Reduction of CD147 surface expression on primary T cells leads to enhanced cell proliferation

Brian Biegler[1] and Watchara Kasinrerk[1,2]

Summary

Background: **CD147 is a ubiquitously expressed membrane glycoprotein that has numerous functional associations in health and disease. However, the molecular mechanisms by which CD147 participates in these processes are unclear. Establishing physiologically relevant silencing of CD147 in primary T cells could provide clues essential for elucidating some aspects of CD147 biology. To date, achieving the knockdown of CD147 in primary T cells has remained elusive.**

Objective: **Utilizing RNA interference and the Nucleofector transfection system, we were able to reduce the expression of CD147 in primary T cells. Comparison of basic functions, such as proliferation and CD25 expression, were then made between control populations and populations with reduced expression.**

Results: **Up-regulation of CD147 was found upon T-cell activation, indicating a role in T-cell responses. To better understand the possible importance of this up-regulation, we knocked down the expression of CD147 using RNA interference. When compared to control populations the CD147 knockdown populations exhibited increased proliferation. This alteration of cell proliferation, however, was not linked to a change in CD25 expression.**

Conclusions: **We achieved reduction of CD147 surface expression in primary T cells by siRNA-mediated gene silencing. Our results point to CD147 having a possible negative regulatory role in T cell-mediated immune responses.** ***(Asian Pac J Allergy Immunol 2012;30:259-67)***

Key words: *CD147, Electroporation, siRNA, T cell activation, Immunoregulation*

From 1. Division of Clinical Immunology, Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai 50200, Thailand.

2. Biomedical Technology Research Center, National Center for Genetic Engineering and Biotechnology, National Sciences and Technology Development Agency at the Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai 50200, Thailand.

Corresponding author: Watchara Kasinrerk

E-mail: watchara.k@cmu.ac.th

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Introduction

CD147 is a 35–60 kDa type 1 integral membrane glycoprotein that belongs to the immunoglobulin (Ig) superfamily[1] and is expressed on a variety of cell types, including: haematopoietic cells, epithelial cells, endothelial cells and leukocytes.[1,2] The CD147 molecule is 248-269 amino acids long and contains two extracellularly-located Ig-like domains. Since its discovery, many studies have probed the myriad functional associations of this molecule and found it to be involved in a number of disparate processes.

CD147 expression is highly up-regulated on tumor cells and found to influence the induction of various types of matrix metalloproteinases (MMPs) leading to the degradation of surrounding matrix and advancement of tumor metastasis.[3-7] Moreover, CD147 has been found to mediate angiogenesis of tumor cells via stimulation of vascular endothelial growth factor (VEGF).[8] Additionally CD147 has been shown to associate with a plethora of molecules, such as monocarboxycylic acid transporters,[9] α3β1 and α6β1 integrins,[10] and even itself.[11] Reduced CD147 in hepatocellular carcinoma cells induces a reduction in cyto-skeletal adhesion kinases which are important for cell-to-cell contact and cell migration.[12] Although CD147 has been shown to be involved in tumor growth and metastasis and even recently recognized as a target molecule for tumor-therapy, the intracellular pathways by which CD147 induces MMPs and other events are not yet clear.[13]

CD147 has also been shown to be involved in the regulation of immune responses.[14,15,16,17] CD147 is expressed on T cells and is up-regulated upon cell

activation.[1,2,18] This up-regulation occurs whether the T cell is activated through its TCR or by mitogens such as PHA. Monoclonal antibodies (mAbs) specific for certain epitopes on CD147 strongly inhibit T-cell proliferation induced by anti-CD3 mAb.[15,19] In a recent study using the Jurkat T Lymphoma cell line, one group reports decreased proliferation, decreased adhesion to fibronectin, cell cycle arrest, and reduced trans-endothelial migration when CD147 surface expression was knocked down with RNAi technology.[20] However, contradictory to this report, a more detailed analysis by another group demonstrated that CD147 contributes to the negative regulation of T-cell responses as the knockdown of CD147 in Jurkat cells promotes higher levels of NF-AT stimulation and Pak1 phosphorylation upon T-cell activation.[21] Also another study reports the expression of CD147 on T lymphocytes is higher in SLE patients than healthy controls. In contrast to healthy donor cells, the T cells from the SLE patients showed a reduction in tyrosine phophorylation levels when stimulated with anti-CD3, anti-CD28 and anti-CD147 monoclonal antibodies.[22]

Although, several functions of CD147 have been demonstrated, the molecular mechanisms by which CD147 participates in many processes are unclear. Establishing physiologically relevant silencing of CD147 in primary T cells has remained elusive, with the entirety of published gene suppression research coming from T-cell lines. Utilizing siRNA-mediated gene silencing coupled with the Nucleofector® transfection system, we were able to reduce the expression of CD147 in primary T cells. We found that T cells with reduced expression of CD147 exhibited increased proliferation. Our results indicate that CD147 plays a role in the negative regulation of T-cell responses.

