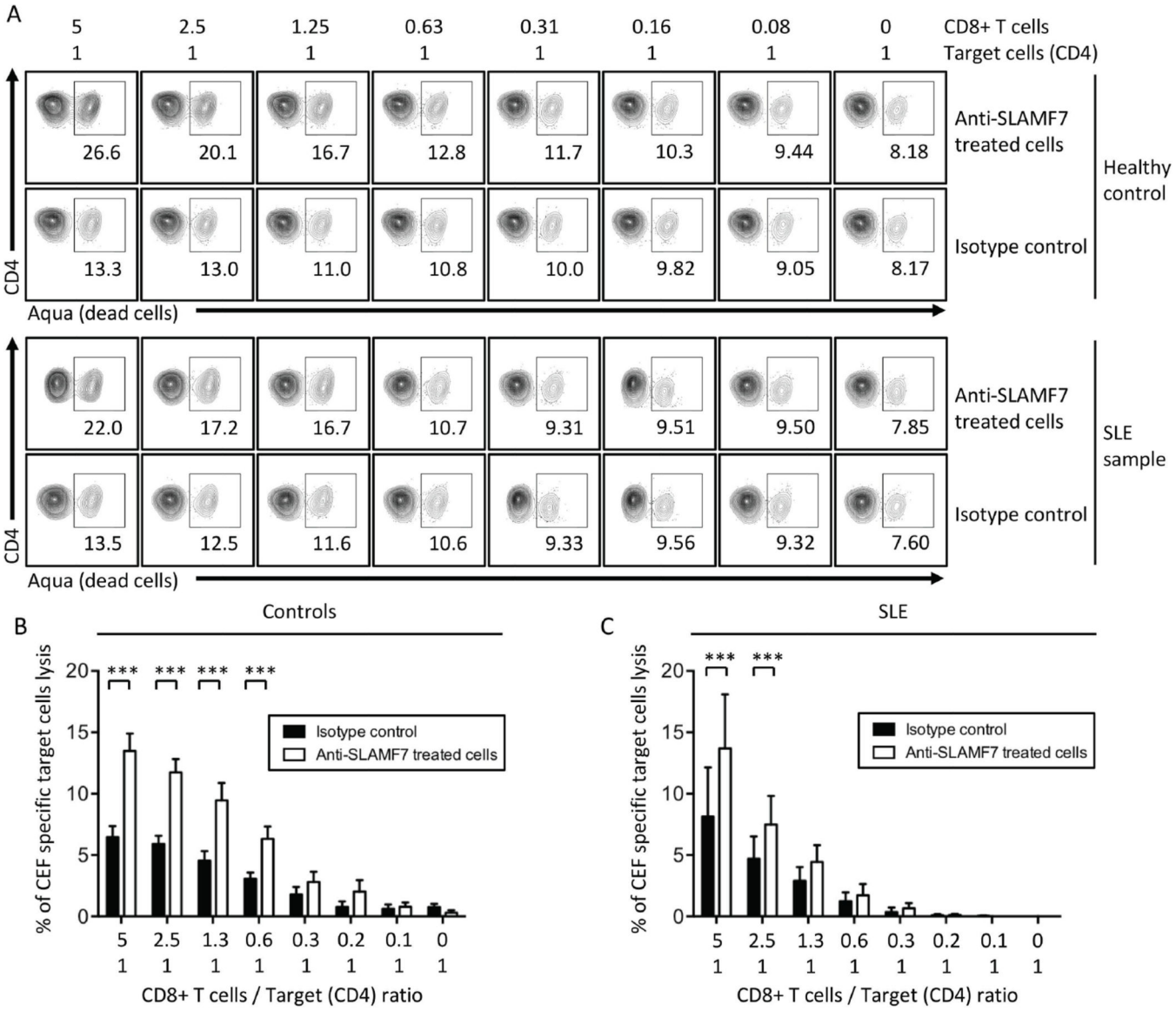

Figure 5. SLAMF7 ligation enhances cytotoxic activity of healthy and SLE CD8+ T cells in response to viral antigens

CEF stimulated PBMC were enriched for CD8+ T cells. CD8+ T cells were treated with anti-SLAMF7 mAb or an isotype control (ISO), and co-cultured for 6h with autologous CEF pulsed CD4+ T cells and un-pulsed CD4+ T cells. CEF specific killing of target CD4+ T cells was expressed as Aqua positive cells (dead cells marker) in response to different ratios of CD8+ T cells. Un-pulsed CD4+ T cells were used as control to determine target cell death background. (A) Representative dot plots showing CD4+ T cells killing (%) in response to SLAMF7 or ISO treated CD8+ T cells. Cumulative healthy healthy controls (n=4) and SLE (n=5) data are depicted in (B) and (C). Results are expressed as [Aqua+ pulsed CD4+ T cells] – [Aqua+ un-pulsed CD4+ T cells] (mean ± SEM).

Table 1

Characteristics of SLE patients included in the study

Characteristics of SLE patients	(N=79)
Age - yr	
Median	41.3
Range	21–72
Gender	
Female - (%)	72 (91.1)
Male - (%)	7 (8.9)
Ethnicity	
Afro-American - (%)	22 (27.8)
Asian - (%)	5 (6.3)
Hispanic - (%)	7 (8.9)
Caucasian - (%)	40 (50.6)
Other - (%)	5 (6.3)
SLE disease activity index (SLEDAI)	
Median	3.6
Range	(0–21)
Treatments	
Prednisone - (%)	50 (63.3)
Hydroxychloroquine - (%)	59 (74.7)
Mycophenolate Mofetil - (%)	30 (38.0)
Azathioprine - (%)	10 (12.7)
Methotrexate - (%)	5 (6.3)
Belimumab - (%)	2 (2.5)
Tacrolimus - (%)	1 (1.3)
I.V. Immunoglobulins - (%)	1 (1.3)