The role of 4-1BB-4-1BBL *cis*-interaction and lactate in the survival and proliferation of $CD8^+$ T cells

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The role of 4-1BB-4-1BBL *cis*-interaction and lactate in the survival and proliferation of $CD8^+$ T cells

Directed by Professor Ki Taek Nam

The Master's Thesis
submitted to the Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Master of Medical Science

Eunjung Cho

December 2023

This certifies that the Master's Thesis
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The Graduate School
Yonsei University

December 2023

ACKNOWLEDGEMENTS

I am deeply grateful for the invaluable help and unwavering support I received from many individuals during my journey to complete my master's degree. I wish to express my heartfelt appreciation in writing. At the core of my academic progress was my dedicated advisor, Professor Ki Taek Nam. His constant guidance, mentorship, and genuine interest in my growth as a scholar have significantly influenced my academic path. His passion for teaching and commitment to expanding knowledge have served as a true inspiration. I am profoundly grateful for his mentorship, which has been instrumental in shaping my understanding and encouraging my aspirations. I am also appreciative of the doctors whose insights and encouragement broadened my perspective and understanding of the field. Engaging in intellectual debates and sharing knowledge with all of you have been enlightening experiences that expanded my academic horizons. Additionally, I extend my heartfelt appreciation to my lab members for their moral support and assistance during my degree pursuit and graduation preparations. Furthermore, to my parents, whose unwavering love and support have been the foundation of my achievements, I express my deepest gratitude.

Lastly, I wish to convey my sincere appreciation to Professor Jun-Young Seo and Dr. Beom Kyu Choi, who generously dedicated their time amid busy schedules to review and provide valuable insights on my thesis.

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ABSTRACT

The role of 4-1BB-4-1BBL *cis*-interaction and lactate in the survival and proliferation of CD8$^+$ T cells

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4-1BB (CD137) plays a crucial role in influencing T cell activity, survival, differentiation, and proliferation. Agonistic anti-4-1BB antibodies have demonstrated the ability to enhance T cell activity by triggering 4-1BB signaling.[1,2] Despite the robust induction of CD8$^+$ T cell responses *in vivo* by agonistic anti-4-1BB mAb, only a relatively small population of 4-1BB$^+$CD8$^+$ T cells was observed in blood or lymphoid organs, such as draining lymph nodes. This paradox prompts a critical inquiry into how agonistic anti-4-1BB mAb induces a massive immune response of CD8$^+$ T cells *in vivo*, especially in the presence of a limited population of 4-1BB$^+$CD8$^+$ T cells.[3,4]

4-1BB is expressed on the surface of activated T cells and is known to engage in *trans*-interactions with 4-1BB ligands (4-1BBL; CD137L) found on antigen-presenting cells (APCs).[5,6,7] However, it's essential to note that 4-1BBL is not limited to APCs alone; they are also expressed on the surface of T cells, leading to the expectation of signaling through *cis*-interactions.[4,7,8] The experiment with agonistic antibodies against 4-1BB and 4-1BBL revealed that triggering 4-1BBL facilitated the egress of CD8$^+$ T cells activated by 4-1BB in the lymph nodes. Given the expression of 4-1BBL by CD8$^+$ T cells, both inside and outside the cell, *cis*-interactions are plausible. Subsequent experiments using 4-1BB$^{-/-}$ and 4-1BBL$^{-/-}$ pmel-1 CD8$^+$ T cells *in vivo* confirmed that the interaction between 4-1BB and 4-1BBL occurs not only in *trans* but also in *cis*, resulting in a more robust increase in CD8$^+$ T cells than *trans*-interactions.[4] These findings elucidate how agonistic anti-4-1BB mAb

robustly induces $CD8^+$ T cell responses i*n vivo* despite the relatively small population of 4-$1BB^+CD8^+$ T cells.

The extensive expansion of $CD8^+$ T cells triggered by agonistic anti-4-1BB mAb cannot be solely explained by the *cis*-interaction of 4-1BB and 4-1BBL. The Warburg effect, characterized by heightened glucose absorption and lactate production in rapidly proliferating cells, challenges the traditional view of lactate as metabolic waste.[10] Instead, it emerges as a crucial energy source for these cells.[11] Recent studies reveal that the agonistic anti-4-1BB mAb not only boosts glucose and fatty acid metabolisms but also enhances lactate synthesis.[12] As 4-1BB triggering amplifies lactate production in $CD8^+$ T cells,[8,13] we investigate whether lactate contributes to the proliferation, survival, and differentiation of $CD8^+$ T cells triggered by agonistic anti-4-1BB mAb, potentially extending the impact of agonistic anti-4-1BB mAb.[12] To validate this hypothesis, a combined treatment involving agonistic anti-4-1BB mAb and lactate was administered to mice bearing MC38 mouse colorectal cancer. The objective was to determine if lactate treatment could enhance proliferation of tumor-specific $CD8^+$ T cells and amplify the anti-tumor effects of agonistic anti-4-1BB mAb. In the MC38 tumor model, concurrent administration of sodium lactate (NaLac) exhibited an augmented anti-tumor effect in combination with the agonistic anti-4-1BB mAb. Importantly, this combined effect was observed only in "hot tumors," characterized by a high infiltration rate of immune cells, and not in "cold tumors" with low immune cell infiltration. These findings strongly suggest that lactate supports the survival and proliferation of $CD8^+$ T cells triggered by agonistic anti-4-1BB mAb.

The study highlights the significance of *cis*-interaction between 4-1BB and 4-1BBL in enhancing $CD8^+$ T cell responses. Additionally, it reveals that lactate plays a crucial role in supporting the survival and proliferation of $CD8^+$ T cells triggered by agonistic anti-4-1BB mAb.

Key words : $CD8^+$ T cell, 4-1BB (CD137), 4-1BBL (CD137L), lactate, metabolism

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I. INTRODUCTION

T cells undergo activation via three fundamental signals: 1) antigen recognition, 2) costimulatory signals, and 3) cytokines, which sequentially orchestrate cellular growth.[9] 4-1BB, a costimulatory receptor expressed on T cell surfaces, transmits signals by engaging with 4-1BB ligand (4-1BBL) expressed on antigen-presenting cells (APC). The 4-1BB-4-1BBL interaction induces the downregulation of TRAF1, TRAF2, and TRAF3, subsequently enhancing T cell differentiation, proliferation, effector function, and survival.[13,14] Agonistic anti-4-1BB mAb have been demonstrated to robustly amplify these signals, leading to potent T cell responses *in vivo* and eliciting anti-tumor effects. Nevertheless, the presence of $4\text{-}1BB^+CD8^+$ T cells *in vivo* remains scarce, raising questions about boosting immune responses and sustaining the activity of $CD8^+$ T cells activated by agonistic anti-4-1BB mAb.[2]

While 4-1BB is known to positively impact T cell growth, the effects of 4-1BBL signaling on T cells have been less understood. Traditionally, 4-1BB and 4-1BBL interaction understood as a *trans*-interaction between activated T cell and APC,[4,15] but recent findings challenge this notion by revealing that 4-1BBL is expressed by T cells themselves. This raises questions about the predominant mediation of 4-1BB and 4-1BBL interaction -whether it occurs through the conventional *trans*-interaction or a novel *cis*-interaction within T cells. The intricate interplay between these receptors, particularly in the context of T cell growth, adds a layer of complexity to our understanding of 4-1BB signaling. Further elucidating these mechanisms holds promise for uncovering novel

aspects of T cell regulation and may have implications for therapeutic interventions targeting 4-1BB pathways.

