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Streptavidin Suppresses T Cell Activation and Inhibits IL-2 Production and CD25 Expression

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Abstract

Streptavidin is widely used as a detection tool in biology research because of its high affinity and specificity binding to biotin. Biotin-streptavidin system has also been explored for detection of infection and tumor in clinical medicine. Here, we show immunosuppressive property of streptavidin on T cell activation and proliferation. Upon CD3 and CD28 stimulation, CD4⁺ T cells produce interleukin 2 (IL-2) and express IL-2 receptor α chain (CD25). Addition of streptavidin in T cell culture suppressed IL-2 synthesis and CD25 expression with no cytotoxicity. The immunosuppressive effect of streptavidin was reversed by excessive biotin. Conjugated to a single chain anti-CD7 variable fragment (scFvCD7), streptavidin was directly delivered to T cells and showed substantially more profound suppressive effect on T cell activation. These results suggest that streptavidin could potentially be used as a novel immunomodulator.

Keywords

streptavidin; biotin; T cell activation; interleukin 2; interleukin 2 receptor

1. Introduction

Streptavidin is a 60 KD protein isolated from *streptomyces avidinii* and has strong biotin binding property similar to an egg-white protein, avidin [1]. Compared to avidin, streptavidin lacks carbohydrate and has less nonspecific interaction besides biotin [2]. Its high affinity for biotin has been used for a variety of biological and analytical research by coupling with various reporter probes, such as fluorescence dyes, radioactive elements or enzymes. In addition, biotin-streptavidin system is also applied to human clinical medicine for cancer diagnosis, radioactive imaging and pre-targeted immunotherapy [3,4,5,6]. Previous studies reported that administration of streptavidin after heart transplant prolonged survival and streptavidin suppressed T cell proliferation in mixed splenocyte culture [7,8]. These findings suggest immunosuppressive effect of streptavidin and its potential

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5. Conflict of interest None.

application of streptavidin in suppressing transplant rejections. Here we investigated the mechanisms of immunosuppressive effect of streptavidin with a focus on CD4⁺ T cell activation and proliferation.

T cells play an important role in pathogenesis of many immune mediated inflammatory diseases and rejection of transplanted organs [9,10,11,12]. Thus, T cell functions are often modulated in the treatment of those autoimmune diseases and in suppression of rejection of transplants [13,14,15,16,17]. Since interleukin 2 (IL-2) is required to initiate and support T cell activation [18], IL-2 and IL-2 signal transduction are favorite target for T cell suppression. For instance, cyclosporine and FK506 inhibit intracellular calcineurin which stimulates IL-2 expression during T cell immune response [19,20,21], and rapamycin dampens IL-2 signaling [22]. In addition to inhibiting IL-2 synthesis and IL-2 signal transduction, cytostatic drugs, such as azathioprine and methotrexate, are also commonly used as immunosuppressant [23,24,25]. Current immunosuppression regimens often include combination of these drugs [26].

We found that streptavidin suppresses T cell activation and proliferation upon T cell receptor (TCR) stimulation. In addition, IL-2 synthesis and IL-2 receptor (CD25) expression were also reduced with streptavidin treatment. CD25 is α chain of IL-2 receptor and has high affinity for IL-2 when expressed with β (CD122) and γ chain (CD132) [18]. In response to CD3/CD28 stimulation, CD25 is highly expressed on T cell surface and support T cell activation by IL-2 signal transduction. The immunosuppressive capacity of streptavidin was diminished by excessive amount of biotin. Because of its high affinity binding to biotin, biotin deprivation induced by streptavidin could be one of potential adverse effects when streptavidin is administrated *in vivo*. In order to avoid the global biotin depletion, we designed a T cell specific delivery system by using single chain anti-CD7 variable fragment (scFvCD7) conjugated with streptavidin. This scFvCD7-streptavidin (scFvCD7-SA) system is more efficient in suppressing T cells activation.

2. Material and methods

2.1 Reagents

Streptavidin (#S4762) and biotin (#B4501) were purchased from Sigma-Aldrich. Streptavidin was dissolved in sterile de-ionized water at final concentration of 2 mg/ml. Biotin was dissolved in DMSO at 100 mg/ml and further diluted in culture medium for use in experiments.