Methods

Cell cultures

Peripheral blood mononuclear cells (PBMC) were isolated from the heparinized whole blood of healthy donors by Ficoll-Hypague density gradient centrifugation. The isolated PBMC fraction was suspended at $2x10^6$/ml in RPMI 1640 medium (GIBCO BRL, Grand Island, NY, USA) supplemented with glutamine, 10% fetal calf serum, penicillin and streptomycin (complete RPMI). For cell activation, PBMC were stimulated with 4 µg/ml phytohemagglutinin (PHA) and incubated in a CO_2 incubator at 37°C for 5 days. Human T lymphoma Jurkat cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; GIBCO BRL) supplemented with 10% fetal calf serum, penicillin and streptomycin. Cells were cultured in a CO_2 incubator at 37°C.

siRNA for knockdown CD147

Two pre-designed siRNAs targeting the coding region of the BSG (CD147) gene (Ambion Silencer siRNA ID# 10732 and 215973)(Life Technology, Grand Island, NY, USA) were used in this study. The sequences are as follows: #10732 – 5'>3' GGUUCUUCGUGAGUUCCUCtt , #215973 – 5'>3' GCUACACAUUGAGAACCUGtt. Both sequences target the C2 Ig domain of the CD147 molecule. Silencer select negative control siRNA #1 (cat #4390843) (Life Technology) was used as a control siRNA targeting no known human gene.

Transfection

Electroporation reactions were conducted with the Nucleofector® transfection system (Lonza, Basel, Switzerland). Each Nucleofection reaction contained: 3-5 $x10^6$ cells, 2 µg pmaxGFP vector or 30-300 nM siRNA (3-30 pmol/sample), 100 µl Human T-cell Nucleofector solution that has been supplemented with the included supplement solution. Six or 12-well plates were prepared by filling them with 1.5-3.0 ml of culture media and were placed in a cell-culture incubator to equilibrate to 37°C. Cells were then soft-spun at 90 x g for 10 minutes at room temperature and the supernatant was aspirated off until no residual media remained. The pellet was then resuspended in 100 µl room temperature Nucleofector solution per sample, or PBS in the case of control samples. The suspension was then combined with siRNA, Pmax GFP, or PBS. The cell suspension was then transferred to a Nucleofector cuvette, covered, tapped and placed in the electroporation machine. The samples were then transfected using protocols U-014 or V-024 for unactivated T cells or X001 for the Jurkat cell line. Once the transfection was complete 500 µl of the pre-equilibrated culture media was added to the cuvette and then redrawn with the sample to add to the culture plate. The cells were then incubated in a CO_2 incubator at 37°C.

Flow Cytometry

Cells were washed 2 times using FACS buffer (PBS containing 1% bovine serum albumin and 0.1% Sodium azide) at 4°C. After the supernatant was decanted, cell pellets were disturbed and fluorochrome-conjugated antibodies were added.

Cells were then incubated for 30 minutes at 4°C in the dark. After incubation the wash procedure was repeated and finally the samples were resuspended in 1% Paraformaldehyde in PBS and analyzed by a flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA).

CFSE-based proliferation assays

siRNA transfected cells (1×10^6 cells/ml in PBS) were incubated with 0.25 μM of carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Eugene, OR, USA) at 37°C for 7 min. The CFSE staining was terminated by adding cold complete RPMI-1640 medium. The cells were then washed twice and resuspended with complete RPMI-1640 medium. To determine the cell proliferation, CFSE labelled PBMCs (2×10^6 cells in complete RPMI medium) were activated with PHA and cultured at 37°C in a 5% CO_2 incubator for 3, 5 or 7 days. Cells were then harvested and determined for cell proliferation by flow cytometry.

Results

CD147 is upregulated upon activation in primary T cells

Activation of PBMC cultures induced an increase in CD147 on the surface of primary T cells. In Figure 1, the histograms show the CD3+ population from the culture activated with PHA compared to a resting culture. The activated culture is confirmed by the CFSE stain showing enhanced proliferation and the up-regulation of CD25. These results indicate that CD147 is clearly up-regulated upon T-cell activation.