Moreover, within the context of cellular proliferation, particularly in the case of T cells, several pivotal prerequisites come to the forefront. Notably, the growth of these cells necessitates the orchestration of DNA synthesis, a robust energy reservoir exemplified by adenosine triphosphate (ATP), and the building blocks such as lipids and cholesterol that support cellular architecture.[16,17] While glucose is widely acknowledged as a potent and ubiquitous energy source, recent studies have shed light on the intricate nuances of cellular metabolism.[18] Glucose is not used as an energy source in other organs except the brain, and lactate and glutamine are used as energy sources.[10] In accordance with previous findings, 4-1BB signaling increased the production of glucose, fatty acids, and lactate. Remarkably, when glucose and fatty acid supplies were simultaneously deprived, activated $CD8^+$ T cells exhibited an inability to survive. Intriguingly, the absence of fatty acids led to a disappearance of key signaling molecules within $CD8^+$ T cells, while a similar effect was not observed upon blocking glucose uptake. These findings collectively suggest distinct and divided roles within the metabolic pathways of growing $CD8^+$ T cells. Fatty acids appear to serve as the primary energy source, vital for cellular survival. Collectively, these findings strongly suggest a sequential connection between lactate and fatty acid metabolism, where lactate production induced by agonistic anti-4-1BB mAb serves as a metabolic precursor for amino acid synthesis and the generation of fatty acids for sustaining energy and building blocks.[11]

Based on our hypothesis, here we demonstrate that the *cis*-interaction of 4-1BB and 4-1BBL within activated $CD8^+$ T cells plays a crucial role in mediating the enhancement of $CD8^+$ T cells triggered by 4-1BB. Additionally, we also reveal that lactate, a byproduct of anti-4-1BB-triggered $CD8^+$ T cells, actively supports the survival and proliferation of these cells.

II. MATERIALS AND METHODS

1. Reagents

Agonistic anti-4-1BB mAb (3H3 clone), antagonistic anti-4-1BB mAb (17B5) and agonistic anti-4-1BBL mAb (TKS-1) were purchased from BioXCell (Lebanon, NH, USA). Sodium lactate was obtained from Deoksan Bio (Seoul, Korea) and CellTrace carboxyfluorescein succinimidyl ester (CFSE) was from Invitrogen (Waltham, MA, USA). Mouse $gp100_{25\text{-}33}$ (mgp100, EGSRNQDWL) and human $gp100_{25\text{–}33}$ (hgp100, KVPRNQDWL) peptides were synthesized by Peptron (Daejeon, Korea). Anti-CD8-PE and anti-4-1BB-PE for flow cytometry and functional grade anti-CD3 mAb were purchased from BioLegend (San Diego, CA, USA), and anti-Thy1.1-PE-Cy5 and anti-CD8-PE-Cy5 were from BD Biosciences (East Rutherford, NJ, USA). Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Welgen (Gyeongsan City, Korea). CD8-microbeads were purchased from Miltenyi Biotec (Cologne, Germany). Anti-CD8β-FITC, anti-CD68-Cy3, anti-MCT1-TR and anti-CD11c-cy3 were obtained from Abcam (Cambridge, United Kingdom). Anti-4-1BB-PE-Cy5 was sourced from BD Bioscience (East Rutherford, NJ, USA), and anti-CD8α-Cy3 was purchased from Thermo fisher Science (Waltham, MA, USA) for confocal microscopy.

2. Mouse

Six-to-eight-week-old C57BL/6 mice and Balb/c mice were purchased from Orient Bio (Gapyeong, Korea), while $Thy1.1^{+}$ $Pmel\text{-}1^{+}$ transgenic (Tg) mice were sourced from Jackson Laboratory (JAX; Bar Harbor, Maine). 4-1BB knockout or 4-1BB ligand knockout Thy1.1pmel-1 Tg mice were obtained by breeding $4\text{-}1BB^{-/-}$ or $4\text{-}1BBL^{-/-}$ B6 mice with Thy1.1pmel-1 Tg mice. All mice were housed in a specific-pathogen-free environment within the animal facility of the National Cancer Center. These animal experiments, carried out in accordance with the guidelines for the use and care of laboratory animals, received approval and review from the animal care and National Cancer Center committees.

3. MC38 tumor model

MC38 adenocarcinoma tumor cells (5×10^5 cells/mice) were injected into the back of C57BL/6 mice through subcutaneous (s.c.) injection. When the tumors reached a diameter of 2~3mm on day 10, rat IgG, anti-4-1BB mAb (3H3), anti-4-1BB mAb (TKS-1) and anti-4-1BBL (17B5) were intraperitoneal (i.p.) injected to the mice 4 times with 5-day intervals. All antibodies were injected at 200 μg/100 μl/mice. The mice were routinely monitored and tumor size were measured using a caliper (width × length × height in mm^3)

Alternatively, MC38 tumor cells (5×10^5 cells/mice) were injected s.c into the back of C57BL/6 mice. Ten days later, rat IgG or anti-4-1BB mAb was i.p. injected to the mice 4 times with 5-day intervals along with the injection of 0.5, 1, 1.5, 2 g/Kg NaLac, every 2 days for 10 days. The tumors were monitored every 4 days, and their size was measured.

4. CMS5a tumors modeling and treatment

CMS5a (5×10^5 cells/mice) cells were s.c. injected into the back of Balb/c mice. When the tumor reached a size of 2~3mm in a diameter on day 10, rat IgG or anti-4-1BB mAb was i.p. injected to the mice 4 times with 5-day intervals along with the injection of 2 g/Kg NaLac, every 2 days for 10 days. Tumor growth was measured using a caliper, calculating width × length × height (mm^3) at intervals of 3 to 4 days. If the tumor size exceeds $2000mm^3$, euthanasia was performed.

5. Adoptive transfer of pmel-1 $CD8^+$ T cells

$CD8^+$ T cells were isolated from lymph nodes and spleens of $Thy1.1^+$pmel-1^+ transgenic (Tg) mice, 4-$1BB^{-/-}$ or 4-$1BBL^{-/-}$ Thy1.1pmel-1 Tg mice using CD8-microbeads and suspended as single cells. The isolated $Thy1.1^+CD8^+$ T cells were labeled using the CellTrace CFSE kit. The CFSE-labeled naïve pmel-1 $Thy1.1^+CD8^+$ T cells were intravenously (i.v.) injected through the tail vein of C57BL/6 mice. Rat IgG, anti-4-1BB mAb 3H3, anti-4-1BB mAb TKS-1, anti-4-1BBL mAb 17B5, and combinations of anti-4-1BB mAb and anti-4-1BBL mAb were administered via i.p. injection on the 1 and 3 days.

In a separate experiment, $CD8^+$ T cells from the LNs and spleens of Thy1.1 pmel-1 transgenic (Tg) mice were intravenous (i.v.) injected into normal C57BL/6 mice. The recipient mice were immunized with a subcutaneous (s.c.) injection of 20 μg of mgp100 or hgp100 peptide emulsified in incomplete Freund's adjuvant (IFA). Rat IgG or anti-4-1BB mAb was injected twice, on days 1 and 3, and/or 1 g/Kg of NaLac was administered i.p. daily from days 1 to 3. On day 5, inguinal lymph nodes (InLNs) were isolated from C57BL/6 mice, and a single-cell suspension was prepared. The suspended cells were counted and suspended at a concentration of 1×10^6 cells/ml. The cells were incubated with Fc blocker (2.4G2 antibody) for 5 minutes. Subsequently, they were stained with anti-CD8-PE and anti-Thy1.1-PE-Cy5 for 30 minutes at 4 °C. The CFSE dilution was assessed using FACSCalibur (BD Biosciences).

Using the single-cell suspension from InLNs, the total cell count was determined using an automated cell counter. Absolute numbers were measured as percentages for each population and the total percentage of live cells. Absolute number (%) = [Total cell numbers × $Thy1.1^+$ %] / 100

6. Proliferation of $CD8^+$ T cells in the presence of NaLac *in vitro*

$CD8^+$ T cells were isolated using micro-CD8 microbeads from LNs and spleens of B6 mice, and labeled with 10 μM CFSE. The CFSE-labeled $CD8^+$ T cells were suspended in RPMI1640 supplemented with 10% FBS at 1×10^6 cells/ml concentration. The cells were seeded in 96 well round-bottom plate that were coated with 1 μg/ml of anti-CD3 and/or anti-4-1BB mAb. Alternatively, the cells were seeded in 96 well round-bottom plate that were coated with 1 μg/ml of anti-CD3 mAb and 0, 1, or 2 g/L NaLac was added to the cells at day 0, 1, and 2. CFSE dilution was determined by FACSCalbur (BD Biosciences) 3 days after the culture and percentages of live cells were calculated by flow cytometry.

