HHS Public Access

Author manuscript

Autoimmunity. Author manuscript; available in PMC 2015 June 14.

Published in final edited form as:

Autoimmunity. 2011 May ; 44(3): 211–218. doi:10.3109/08916934.2010.530627.

SLAMF6-driven co-stimulation of human peripheral T cells is defective in SLE T cells

Madhumouli Chatterjee[1,2,★], **Katalin Kis-Toth**[1,★], **To-Ha Thai**[1], **Cox Terhorst**[2], and **George C. Tsokos**[1]

[1]Division of Rheumatology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115, USA

[2]Division of Immunology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115, USA

Abstract

The CD28 co-stimulatory pathway is well established for T cell activation. However, there is evidence suggesting the existence of additional co-stimulatory pathways. Here we report that a member of the SLAM superfamily, SLAMF6, or CD352 plays an important role in T cell co-stimulation. Cross-linking of SLAMF6 with anti-CD3 primes human T cell to secrete Th1 cytokines. Among the T cell subsets, $CD8^+$ and $CD3^+CD4^-CD8^-$ cells display the highest Th1 production responses. Engagement of SLAMF6 mobilizes the modulation of the same set of NF-κB-associated genes. Although the expression of SLAMF6 on the surface of T cells from patients with systemic lupus erythematosus (SLE) T cells is comparable to that on the normal T cells, engagement of SLAMF6 results in severely reduced Th1 and IL-2 cytokine production. Our results suggest the existence of an additional co-stimulatory pathway in human T cells, which is defective in SLE T cells.

Keywords

SLAMF6; co-stimulation; systemic lupus erythematosus; Th1; NF-κB

Introduction

Although the specificity of T cell antigen recognition is determined by TCR, the biological consequences of this process are regulated by co-stimulatory molecules such as CD28, ICOS, and SLAM family receptors (CD150). The best characterized T cell co-stimulatory pathways involve CD28 and CTLA-4 on the surface of the T cells, and the partner B7 (CD80 and CD86) molecules on the surface of the B cells. B7/CD28 engagement provides signals that augment and sustain T cell activation in the context of TCR/CD3 signaling,

Correspondence: G. C. Tsokos, Harvard Medical School, Harvard Institutes of Medicine, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, CLS 937, Boston, MA 02115, USA. Tel: 617 735 4161. Fax: 617 735 4170. gtsokos@bidmc.harvard.edu.
★M. Chatterjee and K. Kis-Toth contributed equally to this work.

Declaration of interest: The authors report no conflicts of interest.

while B7/CTLA-4 activation provides an inhibitory signal, which reduces immune responses and induces tolerance [1,2].

The co-stimulatory molecules of the SLAM family (SLAM, CD84, Ly108, CD48) and their adaptors were shown to perform important functions in the adaptive immune responses. The SLAM family immunoreceptors are expressed on a wide array of immune cells, including T and B lymphocytes. By virtue of their ability to transduce tyrosine phosphorylation signals through the immunoreceptor tyrosine-based switch motif sequences, they play an important role in regulating both innate and adaptive immune responses.

SLAMF6 (Ly108, human counterpart is NTB-A), a member of the SLAM family receptors is expressed on natural killer, T and B cells. Previous reports suggest that SLAMF6 co-stimulation drives naïve $CD4^+$ T cells toward Th1 phenotype under Th1 polarizing conditions, that is in the presence of IL-2, the differentiating cytokine IL-12 and anti-IL4 antibody [3].

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by loss of tolerance to nuclear antigens and is accompanied by profound deregulation of the immune system. SLE T cells were reported to be defective in the production of the cytokines IFN-γ, TNF-α, and IL-2 [4–6].

Genome-wide linkage scans of families with multiple members affected with SLE have consistently demonstrated the presence of a susceptibility locus on chromosome 1q23, which includes the SLAMF genes [7–13]. Recent studies demonstrated that there is a variation within the human SLAM region that is associated with SLE [14]. Animal model experiments revealed that among the SLAM family members, *SLAMF6* may be causally involved in the pathogenesis of lupus in mice [15,16].

In this paper, we addressed the question whether SLAMF6 co-stimulation affects the ability of T cells to produce cytokines under non-polarizing conditions, and if it does, whether this co-stimulation is defective in SLE T cells. We report that SLAMF6 represents a co-stimulatory molecule which primes T cells to produce Th1 cytokines. The co-stimulatory effect delivered by SLAMF6 was more prominent in $CD8^+$ T cells and $CD3^+$ $CD4^-$ $CD8^-$, double negative (DN) T cells, compared to $CD4^+$ T cells. SLAMF6 co-stimulatory activation does not rescue the defective Th1 cytokine profile of the SLE T cells.

Materials and methods

Patients and controls

Ten SLE patients fulfilling the American College of Rheumatology revised classification criteria for SLE were studied. SLE disease activity index scores ranged from 0 to 20 (mean 7.2). Blood samples were obtained from 10 healthy platelet donors from the Kraft Family Blood Donor Center (Dana-Farber Cancer Institute, Boston, MA, USA). The study was approved by the Institutional Review Board of BIDMC.

Antibodies and reagents

The following antibodies (Abs) were used for flow cytometry: anti-CD4-Pacific Blue, anti-CD8-PerCP, anti-TNF-α-PE, and anti-IFN-γ-Alexa Fluor 647 (Biolegend, San Diego, CA, USA). The Abs used for the co-stimulation assays were anti-CD3 (Clone OKT3, Bio X Cell, West Lebanon, NH, USA) and anti-CD28 (Biolegend). The anti-NTBA Abs were custom made from Genentech (South San Francisco, CA, USA). For the co-stimulation assays we used the anti-NTBA clone 10B4.12.3.