CD147 siRNA induces specific reduction of CD147 on primary T cells

In order to better understand the function of CD147, we developed a system for siRNA-mediated reduction of CD147 expression in T cells. Two pre-designed siRNAs targeting the Ig-like C2 coding region of the BSG (CD147) gene, Ambion Silencer siRNA ID# 215973 and 10732, were selected for the knockdown experiments. We first tested the CD147 siRNA protocol by using the T-cell line, Jurkat. As can be seen in Figure 2A, CD147 expression levels are constitutively very high on the Jurkat cells. Using each siRNA targeting CD147 in a separate transfection reaction, a reduction was seen in CD147 expression at 24 hours when compared to the control siRNA reactions. CD298 was stained in these cultures to show that the siRNA was indeed specific for CD147 as there was no knockdown of CD298 in all tested reactions.

We then performed the experiment using PBMC. The histograms in Figure 2B show representative knockdowns in 3 subject samples, 3 days after being activated with PHA. The untreated (untransfected and unactivated) culture is shown as a baseline, along with the CD147siRNA transfected culture and the non-specific siRNA transfected culture. Successful knockdown of CD147 was achieved by using both CD147 siRNAs in tandem at equal concentrations of 100 nM each. The histograms represent: a 50% knockdown in subject J, a 42% knockdown in subject B and a 40% knockdown in subject H. Our study demonstrated, for the first time to our knowledge, the successful knockdown of CD147 expression in primary T cells using siRNA.

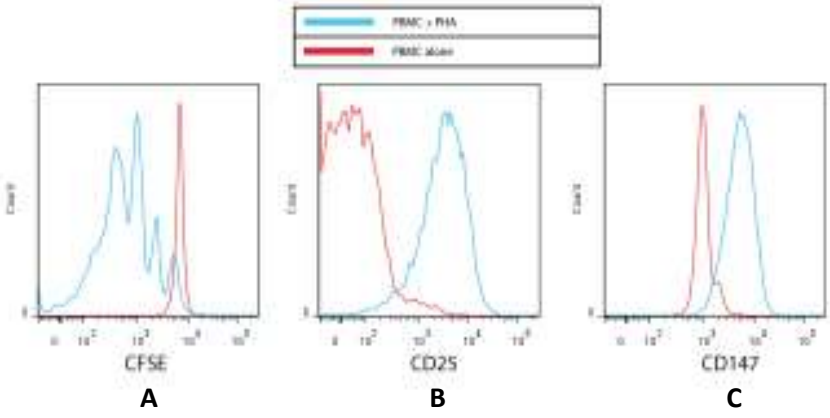
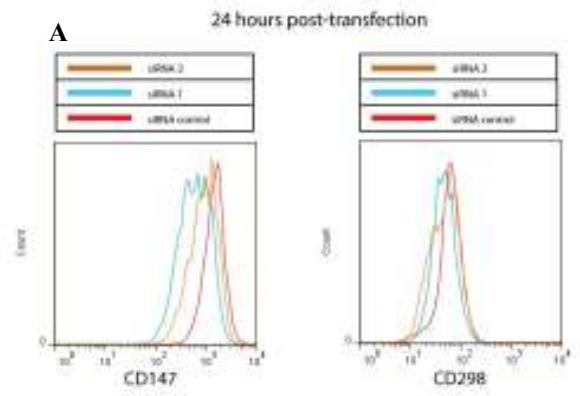


Figure 1. CD147 is up-regulated upon T cell activation. PBMC were cultured with PHA 4 μg/ml or media alone for 5 days. For flow cytometric analysis, cells were harvested and stained with the indicated mAbs in conjunction with CFSE staining for the measurement of proliferation. PBMC populations were analyzed by drawing a lymphocyte gate and then isolating the CD3+ fraction. CFSE staining (A) and CD25 staining (B) indicate successful activation of the culture. CD147 is up-regulated when compared to the non-activated culture (C).