2.2 Human CD4⁺ T cells

Human CD4⁺ T cells were purified by negative selection from peripheral blood mononuclear cells (PBMCs) of healthy donors. PBMCs were incubated with a cocktail of monoclonal antibodies (mAb) to lineage antigens including CD8, CD19, CD56, CD11b and CD11c (BioLegend), followed by Dynabeads-conjugated anti-mouse Ig (Invitrogen #110-31). Those non-CD4⁺ cells were removed by using magnet. The purity of CD4⁺ T cells is typically >90%. This study was approved by Oregon Health & Science University Institutional Review Board.

2.3 T cell activation and proliferation

CD4⁺ T cells were cultured in RPMI 1640 containing 2% human AB serum (Gemini Bio-products #100-512) and seeded into U-bottomed 96-well culture plate at 2×10^5 cells/well. T cells were activated with beads-conjugated anti-CD3 and anti-CD28 mAb in the T cell activation/expansion kit (Miltenyi Biotec Inc. #130-091-441) according to manufacturer's instruction. For proliferation assay, human PBMCs were stained with CFSE (Invitrogen

#C34554) at 1 μ M for 10 minutes at 37 °C, then washed twice with PBS and resuspended in culture medium. After CFSE staining, cells were activated with T cell activation/expansion kit. For T cell suppression assay, CD4⁺ T cells were pre-incubated with 1 – 20 μ g/ml of streptavidin, scFvCD7-SA or scFvCD7 for 16 hours, and subsequently stimulated with anti-CD3/CD28 without replacing medium. For biotin reversal assay, biotin and streptavidin were mixed at various ratio (biotin:streptavidin = 1–100:1) for 30 minutes and biotin-streptavidin complex was added to human CD4⁺ T cells. 24 hours after incubation, supernatant was harvested and IL-2 levels were quantified by ELISA and CD25/CD69 expression on T cells was determined by flow cytometry.

2.4 Flow cytometry

Cell surface staining was performed at 4 °C for 20 minutes in FACS buffer (PBS containing 1% BSA, 0.01% sodium azide). CD25 and CD69 staining was performed with fluorochrome-labeled anti-CD25 (BioLegend #302610) and anti-CD69 antibody (BD Pharmingen #341652).

2.5 Cytotoxicity assay

Human CD4⁺ T cells were incubated with 100 μ g/ml streptavidin and beads conjugated anti-CD3/CD28 mAb. After 48 hour incubation, cells were stained with Annexin V-PE (BD Bioscience #556421) and propidium iodide (BD Bioscience #556463), and cell viability was quantified by flow cytometry.

2.6 Real Time PCR

Human CD4⁺ T cells were stimulated with beads conjugated anti-CD3/CD28 mAb for 24 hours, RNA was extracted by using RNeasy Mini Kits (Qiagen) following standard manufacture's protocol. Purity of RNA was confirmed by NanoDrop. SuperScript III kit (Invitrogen) was used for reverse transcription and StepOnePlus™ system (Applied Biosystem) was used for quantitative PCR. Each sample was amplified in triplicate and target transcripts were normalized to GUSB mRNA expression as an internal control. The relative expression of each gene was calculated by the comparative cycling method. The following probes were used (Applied Biosystem assay identification numbers in parentheses): IL-2 (Hs00174114_m1), CD25 (Hs00907779_m1), GUSB (Hs99999908_m1).

2.7 Streptavidin-conjugated scFvCD7

cDNA encoding scFvCD7 was kindly provided by Dr. George Fey (University of Erlangen-Nuremberg, German) [27]. Recombinant scFvCD7 was produced in E. coli BL21 system and refolded as described [28]. Binding of scFvCD7 was confirmed by pre-incubation of scFvCD7 (5 μ g/ml) to PBMC for 30 minutes on ice and followed by staining with APC-anti-CD3 (BioLegend #300312) and PE-anti-CD7 antibody (BD Pharmingen #55361). scFvCD7 and streptavidin were conjugated by Lightning-Link Streptavidin (Innova Biosciences #708–0010) following manufacture's protocol.