SLAMF6 co-stimulation assay

Total T cells were isolated by negative selection (RosetteSep, Stem Cell Technologies, Vancouver, Canada). T cell purity was always ≥96% monitored by flow cytometry. Then, 24-well plates were coated overnight with 1 or 0.1 μg/ml anti-CD3 Ab and 1, 2, or 5 μg/ml anti-CD28 Ab or 1, 2, or 5 μg/ml anti-SLAMF6 Ab (Genentech), and 1, 2, or 5 μg/ml IgG1. Total T cells were plated at 1.0×10^6 cells per well in RPMI medium supplemented by 10% fetal bovine serum and antibiotics. On day 3, supernatants were removed for cytokine analysis by ELISA and the cells were collected for intracellular cytokine staining. Five hours before collection, brefeldin A (10 μg/ml; Sigma, St Louis, MO, USA) was added to the cell cultures. For detection of IL-2 in the supernatants, the cells were stimulated for 18 h.

Enzyme-linked immunosorbent assay (ELISA)

Secreted cytokine concentrations were measured from cell supernatants using the DuoSet Human Immunoassay Kits for TNF-α and IFN-γ (R&D Systems, Minneapolis, MN, USA) and the Human IL-2 ELISA Kit (eBioscience, San Diego, CA, USA) following the manufacturer's instructions. The optical density of the wells was determined using a microplate reader set at 450 nm.

Flow cytometry

Intracellular staining was performed with the BD Cytofix/Cytoperm Kit, according to the instruction of the manufacturer. Samples were acquired in an LSRII flow cytometer (BD Biosciences, San Jose, CA, USA). Analysis was performed with FlowJo v. 7.2.2 (Tree Star, Ashland, OR, USA).

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

T cells were activated as mentioned above for 4 h. Total RNA was isolated from T cells by RNeasy Mini Kit (Qiagen, Valencia, CA, USA). PCR arrays were performed using the Human NF-κB Pathway PCR Array (SA Biosciences, Frederick, MD, USA) following the Manufacturer's instructions. Reverse transcription was performed from 1μg of total RNA using the RT^2 First, Strand Kit (SA Biosciences). Quantitative real-time PCR were performed (Light Cycler 480, Roche, Indianapolis, IN, USA) with 40 cycles at 94°C for 15 s and 60°C for 60 s. Fold changes were calculated for each gene using the Manufacturer's web-based PCR array data analysis.

Statistical analysis

The paired student two-tailed *t*-test was used for the statistical analyses. Each experiment was repeated at least on five different donors.

Results

SLAMF6 induces cytokine production in T cell subpopulations

Previous reports suggest that SLAMF6 provides co-stimulation signal to naïve $CD4^+$ T cells, induces IFN-γ expression, and directs T cell polarization toward the Th1 lineage [1].

Based on this information, first we analyzed the cell surface expression of SLAMF6 on the different T cell subpopulations ($CD4^+$, $CD8^+$, and DN). The $CD4^+$ and $CD8^+$ T cell subsets expressed equal amount of cell surface SLAMF6, while the expression was lower in the DN cells (Figure 1A–C). Upon activation of T cells with anti-CD3 (aCD3) and aCD28 antibodies (Abs) we noted a significant up-regulation in the expression of SLAMF6 on $CD4^+$ and $CD8^+$ T cells (Figure 1A,B). Expression of SLAMF6 on the surface of DN cells also increased, but not significantly.

Next, we asked whether engagement of the SLAMF6 molecule in conjunction with CD3, under no polarizing conditions, resulted in production of cytokines. T cells were isolated from human peripheral blood and activated with aCD3 Ab at a standard 1 μg/ml concentration and various concentrations of aSLAMF6Ab. Cells treated with aCD3 Ab alone or in conjunction with aCD28 were used as control. Culture of human T cells with aCD3, aCD28, or aSLAMF6 alone did not induce substantial production of IFN-γ, TNF-α, or IL-2. Preliminary experiments revealed that a dose of 5 μg/ml of aSLAMF6 Ab in the presence of 1 μg/ml aCD3 resulted in optimal cytokine production (data not shown). Co-stimulation of $CD4^+$ cells with aSLAMF6 Ab produced comparable amounts of IFN-γ and TNF-α, determined by intracellular staining, to those when stimulated with aCD28 (Figure 2A).

In contrast, $CD8^+$ (Figure 2B) and DN (Figure 2C) cells produced significantly more IFN-γ and TNF-α when co-stimulated with aSLAMF6 compared to aCD28 co-stimulation. Co-stimulation of total peripheral blood T cells with aSLAMF6 resulted in the secretion of higher amounts of IFN-γ and TNF-α compared to co-stimulation with aCD28 (Figure 2D). In contrast, co-stimulation of total peripheral blood T cells with aSLAMF6 resulted in the production of significantly less IL-2 compared to co-stimulation with aCD28 (Figure 2E). Thus, co-engagement of SLAMF6 results in the production of higher amounts of the pro-inflammatory cytokines IFN-γ and TNF-α by $CD8^+$ and DN cells compared to co-stimulation with aCD28, and less amounts of IL-2.