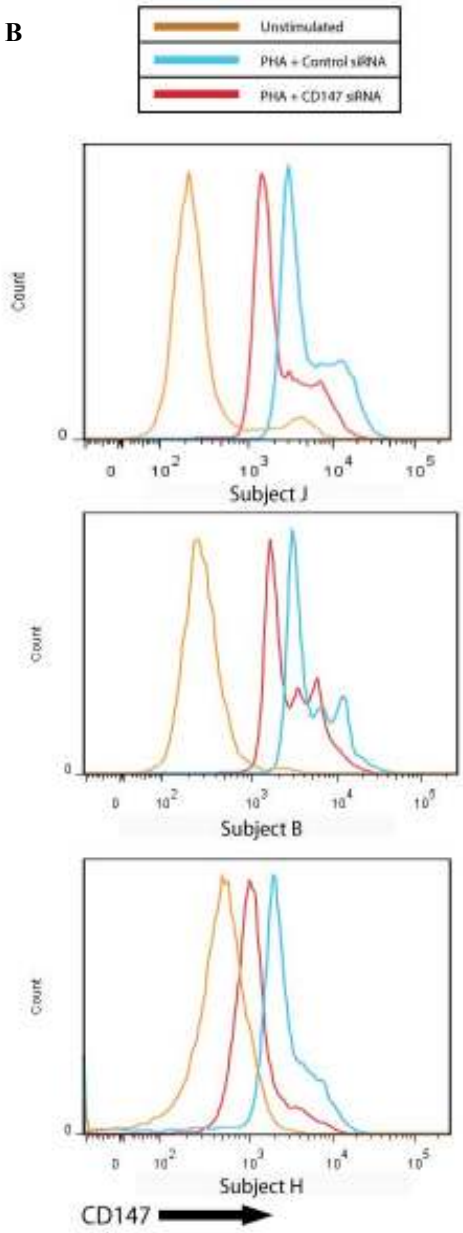


Figure 2. CD147 siRNA induces specific reduction of CD147. **A.** Two siRNAs (siRNA 1 and siRNA 2) targeting CD147 or irrelevant siRNA (siRNA control) were transfected into T lymphoma Jurkat cells which constitutively express high levels of CD147. Twenty-four hours post transfection, surface CD147 and CD298 were monitored by flow cytometry. Using each siRNA CD147 in a separate reaction, a reduction of CD147 expression was observed while levels of the control marker, CD298, remain unchanged.
B. Successful knockdown of CD147 expression in primary T cells from three individual subjects. Histograms show three examples of CD147 knockdown (Subject J, B and H). PBMC were transfected in the Nucleofector® utilizing Program U-014. Two CD147 specific siRNAs (CD147 siRNA; Ambion Silencer siRNA ID# 215973 and 10732) were used in combination (100 nM each) or 200 nM control siRNA. Twenty-four hours post transfection cultures were stimulated with PHA 4 µg/ml. Harvested cells were stained and analyzed by flow cytometry 3 days post-activation. Un-stimulated PBMC are shown for comparison.

Increased proliferation upon CD147 knockdown

Primary T cells transfected with anti-CD147 siRNA that show reduced expression of CD147 on their surface were found to have enhanced proliferation when compared to cultures that were: transfected with non-specific siRNA sequences (Figure 3A), or transfected with anti-CD147 siRNA but no knockdown was achieved (Figure 3B). A comparison between the siRNA-induced successful knockdown of CD147 expression and the unsuccessful knockdown experiments in Figure 3B demonstrate that it is the level of CD147 expression and not merely the addition of the siRNA to the culture that is leading to the enhanced proliferation observed in the CFSE staining.

Correlation between the reduction of CD147 and proliferation

There is a clear correlation between the percent reduction of CD147 expression achieved and the percent increase in proliferation (Figure 4A). The Figure is a composite of replicate experiments conducted in 4 different subjects. In most cases proliferative measurements were taken on day 3 and day 5 post-activation. In one case measurements were also taken on day 7. Interestingly, negative proliferation/reduction corresponds to knockdown experiments that were unsuccessful, where there was no significant difference in CD147 expression or in fact the control culture's expression was lower than the knockdown culture's expression.

