EVALUATION OF TUMOR NECROSIS FACTOR-RELATED APOPTOSIS-
INDUCING LIGAND (TRAIL) EFFECTS ON REGULATORY T CELLS

By

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EVALUATION OF TUMOR NECROSIS FACTOR-RELATED APOPTOSIS-INDUCING LIGAND (TRAIL) EFFECTS ON REGULATORY T CELLS

By: Elaine Hua Situ

ABSTRACT

CD4+CD25+ regulatory T lymphocytes (Treg) are immunosuppressive cells that critically contribute to the maintenance of self-tolerance and to the prevention of autoimmunity in animal and human models. They are also major components of the multiple immunoregulatory processes leading to cancer-induced immune tolerance. Studies in human and animal models have established that the suppression of Treg suppressive activity promotes anti-tumoral immunity.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the Tumor Necrosis Factor (TNF) cytokine family, which relatively triggers apoptosis in tumor cells through engagement with its receptors (DR4 and DR5).

Based on TRAIL’s direct killing effects on tumor cells, its immunomodulatory functions, and the evidence that suppression of Treg’s suppressive effects promotes cancer elimination, it is conceivable that TRAIL may trigger Treg cell death or dampen FoxP3 (unique and critical transcription factor in Treg) expression and Treg immunosuppressive function.

INTRODUCTION

Cancer immunotherapy is an alternate treatment strategy to conventional chemotherapy. The main objective of cancer immunotherapy is to stimulate the immune system to eliminate cancer cells. Immunotherapy-induced cancer elimination can be achieved using vaccines made from tumor components, through immune cell transfer or through administration of tumor-specific antibodies or immunostimulatory cytokines. These approaches assist in tumor specific killing while sparing normal cells from cytotoxicity (Mitchell 2008). The cancer vaccines’ mechanisms of action vary, but the chief goal is to induce antigen-specific T lymphocyte proliferation, cytokine secretion, and killing capacity. However, tumors have evolved mechanisms to escape elimination by the immune system, such as stimulating the proliferation and suppressive activity of regulatory T cells (Treg) (Tang 2004).

Regulatory T cells represent one population of suppressive cells that are necessary for inducing and maintaining peripheral tolerance (Zou 2006, Schwartz 2005) and prevention of autoimmune disease (McHugh 2002). However, Treg’s immunosuppressive function also contributes to the mechanism of tumor escape for the recognition and elimination by the immune system, for cancer cells have subverted Treg to the cancer’s benefit. Treg thus represent major obstacles in strategies for tumor immunotherapy (Nizar 2009).

Treg express a number of extracellular markers including CD25 (the alpha chain of the IL-2 receptor, which is crucial to Treg activation), CTLA4 (Kolar 2009), and GITR. These extracellular markers can be used for Treg depletion and selection. However, there is no specific way to only deplete Treg, for none of extracellular markers are unique to Treg. Such as CD25, which is not only expressed on Treg, but also on activated T cells. On the other hand, the
transcription factor FoxP3 (forkhead box P3), is generally specifically expressed in Treg and is critical to their development and function (Sakaguchi 2006). Treg dampen the immune system by inhibiting cells such as T cells, B cells, NK cells, monocytes, macrophages, and dendritic cells (Larmonier 2008). Treg can specifically prevent activation and proliferation of cytotoxic T cells (CTL) and helper T cells (Th) and affect the differentiation and function of dendritic cells thus impairing tumor immunity (Murphy 2008) through secretion of IL-10 and TGF-β. Treg also prevents transcriptional activation of the IL-2 gene, thereby preventing T cell activation and proliferation via contact-dependent interaction (Thornton 1998). Because of Treg’s extensive effects on immune cells, tracking the expression of FoxP3 is one of the key means of monitoring Treg’s suppressive capabilities. Studies have shown that down-regulation of Treg suppressive activity allows the immune system to overcome cancer-induced immune tolerance (Larmonier 2008), but the exact mechanisms by which Treg suppress the immune system is still not completely clear and is quite controversial.