T cell activation via the CD3/CD28 and CD3/SLAMF6 route activate the NF-κB pathway similarly

To investigate the potential differences between the CD28 and the SLAMF6 co-activation pathways we analyzed 84 specific genes connected with the NF-κB transcription factor activation in T cells using a gene array (Table I). Co-stimulation of T cells with either CD28

or SLAMF6 appeared to regulate the same set of genes. The average fold-change of the 84 genes was significantly higher in the aCD3/aCD28-treated cells compared to the aCD3/aSLAMF6-treated cells (21.4 vs. 18.9, $p < 0.05$). However, the fold-changes of the genes caspase 1, CSF2, and IFN-γ were significantly higher in case of the aCD3/aSLAMF6 treatment compared to the aCD3/aCD28 activation (3.1 vs. 0.002; 113.9 vs. 99.7 and 120.4 vs. 76.6, respectively).

Impaired cytokine production in SLE T cells upon SLAMF6 co-stimulation

In the next set of experiments we investigated the effect of stimulation with aCD3/aSLAMF6 Abs in the production of cytokines by SLE T cells. T cells were isolated from peripheral blood of SLE patients ($n = 10$) and healthy control individuals ($n = 10$) and the cell surface expression of SLAMF6 molecules was investigated. We did not find significant differences in the amount of SLAMF6 molecules expressed on the surface of SLE T cell subsets compared to normal T lymphocyte populations whether unstimulated or after T cell activation with aCD3 and aCD28 Abs (data not shown).

SLE T cells stimulated with aCD3/aSLAMF6 Abs produced significantly less amounts of IFN-γ and TNF-α cytokines as determined by intracellular staining (Figure 3A–C) or in the cell culture supernatants (Figure 3D) compared to control T cells. The production of IL-2 cytokine upon aCD3/a-SLAMF6 Ab treatment was significantly reduced compared to normal T cells (Figure 3E).

Discussion

The engagement of the antigen specific receptor is essential for the activation of T or B lymphocytes. However, triggering the antigen receptor is not the only factor determining lymphocyte activity. Multiple other receptor–ligand interactions are necessary for a productive activation of naive lymphocytes [17,18]. These interactions can deliver co-stimulatory or co-inhibitory signals and are important for the fine-tuning of the immune response. Members of the CD28/B7 family of co-stimulatory molecules are generally considered to be key players in the co-stimulatory pathway. However, there are reports on CD28 deficient T cells indicating the existence of co-stimulatory molecules other than CD28 [19].

Here, we report that SLAMF6 can prime T cells to produce Th1 cytokines IFN-γ and TNF-α by peripheral T cells in the absence of polarizing conditions and it seems to be a stronger co-stimulator compared to the CD28 engagement in terms of Th1 cytokine production in case of the $CD8^+$ and DN T cells. In addition, engagement of SLAMF6 results in the production of lower amounts of IL-2 compared to the engagement of CD28.

These studies prompted us to investigate the role of SLAMF6 co-stimulation in the production of the different Th1 cytokines in SLE patients. SLE T cells have been described previously to produce decreased amounts of IFN-γ, TNF-α, and IL-2 [4–6]. Although some of the molecular events that lead to decreased cytokine production were studied [20,21], it is unclear whether early events associated with the transfer of the signal from the co-

stimulatory molecule inside the cell are defective. It was important to study the ability of additional co-stimulatory molecules to induce the production of cytokines.

The fact, reported herein, that engagement of SLAMF6 provides insufficient co-stimulation to SLE T cells to produce cytokines, in a manner similar to the engagement of CD28, suggests that downstream events account for the defective production of cytokines in SLE T cells. The fact, that most of the NF-κB-associated genes are modulated equally following the engagement of SLAMF6 and CD28 in normal human T cells suggests that in SLE T cells molecular events modifying the NF-κB pathway account for the decreased production of cytokines or that there is an inherent defect in the NF-κB pathway. It was reported that p65, an important component of the NF-κB complex is decreased in SLE T cells [22], for unclear reasons.

A co-stimulatory molecule-driven degradation of p65 which may be enhanced in SLE T cells may account for this. Alternatively, molecular events unrelated to the NF-κB are responsible for the decreased production of cytokines in SLE T cells. Given that the transcription of the IL-2, TNF-α, and IFN-γ is controlled through different mechanisms, the answer to the question may be more complicated. For example, increased expression of the transcriptional repressor may account for the decreased production of IL-2 in SLE T cells [23].

One observation of interest is the fact that $CD8^+$ and DN cells display a higher propensity to produce cytokines following engagement of SLAMF6. This provides a functional link between $CD8^+$ and DN cells that share gene expression profile, and it was shown that at least some of the DN cells may represent $CD8^+$ cells which lost CD8 from the surface membrane.

DN cells are expanded in SLE patients [24] and $CD8^+$ cells were shown repeatedly to display compromised function. For example, $CD8^+$ cells cannot eliminate virally infected self cells [25] and display decreased amounts of CD3ζ on the surface membrane [26]. The defective production of cytokines by SLE T cells, although it appears to involve all T cell subsets, may be more pronounced among $CD8^+$ and DN cells.

Our study has provided first insight into the SLAMF6-mediated co-stimulatory pathway in human T cells and revealed it to be impressively defective in SLE T cells. Our studies have opened a line to further understand the way T cells malfunction in SLE patients.

Acknowledgments

This work was supported by the National Institutes of Health grants [PO1AI 065687, RO1AI42269]; and the Kirkland Scholar Program. The authors alone are responsible for the content and writing of the paper.