2.8 Statistics

The percentages of cell viability between streptavidin treated and control groups was compared using Chi square test. A Student's *t* test was performed to compare the difference of IL-2 and CD25 between streptavidin treated and control groups.

3. Results

3.1 Streptavidin suppressed CD4⁺ T cell activation and proliferation *in vitro*

We first investigated the effect of streptavidin on T cell activation by examining CD25 and CD69 expression. CD25 and CD69 were rapidly expressed following TCR ligation [29,30]. CD4⁺ T cells were first incubated with various concentrations of streptavidin for 16 hours and then activated with beads-conjugated anti-CD3/CD28 mAb without replacing medium. 24 hours after stimulation, CD25 and CD69 expression on T cell surface were determined by flow cytometry. Streptavidin treatment profoundly suppressed expression of both CD25 and CD69 (Figure 1A). Next, we investigated the influence of streptavidin on T cell proliferation. CFSE-labeled PBMCs were incubated with 10 µg/ml streptavidin for 16 hours then followed by stimulation with anti-CD3/CD28 mAb and cultured for 120 hours. As shown in Figure 1B, T cell proliferation was completely inhibited by streptavidin. In order to exclude the direct cytotoxicity of streptavidin to cells, we investigated the T cell viability after streptavidin incubation. As shown in Figure 1C and D, streptavidin did not affect cell viability. Interestingly, Annexin V staining showed that streptavidin, at the highest concentration used in the experiments, had protective role against apoptosis upon CD3/CD28 stimulation. Since T cells are led to apoptosis upon stimulation (activation induced cell death) [31], the protective effect of streptavidin may be a result of its suppressive effect on T cell activation.

3.2 Streptavidin reduced IL-2 synthesis and CD25 expression

We next investigated the mechanism of immunosuppressive effect of streptavidin on T cells. Upon TCR ligation, the initial reaction of CD4⁺ T cells is IL-2 induction followed by expression of IL-2 receptor on cell membrane, especially the α -chain (CD25) [18]. Therefore, we examined the effect of streptavidin on IL-2 production and CD25 expression. IL-2 expression was analyzed by measuring IL-2 level in supernatant and IL-2 and CD25 mRNA expressions were analyzed by quantitative RT-PCR. IL-2 production was inhibited by streptavidin in a dose-dependent manner. mRNA expression of IL-2 and CD25 was significantly suppressed (Figure 2A, B).

3.3 Immunosuppressive effect of streptavidin was reversed by excessive amount of biotin

Streptavidin was first found as antibiotics and the antibiotic effect was diminished by excessive amount of biotin [1]. Therefore, we examined the influence of biotin on immunosuppressive effect of streptavidin. Streptavidin was mixed with biotin at various molar ratio (streptavidin:biotin = 1:0 – 1:100) and incubated for 30 minutes before incubation with T cells. One streptavidin molecule is known to bind four biotin molecules [2], therefore, the excessive ratio is considered to saturate all biotin binding sites of streptavidin. The suppressive effect of streptavidin on IL-2 production (Figure 3A) and on CD25 and CD69 expression was diminished by excessive amount of biotin in a dose dependent manner (Figure 3B).

3.4 Streptavidin conjugated with scFvCD7 inhibited T cell activation more efficiently than free streptavidin

Given the fact that biotin deficiency causes several symptoms including dermatitis or teratogenicity [32,33,34], global biotin depletion by streptavidin should be avoided when streptavidin is applied *in vivo* as an immunosuppressant. We designed a new streptavidin delivery system to T cells using scFvCD7. CD7 is a pan T cell marker. Though its role in T cell is not well known, T cells from CD7 knocked out mouse respond normally to stimuli [35]. In addition, since CD7 rapidly internalizes after antibody binding, CD7 mAb conjugated with drugs has been used for clinical trials [36,37]. Thus, we take advantage of

this system to delivery streptavidin specifically to T cells. Recombinant scFvCD7 was purified and refolded and its binding capacity was confirmed by a competition assay as described [38]. Pretreatment of scFvCD7 with T cells inhibited subsequent CD7 mAb binding to T cells (Figure 4A) which confirmed scFvCD7 specificity. We conjugated scFvCD7 to streptavidin, and incubated the scFvCD7-streptavidin complex to purified CD4⁺ T cells. Compared to free streptavidin, scFvCD7-streptavidin was 10-fold more efficient in suppressing CD25 and CD69 expression. As little as 1 µg/ml of scFvCD7-streptavidin was required to execute T cell suppressive effect, while scFvCD7 alone, even at the highest concentration used in this experiment (20µg/ml), had no suppressive effect (Figure 4B).