In a separate experiment, the cells were seeded in 96 well round-bottom plate that were coated with 1 μg/ml of anti-CD3 and/or anti-4-1BB mAb. CFSE dilution was determined by FACSCalbur (BD Biosciences) 3 days after the culture. The indicated

concentrations of NaLac were added to the cells one days after the culture. The CFSE dilution assay was performed after 3 days using FACSCalibur and percentages of live cells were calculated by flow cytometry.

7. Confocal microscopy of LN

Inguinal TDLNs were isolated ten days after the administration of MC38 colon tumor cells to C57BL/6 mice. Paraffin section of TDLNs were stained with ani-4-1BB and anti-CD8α. Also, TDLNs were stained with anti-MCT1, anti-ER-TR7, and anti-CD68 mAbs or anti-MCT1, anti-CD8β, and anti-CD11c mAbs. The slides were scanned with confocal microscope LSM780 (Carl Zeiss, Oberkochen, Germany).

III. RESULTS

1. Robust induction of draining lymph node swelling by *in vivo* anti-4-1BB stimulation, despite limited 4-1BB$^+$CD8$^+$ T cell presence

4-1BB is widely recognized for its capacity to selectively stimulate activated CD8$^+$ T cells.[3,11] Numerous studies have documented that the administration of agonistic anti-4-1BB mAb inhibits tumor cell growth *in vivo*.[19] As shown in Fig. 1A, when the inguinal tumor-draining lymph nodes (TDLNs) were collected from B6 mice with MC38 mouse colon adenocarcinoma following the fourth injection of the agonistic anti-4-1BB mAb (3H3 clone), the size of inguinal TDLNs was significantly increased (Figure 1B). However, a flow cytometric analysis of TDLNs revealed that 4-1BB$^+$CD8$^+$ T cells were seldom detected within the TDLNs (Figure 1C).

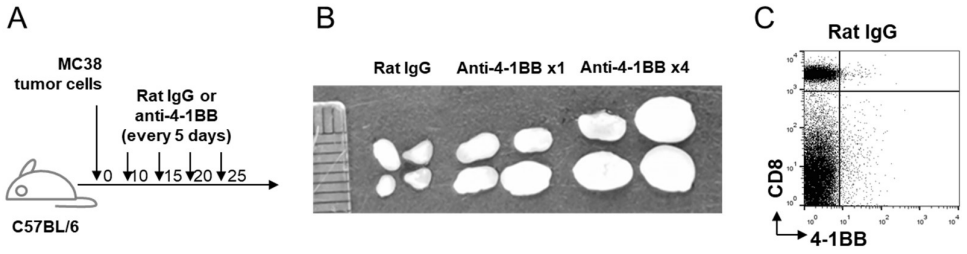

Figure 1. Enhanced *in vivo* response to anti-4-1BB stimulation despite limited presence of 4-1BB$^+$CD8$^+$ T cells. (A) MC38 colon tumor cells were s.c. injected into the back of C57BL/6 mice. Anti-4-1BB mAb or rat IgG as a control was administered to the tumor-bearing mice four times starting from day 10, with injections given every 5 days. The inguinal TDLNs were isolated and subjected to analysis 2 days after the final injection. (B) Macroscopic image of inguinal TDLNs. (C) Single-cell suspensions of TDLNs were prepared, stained with anti-4-1BB-PE and anti-CD8-PE-Cy5, and analyzed for the presence of 4-1BB$^+$CD8$^+$ T cells using FACSCalibur (BD Biosciences).

These findings suggest that the effects of anti-4-1BB treatment extend beyond the direct stimulation of 4-1BB^{+}CD8^{+} T cells and are probably further sustained by secondary immune responses provoked by the initial anti-4-1BB stimulation.

2. Anti-4-1BB and anti-4-1BBL agonists synergistically exert an anti-tumor effect

4-1BB exerts a favorable impact on the proliferation and survival of CD8^{+}T cells, while the functional role of 4-1BBL remains largely unknown. To assess their anti-tumor potential, we conducted an experiment involving the administration of agonistic anti-4-1BB, antagonistic anti-4-1BB, and agonistic anti-4-1BBL monoclonal antibodies into the MC38 tumor-bearing mice. MC38 tumor cells were s.c. injected into the backs of B6 mice, followed by the administration of rat IgG, anti-4-1BB mAb 3H3, anti-4-1BB mAb 17B5, and anti-4-1BBL mAb TKS-1. The mice were i.p. injected with the antibodies at 5-day intervals, commencing on the 10th day when the tumor size reached a diameter of 2-3mm. (Fig. 2A).

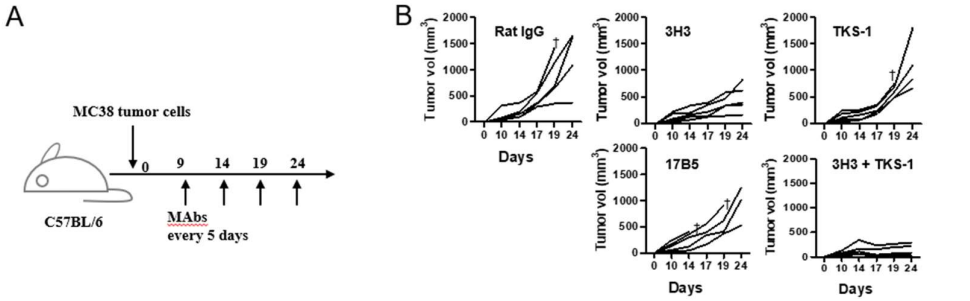

Figure 2. Synergistic Antitumor Effects of Combined Anti-4-1BB and Anti-4-1BBL Agonists in a Mouse Model of Colon Cancer. (A) MC38 colon tumor cells were subcutaneously injected into the backs of C57BL/6 mice. Ten days after the tumor cell injection, four different treatments were administered at 5-day intervals: anti-4-1BB monoclonal antibody (mAb) 3H3, anti-4-1BB mAb 17B5, anti-4-1BBL mAb TKS-1, and a combination of anti-4-1BB mAb and anti-4-1BBL mAb. (B) The tumor growth curve was generated by measuring tumor growth at 3- or 4-day intervals.

During routine monitoring of the tumor growth rate, a noteworthy observation emerged: the simultaneous administration of anti-4-1BB and anti-4-1BBL mAb led to a synergistic effect, resulting in a substantial suppression of tumor growth (Fig. 2B). These findings strongly support the notion that co-administration of anti-4-1BBL mAb enhances the *in vivo* anti-tumor effects of anti-4-1BB mAb.

3. The 4-1BBL agonist augments the egress of anti-4-1BB-stimulated $CD8^+$ T cells from lymph nodes

Tumor growth inhibition was observed when a combination of anti-4-1BB and anti-4-1BBL agonist antibodies was employed. To investigate the direct impact of 4-1BBL on $CD8^+$ T cells, $CD8^+$ T cells isolated from the LNs and spleen of Thy1.1 Pmel-1 Tg were labeled with CFSE and i.v. administered into C57BL/6. All mice were subsequently immunized s.c. with mgp100 peptides emulsified with IFA. Specifically, antibodies 3H3, TKS-1, 17B5, and a combination of 3H3 and TKS-1 were administered i.p. twice on days 0 and 2 (Fig. 3A). For the subsequent analysis, single-cell suspensions from TDLNs of the mice on days 5 and 7 were stained with anti-CD8-PE and anti-Thy1.1-PE-Cy5. Proliferation of the transferred $CD8^+$ T cells were confirmed on days 5 and 7 through the CFSE dilution assay. Notably, the total cell numbers were increased when TKS-1 was used in combination compared to when 3H3 was administered alone. On the 7^{th} day, a substantial population of the transferred $CD8^+$ T cells was still persisted in mice administered with 3H3 alone, whereas the increased $CD8^+$ T cell population was disappeared in mice administered the combination with TKS-1 (Fig. 3B-D). This observation implies that $CD8^+$ T cells activated by the antibody exit the LNs, and anti-4-1BBL facilitates the egress of activated $CD8^+$ T cells.