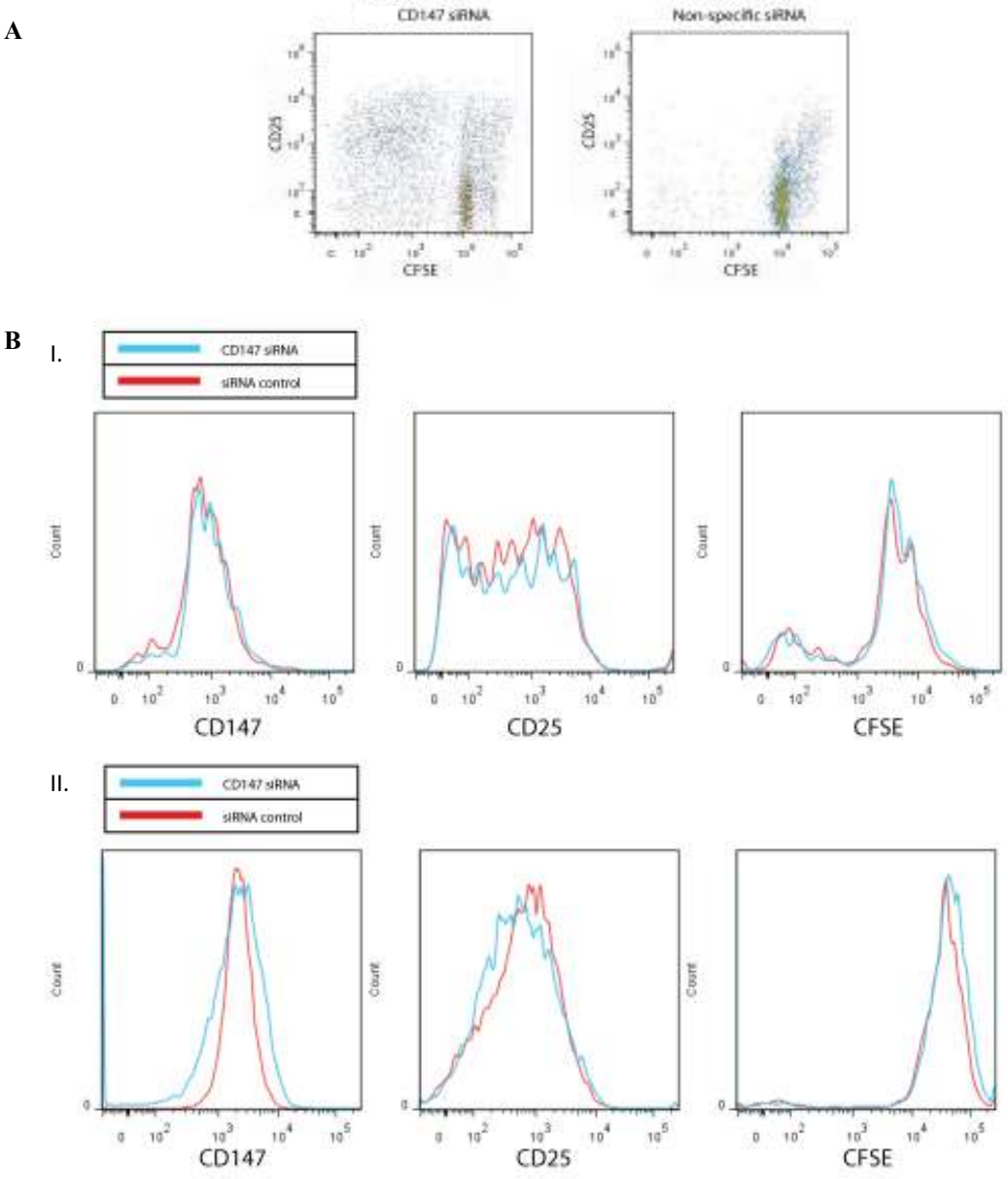


Figure 3. A. Primary T cells with reduced expression of CD147 show enhanced proliferation by CFSE staining. In a representative example (subject J from Figure 2b) CFSE staining shows increased proliferation in the culture transfected with CD147 siRNA compared to the culture transfected with non-specific siRNA. Surface staining for CD25 showed no differences between the knockdown and control cultures. The experimental conditions were the same as described Figure 2B.
B. Experiments where CD147 knockdown was not achieved show comparable levels of proliferation and CD25 expression. Two individual experiments in two subjects (I and II) show comparable levels of both proliferation and CD25 expression when CD147 was found to be expressed at the same level in a failed CD147 knockdown.

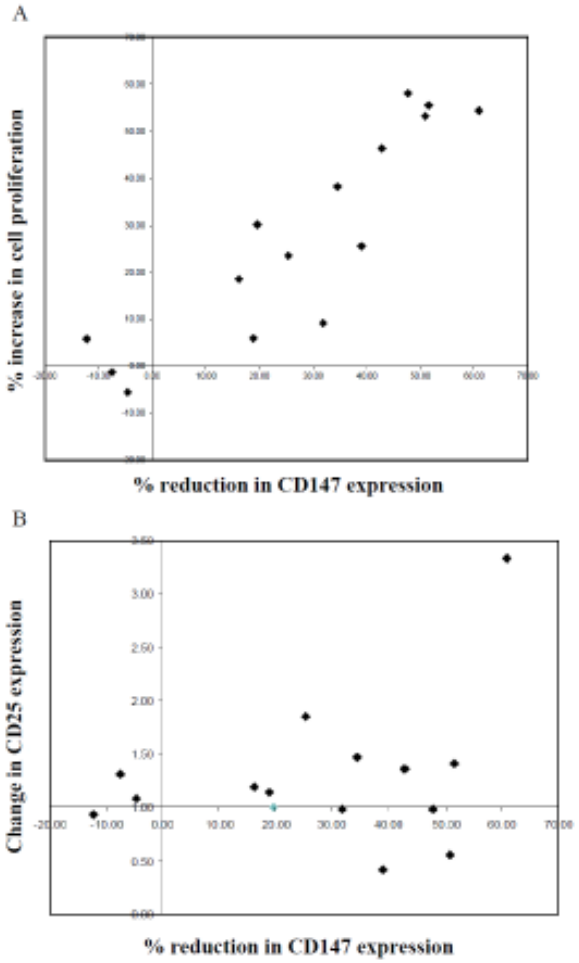


Figure 4. A. Correlation between reduction of CD147 expression and increase in proliferation in primary T cells. The reduction in CD147 expression correlates with a resulting increase in proliferation as measured by CFSE staining. These data represent 7 experiments with 2 time-points each (days 3 and 5), except for one which had 3 time-points (day 3, 5 and 7). Four subjects were tested. Negative proliferation or reduction indicates experiments where the knockdown group showed higher expression/proliferation than the control.