4. Discussion

We demonstrated the profound suppressive effect of streptavidin on CD4⁺ T cell activation and proliferation. This cytostatic effect was associated with a substantial reduction of IL-2 synthesis and CD25 expression. IL-2 signaling is a critical event in CD4⁺ T cell activation and proliferation. It is possible that streptavidin suppressed T cell activation by inhibiting IL-2 production and IL-2 receptor expression. However, the reduced IL-2 and CD25 synthesis could be the consequence of suppressed T cell activation. Further investigation is required to delineate which is the leading event mediated by streptavidin.

The best known property of streptavidin is its high affinity binding to biotin. The reversal of streptavidin suppression on T cells by excessive biotin suggests that blocking biotin pathway involved in such a suppressive action of streptavidin. The role of biotin in T cell biology is poorly understood. A previous study suggests that biotin deficiency impairs IL-2 production and IL-2 metabolism in a T cell lymphoma cell line, Jurkat cells [39]. In Jurkat cells, IL-2 mRNA expression and IL-2 consumption are both reduced in biotin deficient cell culture medium and this effect is attributed to IL-2R γ down-regulation [40]. Our study in primary human CD4⁺ T cells demonstrated that IL-2 production and CD25 synthesis were both profoundly suppressed by streptavidin-mediated deprivation of biotin. How IL-2 and CD25 gene expression is regulated by biotin in T cells remains to be elucidated, but recent studies shed light on the role of biotin in gene transcription. In addition to being a cofactor in carboxylation reactions, biotin also plays an important role in cell signaling, gene expression and chromatin structure [34,41,42,43]. Many genes are dependent on biotin for expression [41], in which biotin is likely involved in epigenetic regulation[44]. Both IL-2 and CD25 gene expression is finely regulated by many transcription factors at transcription level. Biotin affects translocation and DNA binding activity of NF- κ B and Sp1 [45,46] which are both involved in regulation of IL-2 and CD25 gene expression. It is possible that dysfunction of NF- κ B and Sp1 resulted from deficient supply of biotin might impair IL-2 production and CD25 expression and subsequently negatively affect T cell activation in streptavidin treated CD4⁺ T cells.

Immunosuppressants isolated from *Streptomyces* species in clinical use include FK506 and rapamycin. FK506 inhibits intracellular calcium release upon T cell receptor signaling by binding to FKBP [21] and IL-2 synthesis is suppressed. In contrast, rapamycin does not inhibit IL-2 production but blocks IL-2 signal transduction by binding to mammalian target of rapamycin (mTOR) [22]. The remarkably enhanced suppression by streptavidin that is directly delivered to T cells may be due to its binding to intracellular biotin. Alternatively, other than binding to biotin, it is possible that streptavidin which is internalized by scFvCD7 also binds to an unknown intracellular ligand(s) that involves in T cell activation or IL-2 and CD25 production.

Finally, our study uncovered an important novel property of streptavidin – suppression of T cell activation and proliferation. Further studies to delineate the molecular mechanisms of

action by streptavidin on T cells will yield useful information for future exploration of streptavidin as a potential therapeutic immunosuppressant for immune mediated inflammatory diseases and rejection of transplants. However, the potential immunogenicity and biotin depletion will limit the direct use of streptavidin as a therapeutic agent and molecular modification will be required. The high effectiveness of T cell specific delivery of streptavidin can be further explored for developing T cell targeted immunosuppression to avoid global deprivation of biotin.