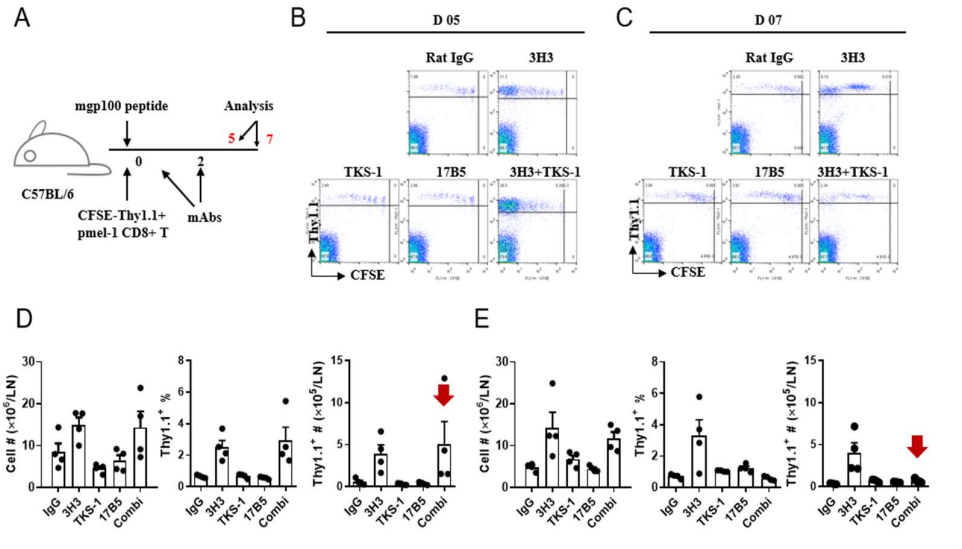

Figure 3. Characterizing the Impact of 4-1BB and 4-1BBL Agonists on CD8$^+$ T Cell Dynamics and Anti-tumor Synergy. (A) CD8$^+$ T cells were isolated from the lymph nodes (LNs) and spleen of Thy1.1 Pmel-1 transgenic mice, labeled with 10 μM CFSE, and intravenously injected into C57BL/6 mice via the tail vein. The B6 mice were subsequently subcutaneously immunized with 20 μg of mgp100 in an IFA (Incomplete Freund's Adjuvant) emulsion. Antibodies were administered twice, on days 0 and 2. (B ~ D) On day 5 and 7, the inguinal LNs were harvested from the mice and processed into single-cell suspensions. These cells were then stained with anti-CD8-PE and anti-Thy1.1-PE-Cy5, and a CFSE dilution analysis was conducted on Thy1.1$^+$CD8$^+$ T cells using a FACSCalibur flow cytometer. The single-cell suspension in LNs was quantified automatically using a cell counter (ADAM). The percentage of Thy1.1$^+$ cells was determined using FACSCalibur, and the absolute number of Thy1.1$^+$ cells was calculated by multiplying the total live cells by the Thy1.1% value.

4. Both 4-1BB and 4-1BBL of $CD8^+$ T cells are required for the enhancement of $CD8^+$ T cell proliferation

By isolating $CD8^+$ T cells from 4-1BB$^{-/-}$ or 4-1BBL$^{-/-}$ Thy1.1 Pmel-1 Tg mice, $CD8^+$ T cells lacking 4-1BB or 4-1BBL were prepared. These cells were labeled with 10 μM CFSE and transferred into B6 mice. Subsequently, B6 mice were i.p. injected with rat IgG, 3H3, and a combination of 3H3 and TKS-1 on days 0 and 2, followed by analysis on day 7 (Fig. 4A). Single-cell suspensions of TDLNs were prepared and stained with anti-CD8-PE and anti-Thy1.1-PE-Cy5, and subjected to a CFSE dilution assay.

In mice receiving WT Pmel-1 $CD8^+$ T cells, 3H3 treatment increased the ratio of CFSE-negative Pmel-1 $CD8^+$ T cells, a response attenuated by combining anti-4-1BBL mAb TKS-1 (Fig. 4B). In contrast, 4-1BB$^{-/-}$ Pmel-1 $CD8^+$ T cells displayed poor proliferation regardless of 3H3 or combined treatment with TKS-1 (Fig. 4B), indicating the essential role of 4-1BB signaling for the survival of dividing $CD8^+$ T cells. 4-1BBL$^{-/-}$ Pmel-1 $CD8^+$ T cells showed increased CFSE-negative cells with 3H3 treatment, albeit less than WT Pmel-1 $CD8^+$ T cells, and TKS-1 did not decrease CFSE-negative cells (Fig. 4B). Statistical analysis confirmed that 3H3 treatment significantly increased total cell numbers of TDLN in mice receiving WT and 4-1BBL$^{-/-}$ Pmel-1 $CD8^+$ T cells, but not 4-1BB$^{-/-}$ Pmel-1 $CD8^+$ T cells (Fig. 4C). TKS-1 reduced total cell numbers of TDLNs in the mice received WT Pmel-1 $CD8^+$ T cells but not 4-1BB$^{-/-}$ and 4-1BBL$^{-/-}$ Pmel-1 $CD8^+$ T cells (Fig. 4C).

Statistical analysis indicates that percentages of Pmel-1 $CD8^+$ T cells were increased with 3H3 in mice receiving WT and 4-1BBL$^{-/-}$ Pmel-1 $CD8^+$ T cells, but not in the mice received 4-1BB$^{-/-}$ Pmel-1 $CD8^+$ T cells due to poor proliferation (Fig. 4C). Although the combined treatment with TKS-1 decreased the numbers of anti-4-1BB-treated Pmel-1 $CD8^+$ T cells, it was not statistically significant (Fig. 4C). Similar trends were observed in absolute numbers of Pmel-1 $CD8^+$ T cells, with a significant decrease in anti-4-1BB-treated cells by TKS-1 only in mice receiving WT Pmel-1 $CD8^+$ T cells (Fig. 4C).

These results underscore the essential role of 4-1BB signals for the survival of dividing $CD8^+$ T cells, with 4-1BBL signals of $CD8^+$ T cells further enhancing the

proliferation and differentiation of anti-4-1BB-treated CD8$^+$ T cells.