References

1. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. Annu Rev Immunol. 2005; 23:515–548. [PubMed: 15771580]
2. Teft WA, Kirchhof MG, Madrenas J. A molecular perspective of CTLA-4 function. Annu Rev Immunol. 2006; 24:65–97. [PubMed: 16551244]

3. Valdez PA, Wang H, Seshasayee D, van Lookeren Campagne M, Gurney A, Lee WP, Grewal IS. NTB-A, a new activating receptor in T cells that regulates autoimmune disease. J Biol Chem. 2004; 279:18662–18669. [PubMed: 14988414]
4. Tsokos GC, Balow JE. Cellular immune responses in systemic lupus erythematosus. Prog Allergy. 1984; 35:93–161. [PubMed: 6385016]
5. Kammer GM. Altered regulation of IL-2 production in systemic lupus erythematosus: An evolving paradigm. J Clin Invest. 2005; 115:836–840. [PubMed: 15841173]
6. Tsokos GC, Boumpas DT, Smith PL, Djeu JY, Balow JE, Rook AH. Deficient gamma-interferon production in patients with systemic lupus erythematosus. Arthritis Rheum. 1986; 29:1210–1215. [PubMed: 2429672]
7. Moser KL, Gray-McGuire C, Kelly J, Asundi N, Yu H, Bruner GR, Mange M, Hogue R, Neas BR, Harley JB. Confirmation of genetic linkage between human systemic lupus erythematosus and chromosome 1q41. Arthritis Rheum. 1999; 42:1902–1907. [PubMed: 10513806]
8. Shai R, Quismorio FP Jr, Li L, Kwon OJ, Morrison J, Wallace DJ, Neuwelt CM, Brautbar C, Gauderman WJ, Jacob CO. Genome-wide screen for systemic lupus erythematosus susceptibility genes in multiplex families. Hum Mol Genet. 1999; 8:639–644. [PubMed: 10072432]
9. Tsao BP, Cantor RM, Grossman JM, Kim SK, Strong N, Lau CS, Chen CJ, Shen N, Ginzler EM, Goldstein R, Kalunian KC, Arnett FC, Wallace DJ, Hahn BH. Linkage and interaction of loci on 1q23 and 16q12 may contribute to susceptibility to systemic lupus erythematosus. Arthritis Rheum. 2002; 46:2928–2936. [PubMed: 12428234]
10. Tsao BP, Cantor RM, Kalunian KC, Chen CJ, Badsha H, Singh R, Wallace DJ, Kitridou RC, Chen SL, Shen N, Song YW, Isenberg DA, Yu CL, Hahn BH, Rotter JI. Evidence for linkage of a candidate chromosome 1 region to human systemic lupus erythematosus. J Clin Invest. 1997; 99:725–731. [PubMed: 9045876]
11. Johanneson B, Lima G, von Salome J, Alarcon-Segovia D, Alarcon-Riquelme ME. A major susceptibility locus for systemic lupus erythemathosus maps to chromosome 1q31. Am J Hum Genet. 2002; 71:1060–1071. [PubMed: 12373647]
12. Gray-McGuire C, Moser KL, Gaffney PM, Kelly J, Yu H, Olson JM, Jedrey CM, Jacobs KB, Kimberly RP, Neas BR, Rich SS, Behrens TW, Harley JB. Genome scan of human systemic lupus erythematosus by regression modeling: Evidence of linkage and epistasis at 4p16-15.2. Am J Hum Genet. 2000; 67:1460–1469. [PubMed: 11078476]
13. Wakeland EK, Liu K, Graham RR, Behrens TW. Delineating the genetic basis of systemic lupus erythematosus. Immunity. 2001; 15:397–408. [PubMed: 11567630]
14. Vyse TJ, Richardson AM, Walsh E, Farwell L, Daly MJ, Terhorst C, Rioux JD. Mapping autoimmune disease genes in humans: Lessons from IBD and SLE. Novartis Found Symp. 2005; 267:94–107. discussion 107–12. [PubMed: 15999803]
15. Wandstrat AE, Nguyen C, Limaye N, Chan AY, Subramanian S, Tian XH, Yim YS, Pertsemlidis A, Garner HR Jr, Morel L, Wakeland EK. Association of extensive polymorphisms in the SLAM/CD2 gene cluster with murine lupus. Immunity. 2004; 21:769–780. [PubMed: 15589166]
16. Kumar KR, Li L, Yan M, Bhaskarabhatla M, Mobley AB, Nguyen C, Mooney JM, Schatzle JD, Wakeland EK, Mohan C. Regulation of B cell tolerance by the lupus susceptibility gene Ly108. Science. 2006; 312:1665–1669. [PubMed: 16778059]
17. Sharpe AH, Freeman GJ. The B7-CD28 superfamily. Nat Rev Immunol. 2002; 2:116–126. [PubMed: 11910893]
18. Veillette A, Latour S, Davidson D. Negative regulation of immunoreceptor signaling. Annu Rev Immunol. 2002; 20:669–707. [PubMed: 11861615]
19. Lucas PJ, Negishi I, Nakayama K, Fields LE, Loh DY. Naive CD28-deficient T cells can initiate but not sustain an *in vitro* antigen-specific immune response. J Immunol. 1995; 154:5757–5768. [PubMed: 7751626]
20. Crispin JC, Liossis SN, Kis-Toth K, Lieberman LA, Kyttaris VC, Juang YT, Tsokos GC. Pathogenesis of human systemic lupus erythematosus: Recent advances. Trends Mol Med. 2010; 16:47–57. [PubMed: 20138006]
21. Lieberman LA, Tsokos GC. The IL-2 defect in systemic lupus erythematosus disease has an expansive effect on host immunity. J Biomed Biotechnol. 2010; 740619

22. Wong HK, Kammer GM, Dennis G, Tsokos GC. Abnormal NF-kappa B activity in T lymphocytes from patients with systemic lupus erythematosus is associated with decreased p65-RelA protein expression. J Immunol. 1999; 163:1682–1689. [PubMed: 10415075]