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Abbreviations

SA	streptavidin
scFv	single chain variable fragment
NT	no treatment

Reference

- [1]. Tausig F, Wolf FJ. Streptavidin--a substance with avidin-like properties produced by microorganisms. *Biochem Biophys Res Commun.* 1964; 14:205–209. [PubMed: 5319841]
- [2]. Gitlin G, Khait I, Bayer EA, Wilchek M, Muszkat KA. Studies on the biotin-binding sites of avidin and streptavidin. A chemically induced dynamic nuclear polarization investigation of the status of tyrosine residues. *Biochem J.* 1989; 259:493–498. [PubMed: 2719662]
- [3]. Forero A, Weiden PL, Vose JM, Knox SJ, LoBuglio AF, Hankins J, Goris ML, Picozzi VJ, Axworthy DB, Breitz HB, Sims RB, Ghalie RG, Shen S, Meredith RF. Phase 1 trial of a novel anti-CD20 fusion protein in pretargeted radioimmunotherapy for B-cell non-Hodgkin lymphoma. *Blood.* 2004; 104:227–236. [PubMed: 14996706]
- [4]. Kalofonos HP, Rusckowski M, Siebecker DA, Sivolapenko GB, Snook D, Lavender JP, Epenetos AA, Hnatowich DJ. Imaging of tumor in patients with indium-111-labeled biotin and streptavidin-conjugated antibodies: preliminary communication. *J Nucl Med.* 1990; 31:1791–1796. [PubMed: 2230992]
- [5]. Shen S, Forero A, LoBuglio AF, Breitz H, Khazaeli MB, Fisher DR, Wang W, Meredith RF. Patient-specific dosimetry of pretargeted radioimmunotherapy using CC49 fusion protein in patients with gastrointestinal malignancies. *J Nucl Med.* 2005; 46:642–651. [PubMed: 15809487]
- [6]. Lazzeri E, Pauwels EK, Erba PA, Volterrani D, Manca M, Bodei L, Trippi D, Bottoni A, Cristofani R, Consoli V, Palestro CJ, Mariani G. Clinical feasibility of two-step streptavidin/111In-biotin scintigraphy in patients with suspected vertebral osteomyelitis. *Eur J Nucl Med Mol Imaging.* 2004; 31:1505–1511. [PubMed: 15241627]
- [7]. Fawwaz RA, Oluwale OO, DePaz HA, Jin MX, Wang TS, Hardy MA, Oluwale SF. Peritransplant streptavidin recipient treatment prolongs rat cardiac allograft survival. *Transplantation.* 2002; 73:1954–1956. [PubMed: 12131695]
- [8]. Witkowski P, Fawwaz RA, Jin MX, DePaz HA, Oluwale OO, Hardy MA, Oluwale SF. Effect of streptavidin on cardiac allograft prolongation is due to host T-Cell suppression. *Transplant Proc.* 2005; 37:116–118. [PubMed: 15808566]
- [9]. Cornell LD, Smith RN, Colvin RB. Kidney transplantation: mechanisms of rejection and acceptance. *Annu Rev Pathol.* 2008; 3:189–220. [PubMed: 18039144]
- [10]. Felix NJ, Allen PM. Specificity of T-cell alloreactivity. *Nat Rev Immunol.* 2007; 7:942–953. [PubMed: 18007679]
- [11]. Davidson A, Diamond B. Autoimmune diseases. *N Engl J Med.* 2001; 345:340–350. [PubMed: 11484692]