B. Correlation between reduction of CD147 expression and change in CD25 expression. Changes in surface expression of CD147 did not produce a trend in expression of CD25 in activated T lymphocytes. A value of 1.00 on the Y-axis denotes no difference between the knockdown and control culture. Data points greater than 1.00 show comparatively higher levels of CD25 expression in the knockdown culture, while data points below 1.00 show lower levels of CD25 expression compared to the control culture. These data represent 7 experiments with 2 time-points each (days 3 and 5), except for one which had 3 time-points (day 3, 5 and 7). Four subjects were tested.

Correlation between the reduction of CD147 and expression of CD25

The level of CD25 expression was measured by flow cytometry in each knockdown experiment. As can be seen in Figure 4B, no meaningful correlation could be found between the total expression of CD25 and the percent reduction of CD147. While more often than not a reduction in CD147 produced a slight increase in CD25 expression, there were also cases where that trend was reversed.

Discussion

CD147 is a pleiotropic molecule that is widely expressed in a variety of cell types. Perhaps some of the difficulty in illuminating the myriad possible functions and associations of CD147 lies in the very fact that they are so numerous. In this study we shed some light on the possible functions of CD147 in primary T cells. As this is the first report showing a knockdown of CD147 in primary T cells that we are aware of, we assayed the most basic parameters with regard to T-cell function; proliferation upon activation and expression of CD25.

The expression levels of CD147 were clearly up-regulated when T cells were activated and expression levels of CD25 and CFSE proliferation confirmed the activation state of the T cells (Figure 1). The increased expression levels upon activation may be linked to some function of CD147 dependent upon the formation of homo-dimers. It has been shown that CD147 will accumulate in lipid rafts upon T-cell activation and form homo-dimers by way of the N-terminally linked Ig-like domain of the extracellular portion.[11] Further evidence for the significance of the CD147 homo-dimer comes from a study which found that a certain anti-CD147 mAb, MEM-M6/6, is able to significantly reduce anti-CD3 mediated activation of T cells (up to 80%) only when administered in a certain dosage range. High dosage and low dosage do not produce the effect. The author postulates that this is because at high concentrations the antibody may bind monovalently due to the saturating conditions and at a low dose the antibody will bind but there may not be enough bound to pull together a significant number of CD147 dimers.[19]

Achieving a reduction in CD147 surface expression in primary T cells proved to be a challenge. However we believe these experiments are important because all previous reports of CD147 knockdown in T cells were performed in cell lines. Of major concern in the electroporation of primary

T cells are the effects of the harsh reaction conditions which can cause considerable cell death. This is the primary reason why the immortalized cell line proves a better candidate for gene-silencing. However, it is imperative that the investigation eventually lead to the healthy, normal primary cell. Other researchers have reported similar difficulties with cell death, sometimes running as high as 50% when using the Nucleofector,[24] however there have been reports of gene transfer into T cells causing apoptosis mediated by the TNF alpha receptor pathway even when other methods are involved.[25] The documentation provided by Lonza cites expected transfection efficiencies in the range of 40%-60%. Therefore the reduction in CD147 we were able to achieve (Figure 2B) is well within the expected capabilities of the Nucleofector system and in fact quite good.

Our finding that a reduction in CD147 expression resulted in enhanced proliferation of T cells is indeed very interesting. Currently there are limited reports that comment on this very subject and they have achieved opposing results. In fact, Igakura et al. reported that CD147 knockout mice are characterized by an enhanced mitogenic response of T lymphocytes in mixed lymphocyte reactions.[23] In addition to the support this report lends to our findings, another study concludes that CD147 is an integral component of the T-cell immune synapse and that its over expression leads to the inhibition of NF-AT and this phenomenon is mediated by the intracellular tail of CD147. Also, CD147 knockdown in Jurkat cells promoted higher levels of NF-AT stimulation and Pak1 phosphorylation upon TCR cross-linking. They concluded that CD147, via selective inhibition of specific downstream elements of the Vav1/Rac1 route, contributes to the negative regulation of T-cell responses.[21] In yet another study, T cells from SLE patients were found to have increased expression of CD147 and these T cells showed a reduction in tyrosine phosphorylation levels when activated.[22] In contrast to these findings, another study claims that CD147 knockdown reduced proliferation and activation of the Jurkat T-cell lymphoma.[20] We cannot speculate as to why these results are contradictory, but the experimental systems were different in each example.