The ligand of interest to possibly down-regulate the suppressive effects of Treg in our study is the tumor necrosis factor (TNF)-related apoptosis-inducing ligand, also known as TRAIL. Studies have shown that TRAIL has a direct killing effect on tumor cell lines and is involved in immunosurveillance (Newsom-Davis 2009). In addition to tumor cells, TRAIL has also been seen to inhibit T cell activation, proliferation, and survival (Martinez 1998, Lünemann 2002, Zhang 2003) and has similar effects on other immune cells as well (Griffiths 2009, Ehrlich 2003). Another TNF-family ligand, Fas Ligand has shown to cause apoptosis to Treg (Reardon 2008). However, there are currently no studies on TRAIL’s effects on human Treg. Therefore, it is conceivable that TRAIL may cause Treg apoptosis or down-regulate Treg suppressive function.

The mechanisms by which TRAIL induces apoptosis in human cells, involves multiple TRAIL receptors, and the main receptors pertinent to this study are death receptor 4 (DR4) and death receptor 5 (DR5) (Newsom-Davis 2009). Upon binding to the agonistic receptors DR4 and DR5 (Pan 1997), TRAIL induces heterotrimerization of the receptors (Newsom-Davis 2009). The apoptotic signaling then begins with the recruitment of the adaptor protein FADD (Fas-associated death domain) followed by the recruitment of pro-caspase-8 and -10 (Thorburn 2004). The interactions between FADD and the procaspases form the macromolecule complex called DISC (death-inducing signaling complex), ultimately inducing the caspase-triggered apoptosis (Thorburn 2004). However, there are also two antagonistic TRAIL receptors, Decoy Death Receptors 1 and 2 (DcR1 and DcR2), which are unable to induce apoptotic signals. When TRAIL binds to DcR1 and DcR2, no apoptotic signals are transduced due to the receptors’ inability to recruit FADD, directly interfering with the formation of DISC (Mérino 2006). DcR1 lacks a death domain, whereas DcR2 has a truncated death domain (Schneider 2003). The exact mechanism by which DcR1 and DcR2 prevents TRAIL-induced apoptosis remains unelucidated (Almasan 2003, Griffith 1999).

In the current study, we explore the possibility of TRAIL negatively modulating human Treg cell numbers or suppressive function due to TRAIL’s significant role in immune cell regulation (Smyth 2003). Part of the Tumor Necrosis Factor family, TRAIL not only mediates cell death in select tumor cells, but because it is also in the same family as Fas Ligand, TRAIL may also play a role in the negative modulation of Treg cell viability or suppressive functions. It has been
shown that depletion or suppression of Treg suppressive function allows for tumor cell recognition and elimination by the immune system.

![Figure 1. TRAIL-induced apoptosis.](image)

**MATERIALS AND METHODS**

**HeLa cell line**

The human epithelial carcinoma cell line (HeLa) is a cervical cancer cell line, expressing DR4 and DR5, while HDcR1 and HDcR2 cells, Hela cells in which DcR1 and DcR2 expression was induced by retroviral infection, were used as positive controls for DcR1 and DcR2 expression.

**Sources of human Treg**

A. *Human pheresis*. Human pheresis from anonymous patients was one of the sources of the human regulatory T cells for the experiments. Five percent bovine serum albumin (BSA) was added to the pheresis to prevent blood coagulation. B. *Freshly donated whole blood*. Certain experiments were conducted with freshly donated human blood or peripheral blood mononuclear cells (PBMC). The blood was diluted using a 1:2 dilution, 30mL blood and 30mL 1x PBS. Then 30mL of diluted blood was transferred into tubes containing 15mL of Ficoll. The tubes
were centrifuged for 20 minutes at 2000rpm with no brake. The cell pellets were then washed twice with 1X PBS. At this point, the cells were ready for automated magnetic cell sorting.

**TRAIL**

The human TRAIL was kindly donated to our laboratory by Dr. Olivier Micheau from the University of Bourgogne, Dijon, France.