- [12]. Goodnow CC, Sprent J, Fazekas de St Groth B, Vinuesa CG. Cellular and genetic mechanisms of self tolerance and autoimmunity. *Nature*. 2005; 435:590–597. [PubMed: 15931211]
- [13]. Pascual M, Theruvath T, Kawai T, Tolkoff-Rubin N, Cosimi AB. Strategies to improve long-term outcomes after renal transplantation. *N Engl J Med*. 2002; 346:580–590. [PubMed: 11856798]
- [14]. Meier J, Sturm A. Current treatment of ulcerative colitis. *World J Gastroenterol*. 2011; 17:3204–3212. [PubMed: 21912469]
- [15]. Yoon KH. Efficacy and cytokine modulating effects of tacrolimus in systemic lupus erythematosus: a review. *J Biomed Biotechnol*. 2010; 2010:686480. [PubMed: 20625508]
- [16]. Svensson A, Chambers C, Ganemo A, Mitchell SA. A systematic review of tacrolimus ointment compared with corticosteroids in the treatment of atopic dermatitis. *Curr Med Res Opin*. 2011; 27:1395–1406. [PubMed: 21563877]
- [17]. Menter A, Korman NJ, Elmets CA, Feldman SR, Gelfand JM, Gordon KB, Gottlieb A, Koo JY, Lebwohl M, Lim HW, Van Voorhees AS, Beutner KR, Bhushan R. Guidelines of care for the management of psoriasis and psoriatic arthritis. Section 3. Guidelines of care for the management and treatment of psoriasis with topical therapies. *J Am Acad Dermatol*. 2009; 60:643–659. [PubMed: 19217694]
- [18]. Kim HP, Imbert J, Leonard WJ. Both integrated and differential regulation of components of the IL-2/IL-2 receptor system. *Cytokine Growth Factor Rev*. 2006; 17:349–366. [PubMed: 16911870]
- [19]. Hemenway CS, Heitman J. Calcineurin. Structure, function, and inhibition. *Cell Biochem Biophys*. 1999; 30:115–151. [PubMed: 10099825]
- [20]. Handschumacher RE, Harding MW, Rice J, Drugge RJ, Speicher DW. Cyclophilin: a specific cytosolic binding protein for cyclosporin A. *Science*. 1984; 226:544–547. [PubMed: 6238408]
- [21]. Siekierka JJ, Staruch MJ, Hung SH, Sigal NH. FK-506, a potent novel immunosuppressive agent, binds to a cytosolic protein which is distinct from the cyclosporin A-binding protein, cyclophilin. *J Immunol*. 1989; 143:1580–1583. [PubMed: 2474605]
- [22]. Powell JD, Delgoffe GM. The mammalian target of rapamycin: linking T cell differentiation, function, and metabolism. *Immunity*. 2010; 33:301–311. [PubMed: 20870173]
- [23]. Smolen JS, Landewe R, Breedveld FC, Dougados M, Emery P, Gaujoux-Viala C, Gorter S, Knevel R, Nam J, Schoels M, Aletaha D, Buch M, Gossec L, Huizinga T, Bijlsma JW, Burmester G, Combe B, Cutolo M, Gabay C, Gomez-Reino J, Kouloumas M, Kvien TK, Martin-Mola E, McInnes I, Pavelka K, van Riel P, Scholte M, Scott DL, Sokka T, Valesini G, van Vollenhoven R, Winthrop KL, Wong J, Zink A, van der Heijde D. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs. *Ann Rheum Dis*. 2010; 69:964–975. [PubMed: 20444750]
- [24]. Bergan S, Rugstad HE, Bentdal O, Sodal G, Hartmann A, Leivestad T, Stokke O. Monitored high-dose azathioprine treatment reduces acute rejection episodes after renal transplantation. *Transplantation*. 1998; 66:334–339. [PubMed: 9721802]
- [25]. Nielsen OH, Vainer B, Rask-Madsen J. Review article: the treatment of inflammatory bowel disease with 6-mercaptopurine or azathioprine. *Aliment Pharmacol Ther*. 2001; 15:1699–1708. [PubMed: 11683683]
- [26]. Weimer R, Deisz S, Dietrich H, Renner F, Bodeker RH, Daniel V, Kamali-Ernst S, Ernst W, Padberg W, Opelz G. Impact of maintenance immunosuppressive regimens--balance between graft protective suppression of immune functions and a near physiological immune response. *Transpl Int*. 2011; 24:596–609. [PubMed: 21401729]
- [27]. Peipp M, Kupers H, Saul D, Schlierf B, Greil J, Zunino SJ, Gramatzki M, Fey GH. A recombinant CD7-specific single-chain immunotoxin is a potent inducer of apoptosis in acute leukemic T cells. *Cancer Res*. 2002; 62:2848–2855. [PubMed: 12019163]
- [28]. Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, Trauger S, Bien G, Yao S, Zhu Y, Siuzdak G, Scholer HR, Duan L, Ding S. Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell*. 2009; 4:381–384. [PubMed: 19398399]
- [29]. Sancho D, Gomez M, Sanchez-Madrid F. CD69 is an immunoregulatory molecule induced following activation. *Trends Immunol*. 2005; 26:136–140. [PubMed: 15745855]