The enhanced proliferation seen in a successful CD147 knockdown experiment (Figure 3A) can be compared with cultures where no reduction of CD147 was achieved (Figure 3B). The two samples shown here constitute a failed knockdown as there is no difference in the expression levels of CD147, however not a failed experiment as the CD25 histogram shows relevant activation in the culture and the CFSE staining shows some level of proliferation. And importantly, the proliferation in both samples is the same when the levels of CD147 are the same.

When the entire group of knockdown experiments is plotted with respect to percent reduction of CD147 versus percent increase in proliferation a clear trend becomes visible (Figure 4A); the greater the reduction in CD147, the greater the increase in proliferation. Perhaps CD147 acts in some way to scaffold together elements in the lipid rafts of the immune synapse that act as signaling molecules to put the 'breaks' on the T-cell activation signal.

Paradoxically, reducing the expression of CD147 did not produce a trend in expression of CD25 in activated T cells. The mechanism by which CD147 exerts its regulation of T cell proliferation appears to lie outside the pathways that lead to up-regulation of CD25 on the surface of activated T cells. This result may add weight to the observations of Ruiz et al., who show that CD147 interferes with the activation of a specific subset of T-cell activation elements, but does not have an effect on other signal cascades of the TCR, such as ERK1 and JNK1.[21]

In this study we achieved reduction in CD147 surface expression in primary T cells using the Nucleofector proprietary electroporation device combined with siRNA-mediated gene suppression. We believe these experiments are important because all previous reports of CD147 knockdown were performed in cell lines. In light of the theory that CD147 density on the cell surface may indeed be critical for the anti-CD147 antibody to generate its suppressive effect by signaling through CD147 dimers combined with the findings in the mouse knockout model, the SLE patients and the detailed findings regarding NF-AT phosphorylation, we support a model where CD147 does indeed have some negative regulatory effect on primary T cells.