**Treg isolation using Automated Magnetic Cell Sorter (autoMACS™)**

Human Treg were isolated from frozen pheresis. The pheresis was retrieved and diluted in a sterilely filtered 5% BSA solution to prevent coagulation. The samples were centrifuged and resuspended in 1mL of 1X lysis buffer in order to lyse red blood cells that were in the cell population. After red cell lysis, samples are resuspended in 10mL of complete medium and centrifuged again. After disposal of the supernatant, the cell pellet was resuspended in 900uL of MACS buffer composed of 1000mL of 1x PBS, 4mL of 0.5M EDTA, and 5mL of deactivated fetal bovine serum for the cell count of $10^8$ cells. Then 100uL of the biotin cocktail from the Miltenyi human Treg isolation kit was added to the cells and resuspended. The cells were incubated at 4°C for 10 minutes. After the incubation, the cells were not washed and 200uL of the anti-biotin beads were added and allowed to incubate with the cells at 4°C for 15 minutes. Then 10mL of MACS buffer was added to the tubes and centrifuged. After disposal of the supernatant, the cells were resuspended in 500uL of MACS buffer. At this point, all major cell types except CD4$^+$ cells were labeled with magnetic bead-conjugated antibodies. Thus, when the cells are passed through the magnetic field generated by the autoMACS™, the non-CD4$^+$ cells were trapped in the magnetic field while the non-labeled CD4$^+$ cells freely passed through and were collected. The collected cells were then centrifuged, and the supernatant was disposed. The cells were then resuspended in 900uL of MACS buffer and 100uL of anti-CD25 beads were added to the suspension and incubated at 4°C for 15 minutes. After the incubation, 10mL of MACS buffer was added to the tubes and centrifuged. After centrifugation, the supernatant was disposed and the cell pellet was resuspended in 500uL. At this point, the cells that were CD25$^+$ were labeled with a magnetic bead-conjugated antibody. Therefore, the CD25$^+$ cells were suspended in the magnetic field while the CD25$^-$ were passed through and collected. After the CD25$^-$ cells were passed through, the magnetic field was shut off, which allowed for the passage of CD25$^+$ cells and then they were collected.

**Cell culture**

Both HeLa cells and isolated Treg cells were cultured at 37°C in 5% CO$_2$ in RPMI medium containing 10% deactivated fetal bovine serum, 2mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin sulfate, 0.025 µg/ml amphotericin B, 0.5 x MEM non-essential amino acids, and 1 mM sodium pyruvate.

**Activation of Treg and CD4$^+$CD25$^-$ cells**

Both Treg and CD4$^+$CD25$^-$ were activated with plate-bound anti-CD3 Ab (5µg/ml), soluble anti-CD28 Ab (4µg/ml) and IL-2 (100U/ml). To be triggered, Treg immunosuppressive function requires cell activation, which can be induced by non-specific stimulation, such as with anti-CD3 and anti-CD28. IL-2 is necessary for the activation and proliferation of T cells as well.
Cell staining and flow cytometry

Cells were washed in PBS containing 3% heat-inactivated FBS and 0.09% sodium azide (Sigma-Aldrich) and were first incubated with an Fc receptor-blocking Ab (BD Pharmingen) for 5 min, then with saturating amounts of fluorochrome-bound antibodies from Diaclone: anti-TRAIL, anti-DR4, anti-DR5, anti-DcR1, and anti-DcR2. 40 min. Cells were then washed and analyzed using a FACS calibur (Becton Dickinson for Immunocytochemistry Systems). A minimum of 10,000 events was collected for each sample, and data analysis was performed with the CellQuest software (Becton Dickinson Immunocytochemistry Systems). For FoxP3 detection, CD4⁺CD25⁺ or CD4⁺CD25⁻ T cells purified by magnetic cell sorting were fixed, permeabilized, stained using an allophycocyanin anti-mouse FoxP3 staining set following the provider’s instructions (Clone FJK-16, eBioscience), and analyzed by flow cytometry. For the monitoring of CD4⁺CD25⁺ Treg, cells were first stained with FITC-conjugated anti-CD4 (rat IgG2b; BD Pharmingen) and PE-conjugated anti-CD25 (rat IgG1; BD Pharmingen) Abs. Then cells were stained using eBioscience FoxP3 staining set as described above. Isotype control Abs were purchased from BD Pharmingen (PE-conjugated rat IgG1, FITC-conjugated rat IgG2a), or eBioscience (allophycocyanin-conjugated rat IgG1).