- [30]. Zola H. Markers of cell lineage, differentiation and activation. *J Biol Regul Homeost Agents*. 2000; 14:218–219. [PubMed: 11037056]
- [31]. Hargreaves RG, Borthwick NJ, Gilardini Montani MS, Piccolella E, Carmichael P, Lechler RI, Akbar AN, Lombardi G. Induction of apoptosis following antigen presentation by T cells: anergy and apoptosis are two separate phenomena. *Transplant Proc*. 1997; 29:1102–1104. [PubMed: 9123219]
- [32]. Mock DM. Marginal biotin deficiency is teratogenic in mice and perhaps humans: a review of biotin deficiency during human pregnancy and effects of biotin deficiency on gene expression and enzyme activities in mouse dam and fetus. *J Nutr Biochem*. 2005; 16:435–437. [PubMed: 15992686]
- [33]. Gehrig KA, Dinulos JG. Acrodermatitis due to nutritional deficiency. *Curr Opin Pediatr*. 2010; 22:107–112. [PubMed: 19966568]
- [34]. McMahon RJ. Biotin in metabolism and molecular biology. *Annu Rev Nutr*. 2002; 22:221–239. [PubMed: 12055344]
- [35]. Bonilla FA, Kokron CM, Swinton P, Geha RS. Targeted gene disruption of murine CD7. *Int Immunol*. 1997; 9:1875–1883. [PubMed: 9466315]
- [36]. Lazarovits AI, Rochon J, Banks L, Hollomby DJ, Muirhead N, Jevnikar AM, White MJ, Amlot PL, Beauregard-Zollinger L, Stiller CR. Human mouse chimeric CD7 monoclonal antibody (SDZCHH380) for the prophylaxis of kidney transplant rejection. *J Immunol*. 1993; 150:5163–5174. [PubMed: 7684422]
- [37]. Frankel AE, Laver JH, Willingham MC, Burns LJ, Kersey JH, Vallera DA. Therapy of patients with T-cell lymphomas and leukemias using an anti-CD7 monoclonal antibody-ricin A chain immunotoxin. *Leuk Lymphoma*. 1997; 26:287–298. [PubMed: 9322891]
- [38]. Kumar P, Ban HS, Kim SS, Wu H, Pearson T, Greiner DL, Laouar A, Yao J, Haridas V, Habiro K, Yang YG, Jeong JH, Lee KY, Kim YH, Kim SW, Peipp M, Fey GH, Manjunath N, Shultz LD, Lee SK, Shankar P. T cell-specific siRNA delivery suppresses HIV-1 infection in humanized mice. *Cell*. 2008; 134:577–586. [PubMed: 18691745]
- [39]. Rodriguez-Melendez R, Lewis B, McMahon RJ, Zemleni J. Diaminobiotin and desthiobiotin have biotin-like activities in Jurkat cells. *J Nutr*. 2003; 133:1259–1264. [PubMed: 12730407]
- [40]. Rodriguez-Melendez R, Camporeale G, Griffin JB, Zemleni J. Interleukin-2 receptor-gamma - dependent endocytosis depends on biotin in Jurkat cells. *Am J Physiol Cell Physiol*. 2003; 284:C415–421. [PubMed: 12388078]
- [41]. Zemleni J. Uptake, localization, and noncarboxylase roles of biotin. *Annu Rev Nutr*. 2005; 25:175–196. [PubMed: 16011464]
- [42]. Stanley JS, Griffin JB, Zemleni J. Biotinylation of histones in human cells. Effects of cell proliferation. *Eur J Biochem*. 2001; 268:5424–5429. [PubMed: 11606205]
- [43]. Hassan YI, Zemleni J. Epigenetic regulation of chromatin structure and gene function by biotin. *J Nutr*. 2006; 136:1763–1765. [PubMed: 16772434]
- [44]. Kuroishi T, Rios-Avila L, Pestinger V, Wijeratne SS, Zemleni J. Biotinylation is a natural, albeit rare, modification of human histones. *Mol Genet Metab*. 2011; 104:537–545. [PubMed: 21930408]
- [45]. Rodriguez-Melendez R, Schwab LD, Zemleni J. Jurkat cells respond to biotin deficiency with increased nuclear translocation of NF-kappaB, mediating cell survival. *Int J Vitam Nutr Res*. 2004; 74:209–216. [PubMed: 15296080]
- [46]. Griffin JB, Rodriguez-Melendez R, Zemleni J. The nuclear abundance of transcription factors Sp1 and Sp3 depends on biotin in Jurkat cells. *J Nutr*. 2003; 133:3409–3415. [PubMed: 14608051]