23. Solomou EE, Juang YT, Gourley MF, Kammer GM, Tsokos GC. Molecular basis of deficient IL-2 production in T cells from patients with systemic lupus erythematosus. J Immunol. 2001; 166:4216–4222. [PubMed: 11238674]

24. Crispin JC, Oukka M, Bayliss G, Cohen RA, Van Beek CA, Stillman IE, Kyttaris VC, Juang YT, Tsokos GC. Expanded double negative T cells in patients with systemic lupus erythematosus produce IL-17 and infiltrate the kidneys. J Immunol. 2008; 181:8761–8766. [PubMed: 19050297]

25. Tsokos GC, Magrath IT, Balow JE. Epstein-Barr virus induces normal B cell responses but defective suppressor T cell responses in patients with systemic lupus erythematosus. J Immunol. 1983; 131:1797–1801. [PubMed: 6311898]

26. Liossis SN, Ding XZ, Dennis GJ, Tsokos GC. Altered pattern of TCR/CD3-mediated protein-tyrosyl phosphorylation in T cells from patients with systemic lupus erythematosus. Deficient expression of the T cell receptor zeta chain. J Clin Invest. 1998; 101:1448–1457. [PubMed: 9525988]

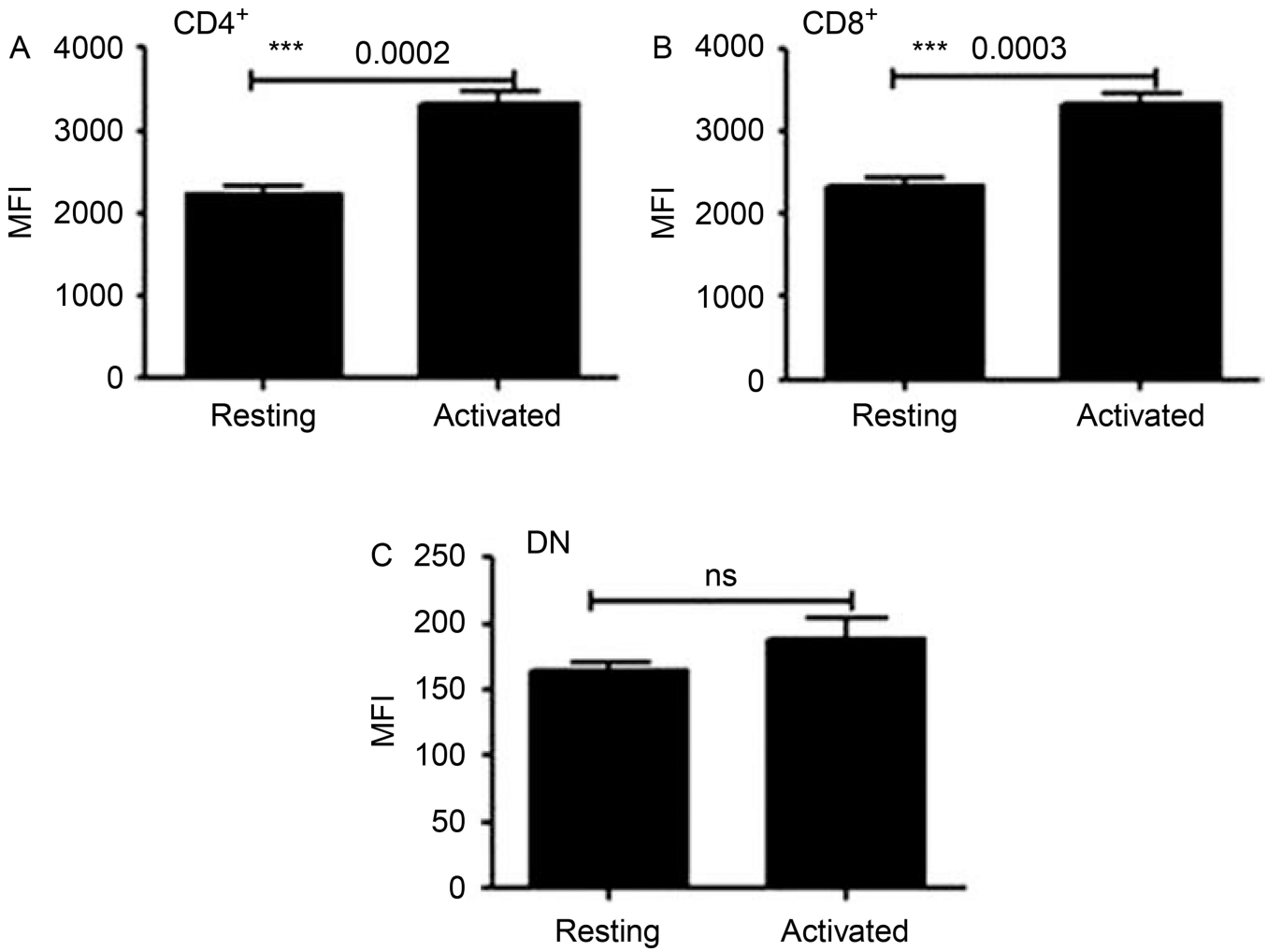

Figure 1.

Cell-surface expression of SLAMF6 is upregulated in activated human T cells. Expression was detected on resting and activated T cells by flow cytometry using a PE-labeled monoclonal antibody against SLAMF6. SLAMF6 expression on (A) $CD4^+$, (B) $CD8^+$ and (C) DN T cells.