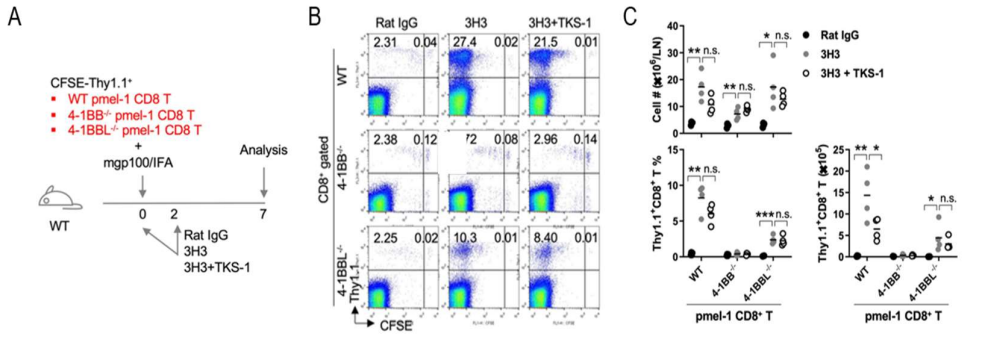

Figure 4. 4-1BBL also plays a crucial role in stimulating CD8$^+$ T cell proliferation induced by the 4-1BB agonist. (A) CD8$^+$ T cells were harvested from Thy1.1 Pmel-1 Tg, 4-1BB $^{-/-}$ Thy1.1 Pmel-1 Tg and 4-1BBL $^{-/-}$ mice. They were labeled with 10 μM CFSE and then intravenously injected into B6 mice. Subsequently, B6 mice were immunized with 20 μg of mgp100 in IFA. Rat IgG, anti-4-1BB, and anti-4-1BB combined with anti-4-1BBL were intravenously injected twice on days 0 and 2. On day 7, lymph nodes (LNs) were isolated and processed into single-cell suspensions. (B) These cells were stained with anti-CD8-PE and anti-Thy1.1-PE-Cy5, and a CFSE assay was conducted using a FACSCalibur instrument. (C) Cell quantification in the single-cell suspensions derived from lymph nodes was conducted via an ADAM cell counter. The proportion of Thy1.1$^+$ cells was evaluated using FACSCalibur (BD Biosciences), and the absolute count of Thy1.1$^+$ cells was determined by multiplying the total count of viable cells by the percentage of Thy1.1$^+$ cells.

5. The interaction between 4-1BB and 4-1BBL: Insights into *Cis*- and *Trans*-Interactions within CD8$^+$ T Cells

CD8$^+$ T cells, traditionally recognized for their interaction with antigen-presenting cells (APC), also exhibit interactions with other T cells. This study explores the investigation of whether the *trans*- and *cis*-interaction between 4-1BB and 4-1BBL contributes to the survival of CD8$^+$ T cells. Flow cytometry analysis was employed to confirm the expression of 4-1BB and 4-1BBL in activated CD8$^+$ T cells derived from B6, 4-1BB$^{-/-}$, and 4-1BBL$^{-/-}$ mice. While 4-1BB was expressed on WT and 4-1BBL$^{-/-}$ CD8$^+$ T cells, and 4-1BBL was not expressed on WT and 4-1BBL$^{-/-}$ CD8$^+$ T cells, it was unexpectedly expressed on 4-1BB$^{-/-}$ CD8$^+$ T cells (Fig. 5A).

Subsequent confocal microscopy examination of 4-1BB expression of CD8$^+$ T cells in inguinal TDLNs revealed not only surface expression but also a more prominent intracellular presence of 4-1BB (Fig. 5B). However, the visualization of 4-1BBL proved challenging (data not shown). Of note, 4-1BB was observed in an aggregated form within a CD8$^+$ T cell, suggesting the transmission of signaling inside of cell. These findings confirm that 4-1BB and 4-1BBL engage not only in *trans*-interactions but also in *cis*-interactions within the cellular context (Fig. 5C).

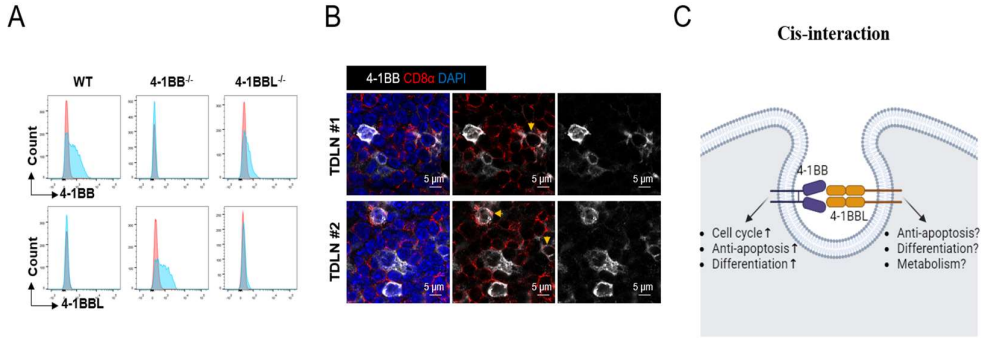

Figure 5. 4-1BB is not only present on the cell surface but also within activated CD8$^+$ T cells. (A) Tumor-draining lymph nodes from MC38 tumor-bearing mice were isolated,

stained with anti-4-1BB and anti-4-1BB antibodies, and subjected to analysis using FACSCalibur. (B) Tumor-draining lymph nodes (TDLNs) isolated from MC38 tumor-bearing mice were stained with 4-1BB mAb and 4-1BBL mAb, and imaged using confocal microscopy. (C) The presence of 4-1BB in activated $CD8^+$ T cells facilitates a *cis*-interaction with 4-1BBL also expressed in activated $CD8^+$ T cells, contributing to cell growth and survival.

6. Enhancing anti-tumor efficacy of anti-4-1BB treatment with optimal sodium lactate supplementation

We have provided an explanation how agonistic anti-4-1BB mAb induces a massive immune response of $CD8^+$ T cells *in vivo*, especially in the presence of a limited population of $4\text{-}1BB^+CD8^+$ T cells, which was confirmed that the 4-1BB of activated $CD8^+$ T cells transmits signals within the cell by *cis*-interacting with the 4-1BBL it expresses. However, it was still not clear how the agonistic 4-1BB antibody continuously maintains $CD8^+$ T cell responses *in vivo*.

One of previous studies has revealed that anti-4-1BB stimulation significantly boosts lactate production of $CD8^+$ T cells.[11] Lactic acid, when produced by actively dividing cells, becomes a crucial energy source fueling their growth, which was challenged the conventional view of lactate as a metabolic waste.[10,18,19,20] This leads to a fundamental question: Can lactate support the proliferation of anti-4-1BB-stimulated $CD8^+$ T cells, vital players in anti-tumor immunity? Therefore, B6 mice were s.c. injected with MC38 tumor cells and subsequently, the mice received i.p. administration of rat IgG or anti-4-1BB mAb every 5 days, starting when the tumor tissue reached a diameter of 2-3 mm on day 10. In addition to the anti-4-1BB mAb, the mice were also treated with varying doses of sodium lactate (NaLac), including 0, 0.5, 1, 1.5, and 2 g/Kg (Fig. 6A). Routine monitoring of tumor growth rates revealed a synergistic suppression of tumor growth when NaLac was co-administered with the anti-4-1BB mAb, particularly within the range of 1 – 1.5 g/Kg NaLac dosage (Fig. 6B).

These findings strongly indicate that co-administration of NaLac enhances the anti-tumor effects of the anti-4-1BB mAb *in vivo*.

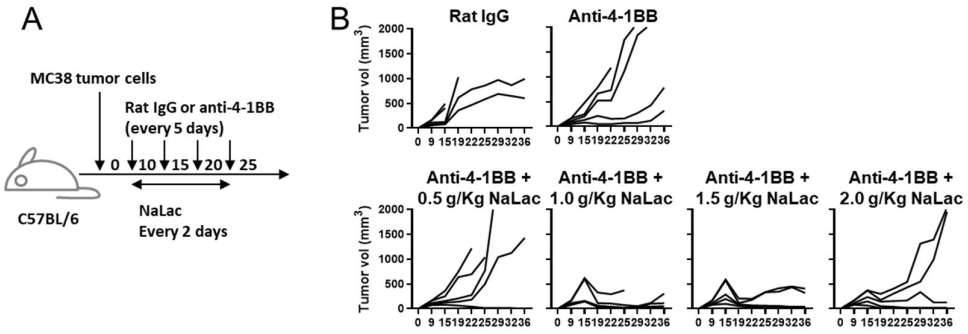

Figure 6. Synergistic anti-tumor effect of combining optimal lactate levels with anti-4-1BB mAb in a tumor model. (A) MC38 colon tumor cells were s.c. injected into the back of C57BL/6 mice. Ten days after tumor cell injection, anti-4-1BB mAb or rat IgG was administered a total of four times at 5-day intervals, accompanied by NaLac injections at concentrations ranging from 0.5 to 2 g/Kg every 2 days. (B) Tumor growth curve generated by measuring tumor growth at 3 or 4-day intervals.