- Streptavidin suppressed CD4⁺ T cell activation and proliferation.
- Interleukin 2 production and interleukin 2 receptor α expression were reduced by streptavidin.
- Excessive biotin reversed immunosuppressive function of streptavidin.
- T cell specific delivery of streptavidin was more efficient in suppressing T cell activation.

Figure 1. Streptavidin suppressed T cell activation and proliferation without cytotoxicity
 (A) Human CD4⁺ T cells were treated with 100 μ g/ml of streptavidin (SA) for 16 hours and subsequently stimulated with anti-CD3/CD28 mAb coated beads. 24 hour after stimulation, CD25 and CD69 on cell surface were stained. (B) CFSE labeled human PBMCs were incubated with 10 μ g/ml of streptavidin and followed by anti-CD3/CD28 mAb coated beads for 120 hours. CFSE dilution was analyzed by flow cytometry (gated on live CD4⁺ T cells). (C, D) Human CD4⁺ T cells were treated as in (A) and cells were stained with PI and Annexin V. PI negative cells were quantified by flow cytometric analysis and shown as mean SEM (n=3). NT: no treatment. $p > 0.05$. All data were representative of three independent experiments.

Figure 2. Streptavidin inhibited IL-2 synthesis and CD25 expression

(A) Human CD4⁺ T cells were treated with 0.5–100 μg/ml of streptavidin for 16 hours and subsequently stimulated with anti-CD3/CD28 mAb coated beads. 24 hour after stimulation, supernatants were harvested and IL-2 protein level was measured by ELISA. * $p < 0.05$ when compared with cells that were not treated with streptavidin. (B) Cells were harvested and RNA was extracted after 24 hours of culture. Relative expression of mRNA of IL-2 and CD25 was quantified by RT-PCR. mRNA expression levels of pre-stimulated CD4 T cells are set as 1. **** $p < 0.0001$ when compared with cells that were not treated (NT) with streptavidin. All data are shown as mean SEM (n=3). Representative of three independent experiments.

Figure 3. Immunosuppressive function of streptavidin was reversed by excessive amount of biotin

Streptavidin (SA) was mixed with biotin at ratio (streptavidin : biotin) of 1:0 – 1:100 for 30 min. Streptavidin-biotin complexes were added to human CD4⁺ T cells and incubated for 16 hours and followed by anti-CD3/CD28 stimulation for 24 hours. (A) IL-2 level in supernatant were quantified by ELISA. (B) For CD25/CD69 staining, streptavidin was conjugated with biotin at a ratio of 1:100 before adding to cells. * $p < 0.05$ when compared with cells that were not treated (NT) with streptavidin. All data are shown as mean SEM (n=3) and representative of three independent experiments.

Figure 4. scFvCD7 conjugated with streptavidin suppressed T cells more efficiently compared to free streptavidin

(A) Human PBMCs were pre-treated with scFvCD7 for 30 minutes and followed by staining with PE-anti-CD7 antibody (clone: M-T701). (B) Human CD4⁺ T cells was pre-treated with scFvCD7 conjugated with streptavidin (scFvCD7-SA) at 0.05 – 20 $\mu\text{g/ml}$, scFvCD7 alone or streptavidin alone (1 – 20 $\mu\text{g/ml}$) and followed by anti-CD3/CD28 stimulation for 24 hours. CD25 and CD69 were determined by flow cytometry. (NT = not treated). Representative of three independent experiments.