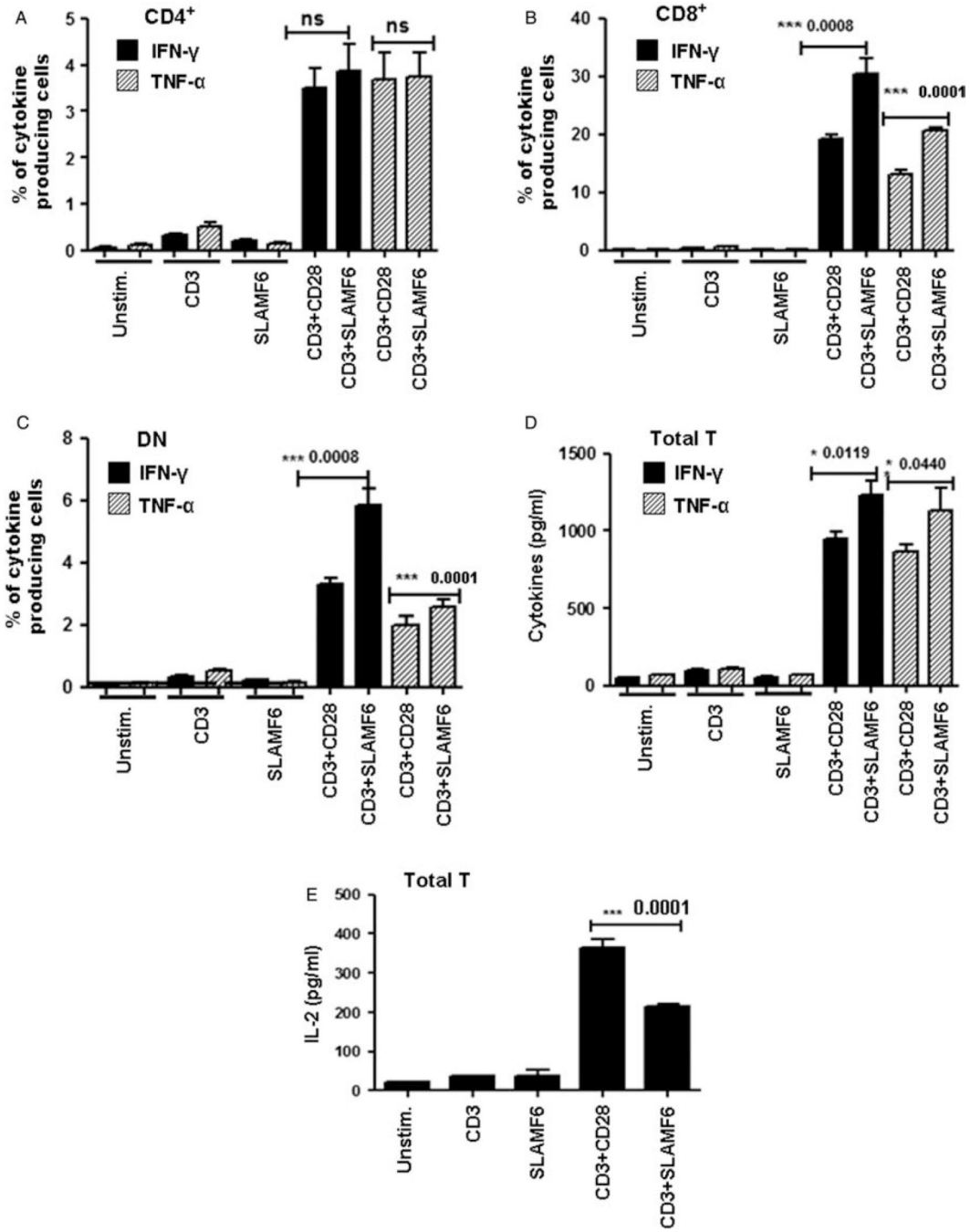

Figure 2.

SLAMF6 co-stimulation leads to production of Th1 cytokines and IL-2 by total T cells. SLAMF6 co-stimulation induces the intracellular production of IFN-γ and TNF-α in (A) $CD4^+$, (B) $CD8^+$ and (C) DN T cells. (D) SLAMF6 co-stimulation leads to IFN-γ and TNF-α secretion by total T cells. Black graphs: IFN-γ; striped graphs: TNF-α. (E) SLAMF6 co-stimulation leads to IL-2 secretion by total T cells.