The anti-TRAIL, anti-DR4, anti-DR5, anti-DcR1, and anti-DcR2 flow cytometry antibodies were kindly donated by Dr. Olivier Micheau from the University of Bourgogne in Dijon, France.

Immunosuppressive assay using [³H]-thymidine incorporation

Both CD4⁺CD25⁺ and CD4⁺CD25⁻ cells were allowed to activate. CD4⁺CD25⁻ T cells were co-cultured for 72 hours in round-bottom 96-well plates with TRAIL-treated or untreated activated CD4⁺CD25⁺ T cells. [³H]-thymidine (ICN Pharmaceuticals) was then added (1 µCi per well) for an additional 18 h. The cells were then harvested using a 96-well cell harvester and the radioactivity measured on a Packard beta counter (Packard Biosciences). Cultures were set up in triplicate.

MTT assay

MTT assays were performed to determine cell viability. The yellow tetrazolium salt (Sigma) is dissolved in 1mL of distilled water to create a 5.0mg/ml solution. Twenty microliters is added to every well and is allowed to incubate at 37°C at 5% CO₂ between 30 minutes to 3 hours. Then 150uL of DMSO (FW 78.13) is added to each well and read with the monochromator-based microplate reader from Molecular Devices at 560nm.

RESULTS

Treg phenotype and immunosuppressive function

Determination of the phenotype of the MACS-isolated cells was necessary to confirm the cells were indeed Treg, which are defined as CD4⁺CD25⁻FoxP3⁺ cells. The human Treg were stained with anti-CD4, anti-CD25, and anti-FoxP3 and analyzed by flow cytometry. The flow cytometry confirmed the phenotype of Treg that is consistent with the literature [Fig. 2A]. To further confirm the identity and function of the cells, an immunosuppressive assay was conducted to
confirm the Treg suppressive activity. Differing ratios of Treg to CD4^+CD25^- cells were cocultured together, and then [^3H]-thymidine was added to incorporate into the DNA of daughter cells. The results show the immunosuppressive effect of Treg on CD4^+CD25^- cells. The higher the number of Treg to CD4^-CD25^-, the more suppression of CD4^-CD25^- proliferation [Fig. 2B].

**Figure 2.** A: Flow cytometry analysis confirms the expression of FoxP3, a specific Treg marker, in human CD4^+CD25^- T cells purified by magnetic cell sorting (Miltenyi). B: Immunosuppressive function of Treg. Human CD4^+CD25^- T cells were activated for 24 hrs. They were then co-cultured with CD4^-CD25^- T cells at the indicated ratios for 72 hrs with plate-bound anti-CD3 Ab and anti-CD28 Ab.[^3H]-thymidine was added for the last 18 hrs.

*Human CD4^+CD25^+FoxP3^+ Treg cells do not express TRAIL receptors*

After confirming the phenotype and immunosuppressive function of the Treg, flow cytometry analysis was conducted to identify any TRAIL receptors it may have on their cell surface, since the apoptotic effects of TRAIL are a result of engagement of DR4 and DR5. In Fig. 3A, both Treg (CD25^+) and CD4^-CD25^- cells were stained with anti-DR4, anti-DR5, anti-DcR1, anti-DcR2, and anti-TRAIL. The results show no expression of any of the TRAIL receptors and no expression of TRAIL on Treg or CD4^-CD25^- cell surface. The positive controls for the TRAIL receptors are in Fig. 3B. The control HeLa cells naturally express DR4 and DR5 receptors, but not the antagonistic DcR1 and DcR2. The HDcR1 and HDcR2 cell lines were retrovirally engineered to express DcR1 (expressed by HDcR1) and DcR2 (expressed by HDcR2). These data show that even if TRAIL does modulate Treg in some manner, it would not be through the engagement of known TRAIL receptors in humans.
Figure 3. A: Freshly isolated human CD4⁺CD25⁺ Treg (CD25⁺) or CD4⁺CD25⁻ T cells (CD25⁻) were stained with the indicated antibodies and analyzed by flow cytometry. Similar results were obtained after activation of the cells for 3 days. B. Hela cells (Hct1) were used as positive control for DR4 and DR5 expression. HDcR1 and HDcR2 cells are Hela cells in which DcR1 and DcR2 expression was induced by retroviral infection. They were used as positive controls for DcR1 and DcR2 expression.