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References

1. Kasinrerk W, Fiebinger E, Stefanova I, Baumruker T, Stockinger H. Human leukocyte activation antigen M6, a member of the Ig superfamily, is the species homologue of rat OX-47, mouse basigin, and chicken HT7 molecule. J Immunol. 1992;149:847–54.
2. Kasinrerk W, Tokrasinwit N, Phunpae P. CD147 monoclonal antibodies induce homotypic cell aggregation of monocytic cell line U937 via LFA-1/ICAM-1 pathway. Immunology. 1999;96:184-92.
3. Ellis SM, Nabeshima K, Biswas C. Monoclonal antibody preparation and purification of a tumor cell collagenase-stimulatory factor. Cancer Res. 1989;49:3385-91.
4. Sameshima T, Nabeshima K, Toole BP. Glioma cell extra-cellular matrix metalloproteinase inducer (EMMPRIN) (CD147) stimulates production of membrane-type matrix metalloproteinases and activated gelatinase A in co-cultures with brain-derived fibroblasts. Cancer Lett. 2000;157:177–84.
5. Kataoka H, DeCastro R, Zucker S, Biswas C. Tumor cell- derived collagenase-stimulatory factor increases expression of interstitial collangenase stomolysin and 72-kDa gelatinase. Cancer Res. 1993;53:3154–8.
6. Tang Y, Kesavan P, Nakada MT, Yan L. Tumor-stroma interaction: positive feedback regulation of extracellular matrix metalloproteinase inducer (EMMPRIN) expression and matrix metalloproteinase-dependent generation of soluble EMMPRIN. Mol Cancer Res. 2004;2:73-80.
7. Kanekura T, Chen X, Kanzaki T. Basigin (CD147) is expressed on melanoma cells and induces tumor cell invasion by stimulating production of matrix metalloproteinases by fibroblasts. Int J Cancer. 2002;99:520-8.
8. Tang Y, Nakada MT, Kesavan P, McCabe F, Millar H, Rafferty P, Bugelski P, Yan L. Extracellular matrix metalloproteinase inducer stimulates tumor angiogenesis by elevating vascular endothelial cell growth factor and matrix metalloproteinases. Cancer Res. 2005;65:3193-9.
9. Gallagher SM, Castorino JJ, Wang D, Philp NJ. Monocarboxylate transporter 4 regulates maturation and trafficking of CD147 to the plasma membrane in the metastatic breast cancer cell line MDA-MB-231. Cancer Res. 2007;67:4182-9.
10. Berditchevski F, Chang S, Bodorova J, Hemler ME. Generation of monoclonal antibodies to integrin-associated proteins. Evidence that alpha3beta1 complexes with EMMPRIN/basigin/OX47/M6. J Biol Chem. 1997;272:29174-80.
11. Yoshida S, Shibata M, Yamamoto S, Hagihara M, Asai N, Takahashi M, Mizutani S, Muramatsu T, Kadomatsu K. Homo-oligomer formation by basigin, an immunoglobulin superfamily member, via its N-terminal immunoglobulin domain. Eur J Biochem. 2000;267:4372-80.
12. Qian AR, Zhang W, Cao JP, Yang PF, Gao X, Wang Z, Xu HY, Weng YY, Shang P. Downregulation of CD147 expression alters cytoskeleton architecture and inhibits gelatinase production and SAPK pathway in human hepatocellular carcinoma cells. J Exp Clin Cancer Res. 2008;27:50.
13. Nabeshima K, Iwasaki H, Koga K, Hojo H, Suzumiyaand J, Kikuchi M. Emmprin (basigin/CD147): Matrix metalloproteinase modulator and multifunctional cell recognition molecule that plays a critical role in cancer progression. Pathol International. 2006;56:359-67.
14. Khunkeawla P, Moonsom S, Kongtawelert P, Kasinrerk W. Engagement of CD147 molecule induced cell aggregation through the activation of protein kinases and reorganization of the cytoskelaton. Immunobiology. 2001;203:659-69.
15. Chiampanichayakul S, Peng-in P, Khunkaewla P, Stockinger H, Kasinrerk W. CD147 contains different bioactive epitopes involving the regulation of cell adhesion and lymphocyte activation. Immunobiol. 2006;211:167-78.
16. Intasai N, Mai S, Kasinrerk W and Tayapiwatana C. Binding of phage displaying multimeric CD147 on U937 cell conducts apoptotic signal. Int J Immunol. 2006;18:1159–69.
17. Intasai N, Tragoolpua K, Pingmuang P, Khunkaewla P, Moonsom S, Kasinrerk W, Lieber A and Tayapiwatana C. Potent inhibition of OKT3-induced T cell proliferation and suppression of CD147 cell surface expression in HeLa cells by scFv-M6-1B9. Immunobiology. 2009;214:410-21.
18. Kirsch, A. H., Diaz, L. A., Jr, Bonish, B., Antony, P. A. and Fox, D. A. 1997. The pattern of expression of CD147/neurothelin during human T-cell ontogeny as defined by themonoclonal antibody 8D6. Tissue Antigens. 1997;50:147-52.
19. Koch C, Staffler G, Hüttinger R, Hilgert I, Prager E, Cerný J, Steinlein P, Majdic O, Horejsí V, Stockinger H. T cell activation-associated epitopes of CD147 in regulation of the T cell response, and their definition by antibody affinity and antigen density. Int Immunol. 1999;11:777-86.
20. Chen X, Su J, Chang J, Kanekura T, Li J, Kuang YH, Peng S, Yang F, Lu H, Zhang JL. Inhibition of CD147 gene expression via RNA interference reduces tumor cell proliferation, activation, adhesion, and migration activity in the human Jurkat T-lymphoma cell line. Cancer Invest. 2008;26:689-97.
21. Ruiz S, Castro-Castro A, Bustelo X. CD147 inhibits the nuclear factor of activated T cells by impairing Vav1 and Rac1 downstream signaling. J Biol Chem. 2008;283:5554-66.
22. Pistol G, Matache C, Calugaru A, Stavaru C, Tanaseanu S, Ionescu R, Dumitrache S, Stefanescu M. Roles of CD147 on T lymphocytes activation and MMP-9 secretion in systemic lupus erythematosus. J Cell Mol Med. 2007;11:339-48.
23. Igakura T, Kadomatsu K, Taguchi O, Muramatsu H, Kaname T, Miyauchi T, Yamamura K, Arimura K, Muramatsu T. Roles of basigin, a member of the immunoglobulin superfamily, in behavior as to an irritating odor, lymphocyte response, and blood–brain barrier. Biochem Biophys Res Commun. 1996;224:33-6.

24. Jurgens L, Khanna R, Weber J, Rimas J. Transduction of primary lymphocytes with Epstein-barr virus latent membrane protein-specific T-cell receptor induces lysis of virus-infected cells. J Clin Immunol. 2006;26:22-32.

25. Ebert O, Finke S, Salahi A, Herrmann M, Trojanek B, Lefterova P, Wagner E. Lymphocyte apoptosis: induction by gene transfer techniques. Gene Ther. 1997;4:296-302.