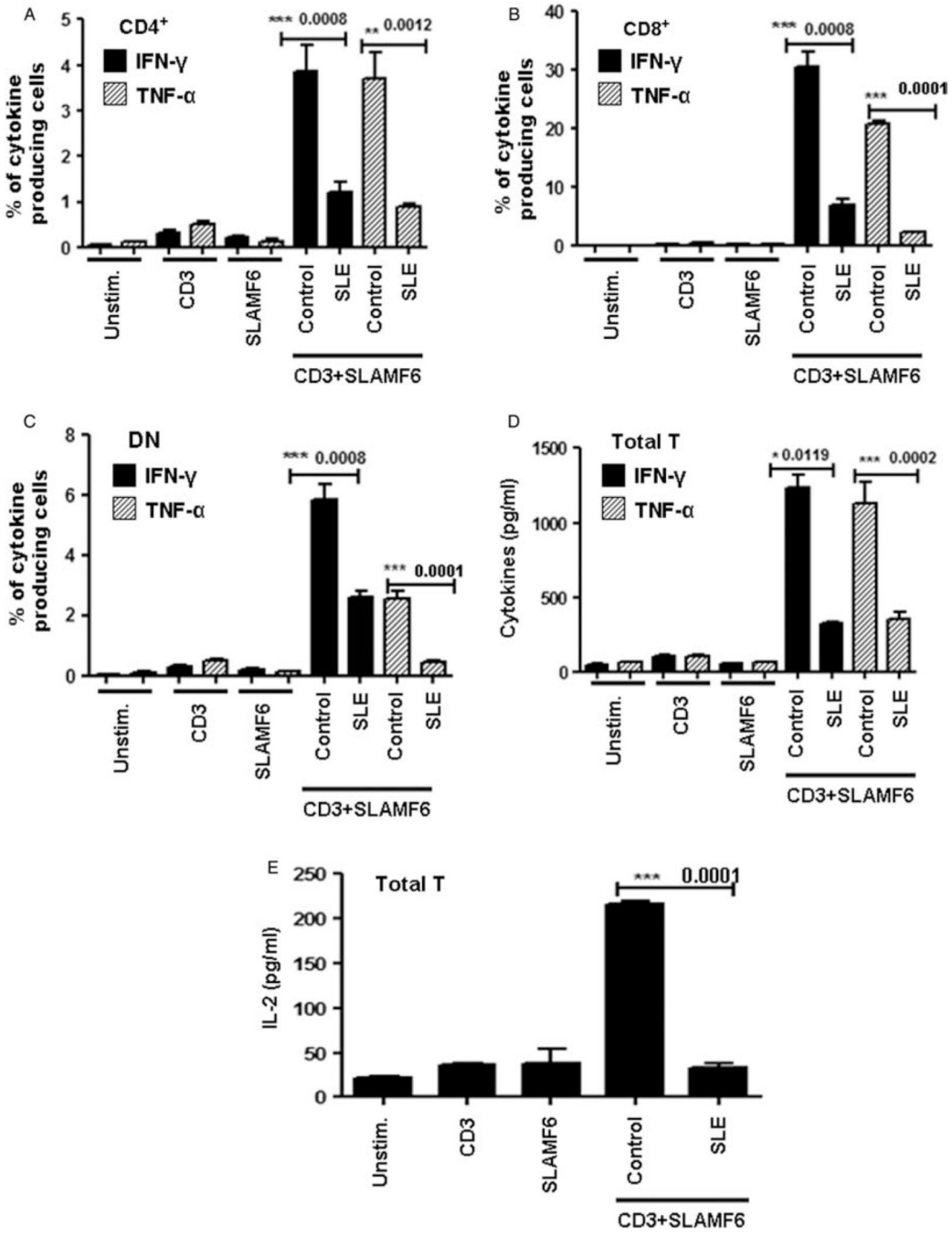

Figure 3.

Cytokine production after SLAMF6 co-stimulation of SLE T cells. SLAMF6 co-stimulation induces the intracellular production of IFN-γ and TNF-α in (A) $CD4^+$, (B) $CD8^+$ and (C) DNSLE T cells. (D) SLAMF6 co-stimulation leads to IFN-γ and TNF-α secretion by total SLE T cells. Black graphs: IFN-γ; striped graphs: TNF-α. (E) SLAMF6 co-stimulation leads to IL-2 secretion by total SLE T cells.

Table I

Gene list of the NF-κB gene array used in this study.

Unigene	Refseq	Symbol	Description
Hs.19383	NM_000029	AGT	Angiotensinogen (serpin peptidase inhibitor, clade A, member 8)
Hs.525622	NM_005163	AKT1	V-akt murine thymoma viral oncogene homolog 1
Hs.648565	NM_005171	ATF1	Activating transcription factor 1
Hs.193516	NM_003921	BCL10	B cell CLL/lymphoma 10
Hs.31210	NM_005178	BCL3	B cell CLL/lymphoma 3
Hs.69771	NM_001710	CFB	Complement factor B
Hs.696238	NM_001166	BIRC2	Baculoviral IAP repeat-containing 2
Hs.405153	NM_006092	NOD1	Nucleotide-binding oligomerization domain containing 1
Hs.2490	NM_033292	CASP1	Caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)
Hs.599762	NM_001228	CASP8	Caspase 8, apoptosis-related cysteine peptidase
Hs.303649	NM_002982	CCL2	Chemokine (C-C motif) ligand 2
Hs.472860	NM_001250	CD40	CD40 molecule, TNF receptor superfamily member 5
Hs.390736	NM_003879	CFLAR	CASP8 and FADD-like apoptosis regulator
Hs.198998	NM_001278	CHUK	Conserved helix-loop-helix ubiquitous kinase
Hs.1349	NM_000758	CSF2	Colony stimulating factor 2 (granulocyte-macrophage)
Hs.2233	NM_000759	CSF3	Colony stimulating factor 3 (granulocyte)
Hs.631631	NM_020428	SLC44A2	Solute carrier family 44, member 2
Hs.352224	NM_080738	EDARADD	EDAR-associated death domain
Hs.126667	NM_057159	LPAR1	Lysophosphatidic acid receptor 1
Hs.326035	NM_001964	EGR1	Early growth response 1
Hs.181128	NM_005229	ELK1	ELK1, member of ETS oncogene family
Hs.482562	NM_001992	F2R	Coagulation factor II (thrombin) receptor
Hs.86131	NM_003824	FADD	Fas (TNFRSF6)-associated via death domain
Hs.2007	NM_000639	FASLG	Fas ligand (TNF superfamily, member 6)
Hs.25647	NM_005252	FOS	V-fos FBJ murine osteosarcoma viral oncogene homolog
Hs.74471	NM_000165	GJA1	Gap junction protein, alpha 1, 43 kDa
Hs.517581	NM_002133	HMOX1	Heme oxygenase (decycling) 1
Hs.421649	NM_000867	HTR2B	5-hydroxytryptamine (serotonin) receptor 2B
Hs.643447	NM_000201	ICAM1	Intercellular adhesion molecule 1
Hs.37026	NM_024013	IFNA1	Interferon, alpha 1
Hs.93177	NM_002176	IFNB1	Interferon, beta1, fibroblast
Hs.856	NM_000619	IFNG	Interferon, gamma
Hs.597664	NM_001556	IKBKB	Inhibitor of kappa light polypeptide gene enhancer in B cells, kinase beta
Hs.321045	NM_014002	IKBKE	Inhibitor of kappa light polypeptide gene enhancer in B cells, kinase epsilon
Hs.43505	NM_003639	IKBKG	Inhibitor of kappa light polypeptide gene enhancer in B cells, kinase gamma
Hs.193717	NM_000572	IL10	Interleukin 10
Hs.1722	NM_000575	IL1A	Interleukin 1, alpha
Hs.126256	NM_000576	IL1B	Interleukin 1, beta
Hs.701982	NM_000877	IL1R1	Interleukin 1 receptor, type I