7. Absence of synergistic effects of anti-4-1BB mAb and NaLac in cold tumors

Tumor tissues can be classified into "cold" or "hot" categories based on their immune microenvironment. Cold tumors typically lack immune cell infiltration, making them less responsive to immunotherapies. In contrast, hot tumors have robust immune cell presence and are more amenable to treatments like immune checkpoint inhibitors, offering promising avenues for cancer therapy. Therefore, we determine the efficacy of combining anti-4-1BB mAb and NaLac in cold tumors. For this investigation, B6 mice were challenged with MC38 hot tumor cells and Babl/c mice with the poorly immunogenic CMS5a cells (murine fibrosarcoma). On day 10, the tumor-bearing mice received i.p. injections of either rat IgG or anti-4-1BB mAb every 5 days, along with an i.p. administration of 1 g/Kg NaLac (Fig. 7A).

Again, while anti-4-1BB or NaLac alone moderately suppressed MC38 tumor cell growth, their combined administration resulted in complete suppression (Fig. 7B). However, in the case of CMS5a tumor cells, neither anti-4-1BB mAb nor NaLac, alone or in combination, demonstrated suppressive effects. These results underscore the efficacy of synergistic anti-tumor effects of anti-4-1BB mAb and NaLac specifically in "hot" tumors.

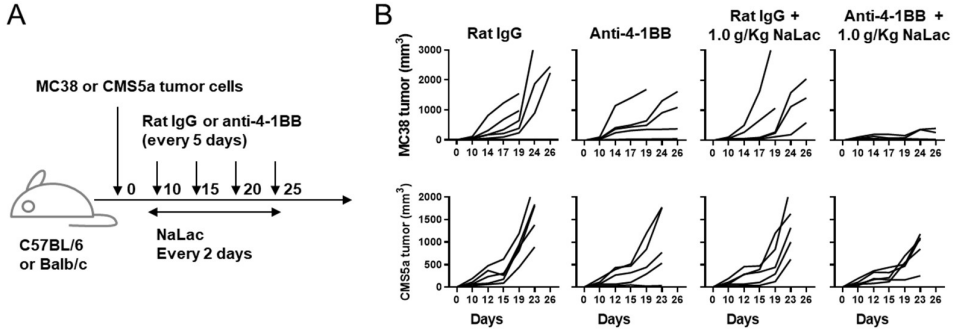

Figure 7. Synergistic effects of anti-4-1BB mAb and NaLac are poorly effective in "cold" tumors. (A) MC38 or CMS5a tumor cells were s.c. injected into the backs of C57BL6 or Balb/c mice. Ten days after tumor cell injection, anti-4-1BB mAb or rat IgG was administered via i.p. injection to the mice at 5-day intervals, and NaLac was given at a dosage of 1g/Kg every 2 days for a total of four administrations. (B) Tumor growth in the mice was regularly monitored at 3- or 4-day intervals.

8. Enhanced proliferation of $CD8^+$ T cells receiving strong TCR signals through NaLac

While NaLac treatment has demonstrated enhanced anti-tumor effects in conjunction with anti-4-1BB mAb (Fig. 6 and 7), it remains uncertain whether NaLac contributes to the proliferation of $CD8^+$ T cells *in vivo*. To address this question, $CD8^+$ T cells were isolated from the lymph nodes and spleens of $Thy1.1^+$ pmel-1 Tg mice, labeled with 10 μM CFSE, and adoptively transferred into B6 mice. Subsequently, all mice were s.c. immunized with either mouse (mgp100) or human gp100 (hgp100) peptides emulsified

with incomplete Freund's adjuvant (IFA). On days 1 and 3, the mice received i.p. injections of either rat IgG or anti-4-1BB mAb, followed by NaLac administration as depicted in Figure 4A. On day 5, single-cell suspensions from inguinal LNs were prepared and stained with anti-Thy1.1-PE-Cy5 and anti-CD8-PE. CD8-gated cells were plotted as Thy1.1 vs. CFSE.

For mice immunized with mgp100 peptide, the division of pmel-1$^+$Thy1.1$^+$CD8$^+$ T cells induced by mgp100 immunization was limited, but significantly accelerated by anti-4-1BB treatment (Fig. 8B). Notably, NaLac alone was significantly altered the division rate of pmel$^+$Thy1.1$^+$CD8$^+$ T cells from rat IgG- or anti-4-1BB-treated mouse (Fig. 8B). Total cell numbers in inguinal LNs and the percentages and absolute numbers of pmel-1$^+$Thy1.1$^+$CD8$^+$ T cells were also not significantly affected by NaLac treatment, compared to rats IgG-treated mice, nor by the combined treatment compared to anti-4-1BB-treated mice (Fig. 8C). These findings align with the observations that NaLac, either alone or in combination with anti-4-1BB mAb, exhibited limited efficacy in treating "cold" tumors (Fig. 7B).

For hgp100-immunized mice, CFSE dilution of pmel-1$^+$Thy1.1$^+$CD8$^+$ T cells was comparably enhanced by anti-4-1BB or NaLac treatment alone but not further enhanced by the combined treatment of anti-4-1BB and NaLac (Fig. 8D). Surprisingly, total cell numbers of inguinal LNs and the percentages and absolute numbers of pmel$^+$Thy1.1$^+$CD8$^+$ T cells were significantly increased by anti-4-1BB treatment compared to rat IgG-treated mice, and remarkably, also by NaLac treatment alone. However, there was no additional increase with the combined treatment compared to anti-4-1BB treatment alone (Fig. 8E).

These results suggest that NaLac treatment supports the proliferation of CD8$^+$ T cells exposed to strong TCR signals and the enhanced effects of anti-4-1BB treatment on CD8$^+$ T cell proliferation likely involve lactic acid-mediated mechanisms.

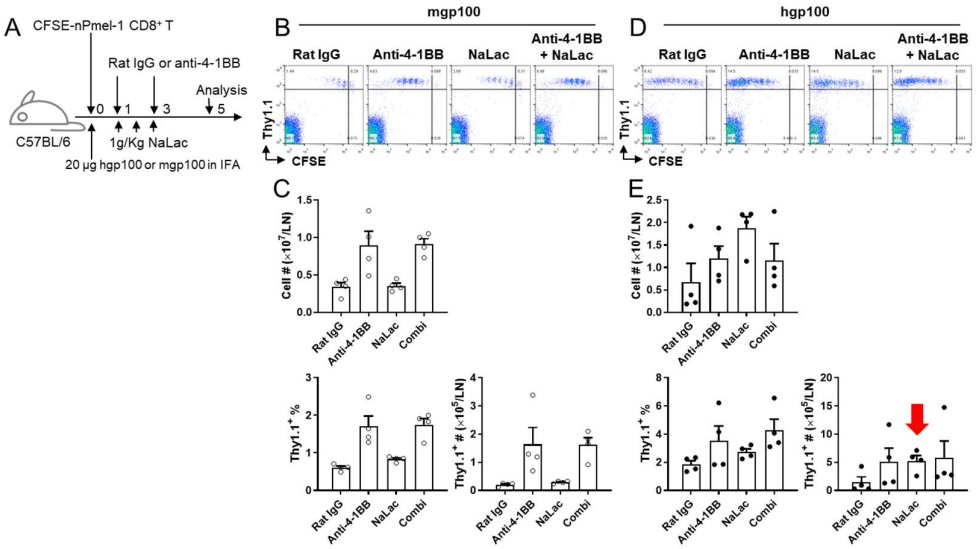

Figure 8. Lactate-induced proliferation is dependent on adequate CD8$^+$ T cell activation. (A) CD8$^+$ T cells, isolated from Thy1.1$^+$ pmel-1 Tg mice, were labeled with 10 μM CFSE and i.v. injected into the tails of C57BL/6 mice, accompanied by s.c. immunization of 20 μg hgp100 or mgp100 in IFA. One day later, NaLac was administered at a dose of 1g/Kg three times daily, and anti-4-1BB mAb was administered twice at two-day intervals. (B and D) On day 5, inguinal LNs from the mice were isolated and processed to prepare a single-cell suspension. These cells were stained with anti-CD8-PE and anti-Thy1.1-PE-Cy5, and the CFSE dilution of Thy1.1$^+$CD8$^+$ T cells was analyzed using FACSCalibur (BD Biosciences). (C and E) Single-cell suspensions of LNs were counted using an automated cell counter (ADAM, NanoEntek, Seoul, Korea). The percentage of Thy1.1$^+$ cells was determined using FACSCalibur (BD Biosciences), and the absolute numbers of Thy1.1$^+$ cells were calculated by multiplying the total count of live cells by the percentage of Thy1.1$^+$ cells.