Human CD4⁺CD25⁺FoxP3⁺ Treg are not sensitive to TRAIL-induced cell death

Although Treg did not express any of the agonistic or antagonistic receptors, cytotoxicity assays were conducted to identify any change in Treg cell viability after treatment with TRAIL. The cytotoxicity assays involved treating freshly isolated human CD4⁺CD25⁺ and CD4⁺CD25⁻ cells with varying concentrations of TRAIL for 24 hours and assessment by MTT. HeLa cells expressing DR4 and DR5 were used as positive controls for this experiment. The percentage of viable Treg cells remained consistent between the untreated Treg and Treg exposed to the highest concentration of TRAIL, 10000ng/mL. These results therefore indicate that TRAIL has no effect on Treg cell viability or CD4⁺CD25⁻ cells [Fig. 4AB].
Figure 4. A: CD4^+CD25^+ Treg (CD25^+) or CD4^+CD25^- cells (CD25^-) were activated for 24 hours with or without TRAIL at the indicated concentrations. MTT assays were performed to determine cell viability. Similar results were obtained when cells were activated for 3 (since the TRAIL receptors may appear on the cell surface over time) days before being treated with TRAIL. B: As controls, Hela cells were treated with increasing concentrations of TRAIL for 24 hours and cell viability was assessed by MTT.

FoxP3 expression is not affected by TRAIL in Treg

FoxP3 is a major transcription factor expressed by Treg. It is responsible for their immune suppressive function and development. Since TRAIL does not affect Treg viability, we reasoned that it may modulate FoxP3 expression. To determine whether TRAIL may modulate FoxP3 expression, Treg were exposed to TRAIL for 24 hours and the cells were stained with anti-FoxP3 and analyzed by flow cytometry. The results show no difference between TRAIL-treated Treg FoxP3 expression and non-treated Treg FoxP3 expression [Fig. 5]. This indicates that TRAIL neither affects Treg viability nor FoxP3 expression.

Figure 5. Human CD4^+CD25^+ Treg were activated for 24 hrs with or without TRAIL. Flow cytometry analysis showed no difference in the expression of FoxP3 between treated and untreated Treg.
TRAIL does not affect Treg immunosuppressive function

As previously described, TRAIL does not affect FoxP3 expression. We sought to further confirm this fact by investigating the effect of TRAIL on Treg immunosuppressive function. To investigate this, we performed an immunosuppressive assay on TRAIL-treated Treg using CD4⁺CD25⁻ cells as targets. Activated TRAIL-treated Treg and activated untreated Treg were co-cultured with CD4⁺CD25⁻ cells for 72 hours, and [³H]-thymidine was added in the last 18 hours. Our results revealed that TRAIL treatment did not affect the ability of Treg to inhibit CD4⁺CD25⁻ Treg proliferation in vitro [Fig. 6]. Our results imply that TRAIL has no negatively modulating effects on Treg cell viability, FoxP3 expression or suppressive function.

**Figure 6.** Human CD4⁺CD25⁺ Treg (CD25⁺) were activated for 24 hours with ([CD25⁺]TRAIL) or without ([CD25⁻]untreated) TRAIL (100ng/ml). CD4⁺CD25⁻ Treg cells were removed, washed, and incubated with CD4⁺CD25⁻ T cells (1:1 ratio) in presence of plate-bound anti-CD3 Ab (5µg/ml) and soluble anti-CD28 Ab (4µg/ml). Cells were cultured for 72 hrs. [³H]-thymidine was added for the last 18 hours.

**DISCUSSION**

Cancer immunotherapy is an alternative to conventional therapy. However, there are obstacles to overcome, such as the suppressive activity of Treg, which impairs cancer vaccination strategies. Thus, Treg should be eliminated or inactivated. The techniques to down-regulate Treg have not been elucidated. TRAIL has shown promise in its direct killing effects against tumor cells (Nagane 2001) and in its immunomodulatory role (Griffiths 2009), but its effects on Treg are not known. Therefore, we sought to determine whether this molecule may be used as a Treg depleting or inactivating agent, in an attempt to identify a strategy to impair these cells. If TRAIL could indeed be used as a Treg inhibiting agent, then it may be integrated into the current strategies of cancer immunotherapy.