Unigene	Refseq	Symbol	Description
Hs.654458	NM_000600	IL6	Interleukin 6 (interferon, beta2)
Hs.624	NM_000584	IL8	Interleukin 8
Hs.522819	NM_001569	IRAK1	Interleukin-1 receptor-associated kinase 1
Hs.449207	NM_001570	IRAK2	Interleukin-1 receptor-associated kinase 2
Hs.714791	NM_002228	JUN	Jun oncogene
Hs.36	NM_000595	LTA	Lymphotoxin alpha (TNF superfamily, member 1)
Hs.1116	NM_002342	LTBR	Lymphotoxin beta receptor (TNFR superfamily, member 3)
Hs.601217	NM_173844	MALT1	Mucosa associated lymphoid tissue lymphoma translocation gene 1
Hs.657756	NM_005921	MAP3K1	Mitogen-activated protein kinase kinase kinase 1
Hs.82116	NM_002468	MYD88	Myeloid differentiation primary response gene (88)
Hs.631573	NM_033297	NLRP12	NLR family, pyrin domain containing 12
Hs.654408	NM_003998	NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B cells 1
Hs.73090	NM_002502	NFKB2	Nuclear factor of kappa light polypeptide gene enhancer in B cells 2 (p49/p100)
Hs.81328	NM_020529	NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, alpha
Hs.130036	NM_177952	PPM1A	Protein phosphatase 1A (formerly 2C), magnesium-dependent, alpha isoform
Hs.159130	NM_002880	RAF1	V-raf-1 murine leukemia viral oncogene homolog 1
Hs.631886	NM_002908	REL	V-rel reticuloendotheliosis viral oncogene homolog (avian)
Hs.502875	NM_021975	RELA	V-rel reticuloendotheliosis viral oncogene homolog A (avian)
Hs.654402	NM_006509	RELB	V-rel reticuloendotheliosis viral oncogene homolog B
Hs.436922	NM_005798	TRIM13	Tripartite motif-containing 13
Hs.247077	NM_001664	RHOA	Ras homolog gene family, member A
Hs.519842	NM_003804	RIPK1	Receptor (TNFRSF)-interacting serine-threonine kinase 1
Hs.187946	NM_005415	SLC20A1	Solute carrier family 20 (phosphate transporter), member 1
Hs.642990	NM_007315	STAT1	Signal transducer and activator of transcription 1, 91 kDa
Hs.505874	NM_013254	TBK1	TANK-binding kinase 1
Hs.710895	NM_021649	TICAM2	Toll-like receptor adaptor molecule 2
Hs.654532	NM_003263	TLR1	Toll-like receptor 1
Hs.519033	NM_003264	TLR2	Toll-like receptor 2
Hs.657724	NM_003265	TLR3	Toll-like receptor 3
Hs.174312	NM_138554	TLR4	Toll-like receptor 4
Hs.662185	NM_006068	TLR6	Toll-like receptor 6
Hs.659215	NM_016562	TLR7	Toll-like receptor 7
Hs.660543	NM_138636	TLR8	Toll-like receptor 8
Hs.87968	NM_017442	TLR9	Toll-like receptor 9
Hs.598832	NM_182547	TMED4	Transmembrane emp24 protein transport domain containing 4
Hs.241570	NM_000594	TNF	Tumor necrosis factor (TNF superfamily, member 2)
Hs.211600	NM_006290	TNFAIP3	Tumor necrosis factor, alpha-induced protein 3
Hs.591834	NM_003844	TNFRSF10A	Tumor necrosis factor receptor superfamily, member 10a
Hs.521456	NM_003842	TNFRSF10B	Tumor necrosis factor receptor superfamily, member 10b
Hs.279594	NM_001065	TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1A
Hs.355307	NM_001242	CD27	CD27 molecule
Hs.478275	NM_003810	TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10

Unigene	Refseq	Symbol	Description
Hs.129708	NM_003807	TNFSF14	Tumor necrosis factor (ligand) superfamily, member 14
Hs.460996	NM_003789	TRADD	TNFRSF1A-associated via death domain
Hs.29344	NM_182919	TICAM1	Toll-like receptor adaptor molecule 1
Hs.534255	NM_004048	B2M	Beta-2-microglobulin
Hs.412707	NM_000194	HPRT1	Hypoxanthine phosphoribosyltransferase 1
Hs.523185	NM_012423	RPL13A	Ribosomal protein L13a
Hs.592355	NM_002046	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
Hs.520640	NM_001101	ACTB	Actin, beta