9. NaLac directly enhances the proliferation of activated CD8$^+$ T cells *in vitro*

In vivo NaLac treatment significantly boosted the proliferation of CD8$^+$ T cells

exposed to strong TCR signaling (Fig. 8E). However, as injected NaLac could potentially impact various immune and tissue cell types, we subsequently investigated its direct influence on $CD8^+$ T cells *in vitro*. When CFSE-labeled $CD8^+$ T cells were stimulated with anti-CD3 alone or in combination with anti-4-1BB mAb for 3 days, $CD8^+$ T cell division was enhanced by anti-CD3 alone and further potentiated by the addition of anti-4-1BB mAb (Fig. 5A). When NaLac was added to $CD8^+$ T cells at 0, 1, and 2 days after anti-CD3 stimulation, $CD8^+$ T cells exposed to NaLac at day 0 exhibited a dose-dependent decrease in viability, whereas NaLac treatment at day 1 or 2 led to a dose-dependent increase in $CD8^+$ T cell proliferation (Fig. 9B ~ C). These findings highlight the necessity for $CD8^+$ T cell activation for NaLac to support their proliferation.

When varying concentrations of NaLac were added to activated $CD8^+$ T cells in the presence of anti-CD3 alone or anti-CD3 plus anti-4-1BB mAb, the division and survival rates of anti-CD3-stimulated $CD8^+$ T cells were notably enhanced within the 0.5 – 1.5 g/L NaLac range, whereas the effect was only partial with 1.5 g/L NaLac in $CD8^+$ T cells stimulated with anti-CD3 pluas anti-4-1BB mAb (Fig. 9D ~ E). These results suggest that NaLac supports the proliferation of activated $CD8^+$ T cells within optimal concentration ranges, and the direct effects of NaLac in enhancing $CD8^+$ T cell proliferation are attenuated in the presence of anti-4-1BB mAb.

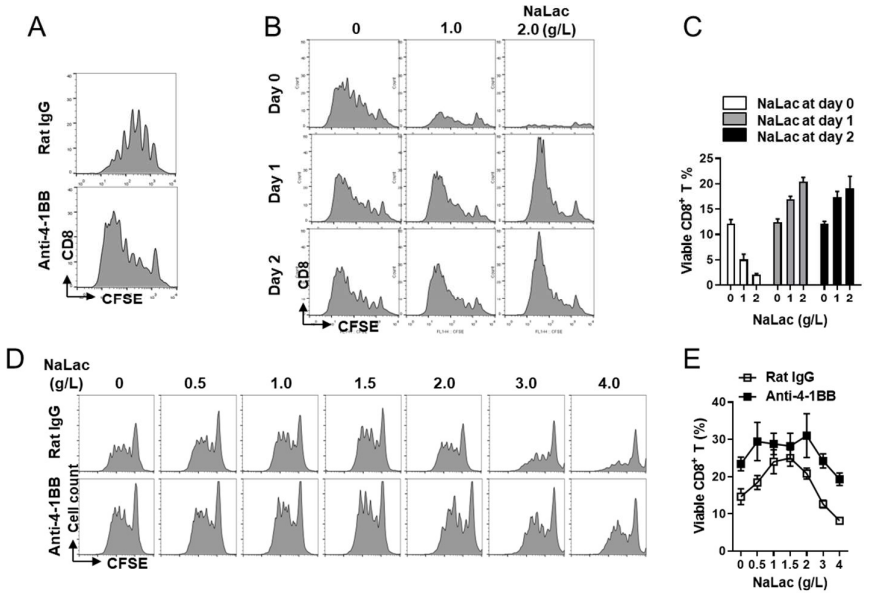

Figure 9. NaLac directly supports the proliferation of activated CD8$^+$ T cells, but not naïve T cells. CD8$^+$ T cells were isolated using micro CD8 beads from LNs and spleens of B6 mice, and labeled with CFSE. The CFSE-labeled CD8$^+$ T cells were suspended in RPMI1640 supplemented with 10% FBS at 1×10^6 cells/ml concentration. (A) The cells were seeded in 96 well round-bottom plate that were coated with 1 μg/ml of anti-CD3 and/or anti-4-1BB mAb. CFSE dilution was determined by FACSCalbur (BD Biosciences) 3 days after the culture. (B-C) The cells were seeded in 96 well round-bottom plate that were coated with 1 μg/ml of anti-CD3 mAb and 0, 1, or 2 g/L NaLac was added to the cells at day 0, 1, and 2. CFSE dilution was determined by FACSCalbur (BD Biosciences) 3 days after the culture (B) and percentages of live cells were calculated by flow cytometry (C). (D ~ E) The cells were seeded in 96 well round-bottom plate that were coated with 1 μg/ml of anti-CD3 and/or anti-4-1BB mAb. CFSE dilution was determined by FACSCalbur (BD Biosciences) 3 days after the culture. The indicated concentrations of NaLac were added to the cells one days after the culture. The CFSE dilution assay was performed after 3 days using FACSCalibur (D) and percentages of live cells were calculated by flow cytometry (E).

10. MCT-1 expression is higher on fibroblast and antigen-presenting cells rather than CD8$^+$ T cells

Monocarboxylate transporter 1 and 4 (MCT1 and MCT4) are integral proteins that play a crucial role in the transport of lactate in and out of cells. These transporters facilitate the exchange of lactate and protons, impacting various physiological processes, including lactate utilization during energy metabolism and its removal from cells. As MCT1 is responsible for cellular lactate uptake, its expression in LNs was examined to determine whether the lactic acid generated by proliferating CD8$^+$ T cells, especially following anti-4-1BB stimulation, is primarily taken up by these T cells or neighboring cells. Inguinal LNs from MC38 tumor-bearing mice were collected, and tissue sections were used to assess MCT1 expression on various cell types, including ER-TR7$^+$ fibroblastic reticular cells (FRCs), CD68$^+$ macrophages, CD8β$^+$ T cells, and CD11c$^+$ dendritic cells (DCs). Confocal microscopy revealed prominent MCT-1 expression on ER-TR7$^+$ FRCs, CD11c$^+$ DCs, and CD68$^+$ macrophages, but minimal expression on CD8β$^+$ T cells (Fig. 10B). These findings suggest that surrounding cells of proliferating CD8$^+$ T cells are more likely to utilize lactic acid than the proliferating CD8$^+$ T cells themselves.

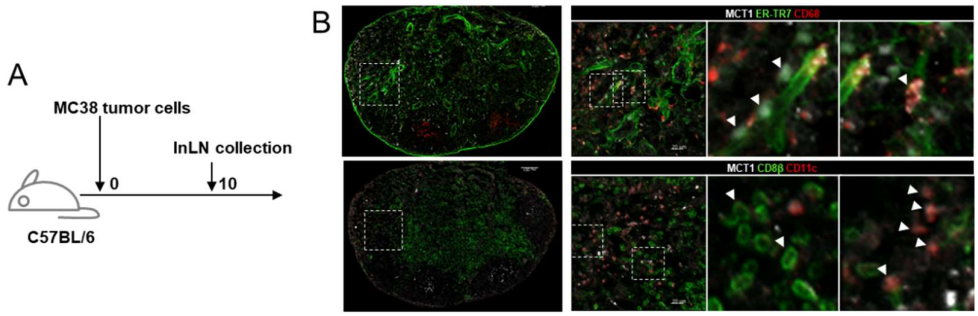

Figure 10. Localization of MCT1 expression in lymph node. (A) TDLNs were isolated ten days after the administration of MC38 colon tumor cells to C57BL/6 mice. (B) Paraffin section of TDLNs were stained with anti-MCT1, anti-ER-TR7, and anti-CD68 mAbs or anti-MCT1, anti-CD8β, and anti-CD11c mAbs. The slides were scanned with confocal microscope (LSM 780 Confocal Microscope, Carl zeiss, Oberkochen, Germany).