The results of this study indicated that Treg do not express DR4 or DR5, the agonistic death receptors that induce apoptotic signaling within the cell. Treg also do not seem to express either
DcR1 or DcR2. TRAIL did not induce Treg cytotoxicity, even at its highest concentration of 10000ng/mL. Neither FoxP3 expression nor Treg immunosuppressive function was down-regulated after exposure to TRAIL. It can be concluded that TRAIL do not express the known TRAIL receptors and do not eliminate Treg or inhibit its suppressive functions through the known mechanisms of TRAIL-induced apoptosis.

Consistent with every study, there were pitfalls in this study as well. First would be the source of the human Treg. There was great difficulty in preventing coagulation of the pheresis after thawing. Every pouch of pheresis had 300mL of blood, and before being advised to resuspend the cells in BSA, complete pouches of pheresis became unfit for Treg isolation. Even a successful harvesting of the blood from the pheresis pouch would frequently only yield 20,000 cells, which is insufficient. It is possible that the isolations could have had a higher yield, but the cell health could have been compromised due to the preparation process of the pheresis and the freezing and thawing before Treg isolation. The other source of Treg comes from fresh whole blood. Only 100mL of fresh blood is used per experiment. Once again, the number of Treg cells isolated is quite low, since Treg only comprises 5-10% of naïve CD4+ cells. Possible solutions to these problems would be increasing the starting amount of blood and being more mindful of the isolation procedures, such as keeping cells on ice, using fresh buffers, the maintenance and sanitation of the autoMACS™, etc.

The activation of Treg cells involves the use of anti-CD3, anti-CD28, and IL-2. The concentrations that are used may not be the optimal concentration for human Treg. Perhaps the Treg cells are under-activated and do not express TRAIL because of that reason or vice versa, the over-activation of Treg inhibits TRAIL receptor expression. It is also possible for the incubation times with these cytokines is not enough. Possible solutions to these issues is conducting an optimization assay that treats Treg cells with varying concentrations of these cytokines and/or varying incubation times.

Another issue that arose during the process of the project was the potency of TRAIL. The initial TRAIL, donated by Dr. Micheau’s laboratory, was limited in supply, and therefore, it was necessary to order more from another company. It is unknown whether or not the two TRAILs were quite the same. In the future, it would be advised to purchase a larger quantity of TRAIL that would last the entire length of the project.

It is also necessary to make sure the positive control cell lines indeed present the correct TRAIL receptors. With retrovirally-engineered cells, it is possible for the transfection to become unstable, inducing the loss of expression of the transfected gene. It is important to confirm phenotypes by flow cytometry analysis.

The analysis process of the cells via flow cytometry may have affected the outcome of the experiments. Once again, the concentrations of the antibodies may not have been optimal for the cell numbers. Perhaps the incubation times were not the appropriate length. It is also possible for the antibodies to have become unstable and not bind as well to the TRAIL receptors that may have been expressed. For this issue, it would be best to test the antibodies periodically on positive controls. All of the aforementioned pitfalls are important for future projects involving TRAIL and Treg interaction.
The future direction of TRAIL and Treg has numerous possibilities. One of the possible directions would be the effect of TRAIL on Treg conversion or perhaps TRAIL can be coupled with other inhibiting agents of Treg, such as imatinib mesylate (Larmonier 2008) to further down-regulate Treg. As the number of studies regarding both TRAIL and Treg increase, the possibilities for more projects involving these two players also increases.

Although TRAIL did not negatively modulate Treg in this study, it is still a ligand of importance in the field of immunotherapy. Because of this study, investigators in the field will not need to utilize precious time on this particular project. The search for a Treg-inhibiting agent continues on, and the potential is high for the discovery of a ligand or mechanism that will facilitate the efficacy of a cancer vaccine in the field of cancer immunotherapy.

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REFERENCES