IV. DISCUSSION

Anti-4-1BB treatment preferentially and robustly induces the proliferation of $CD8^+$ T cells *in vivo* (Fig. 1B), despite the presence of only a small population of $4\text{-}1BB^+CD8^+$ T cells (Fig. 1C). This paradoxical observation prompts questions regarding the underlying mechanisms sustaining their persistence. To address these questions, we investigated agonistic anti-4-1BB antibodies and agonistic anti-4-1BBL antibodies in an *in vivo*.

In *in vivo* tumor model, we observed that anti-4-1BBL, when used in conjunction with anti-4-1BB, exhibited anti-tumor effects, and it facilitated the egress from LNs of anti-4-1BB-stimulated $CD8^+$ T cells (Fig. 3B and C). Furthermore, we found that the induction of $CD8^+$ T cell proliferation by anti-4-1BB is diminished in the absence of 4-1BBL (Fig. 4B and C). Interestingly, we identified the presence of 4-1BB not only on the surface of activated $CD8^+$ T cells but also intracellularly (Fig. 5B), and the interaction with 4-1BBL occurs in *cis*, resulting in a more robust signal compared to *trans*-interactions. This increased activation of $CD8^+$ T cells by anti-4-1BB was identified as the underlying mechanism.

We have first elucidated the mechanism by which agonistic anti-4-1BB mAb elicits a robust immune response in $CD8^+$ T cells *in vivo*, particularly in the context of a limited population of $4\text{-}1BB^+CD8^+$ T cells. This was clarified by demonstrating that activated $CD8^+$ T cells employ *cis*-interactions between the 4-1BB and the 4-1BBL it expresses. However, the persistent maintenance of $CD8^+$ T cell responses by the agonistic 4-1BB antibody *in vivo* remained unclear.

Therefore, we questioned whether lactate, often considered a metabolic waste product, might play a role as an energy source for rapidly dividing cells including $CD8^+$ T cells.[10,21] To test this hypothesis, we conducted *in vivo* and *in vitro* experiments involving the treatment of $CD8^+$ T cells with lactate. Anti-4-1BB treatment not only stimulates $CD8^+$ T cells but also significantly increases the production of lactate, a metabolic byproduct once considered mere waste.[11,22] Lactate's newfound importance lies in its potential as an energy source for rapidly dividing cells,[10,23,24] including $CD8^+$ T cells. *In vivo* tumor model

demonstrate that lactate does enhance the anti-tumor effects of anti-4-1BB treatment, particularly in “hot” tumors (Fig. 7B). Additionally, lactate influenced the increase in anti-4-1BB-stimulated $CD8^+$ T cells only when an activated immune response was present (Fig. 8B and D). *In vitro* experiments with $CD8^+$ T cells isolated from mice confirmed that the efficacy of lactate appeared after $CD8^+$ T cells were activated, enhancing their viability (Fig. 9C). Notably, the positive effect on $CD8^+$ T cell proliferation activated by anti-4-1BB was only observed when an appropriate concentration of lactate was used (Fig. 9D and E).

In conclusion, our study underscores the significance of *cis*-interactions between 4-1BB and 4-1BBL in fostering the proliferation and differentiation of $CD8^+$ T cells. Furthermore, our findings suggest the potential use of lactate, a by-product of glucose metabolism, as a supportive energy source for dividing $CD8^+$ T cells when administered concomitantly with anti-4-1BB mAb. Considering the higher expression of MCT-1 in fibroblasts and APCs compared to $CD8^+$ T cells, we hypothesize that lactate produced by proliferating $CD8^+$ T cells is likely taken up by surrounding fibroblasts and APCs in the lymph nodes, undergoing modification to aid $CD8^+$ T cell proliferation - an avenue that warrants further investigation.

V. CONCLUSION

Here we demonstrate that the activation of anti-4-1BB results in the migration of $CD8^+$ T cells from lymph nodes and elicits an anti-tumor response in the MC38 tumor model. Interestingly, it was observed that 4-1BB activation takes place not only on the cell surface of activated $CD8^+$ T cells but also intracellularly. Furthermore, this investigation confirmed that the interaction between 4-1BB and 4-1BBL occurs in both *trans* and *cis* configurations. Additionally, the study demonstrated that treatment with NaLac significantly enhanced the 4-1BB-mediated anti-tumor response in the MC38 tumor model, ultimately leading to improved survival rates among activated $CD8^+$ T cells (Fig.11).

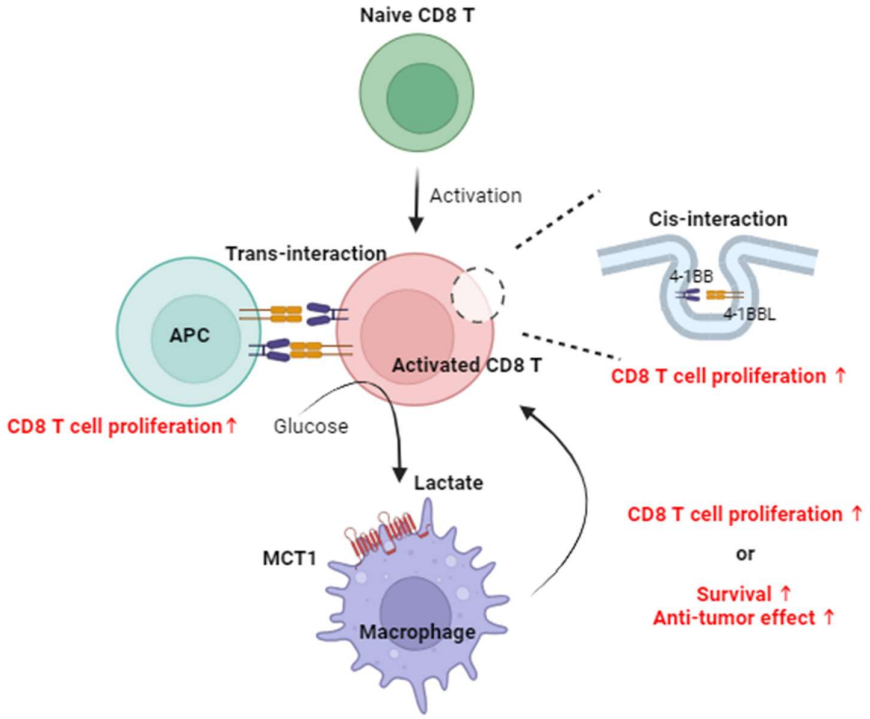

Figure 11. Graphic summary of 4-1BB-mediated enhancement of $CD8^+$ T cells

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ABSTRACT(IN KOREAN)

CD8$^+$ T 세포의 증식과 생존에 4-1BB-4-1BBL 시스상호작용과 젖산의 기여

<지도교수 남기택>

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조 은 정

4-1BB (CD137)는 T 세포의 활성, 생존, 분화 및 증식에 긍정적인 영향을 미치며, agonistic anti-4-1BB 항체는 4-1BB 신호전달을 강화시켜 T 세포의 활성을 촉진시킨다. 4-1BB는 활성화된 T 세포 표면에서 발현하며 항원제시세포 (antigen-presenting cell; APC)가 세포표면에 발현한 4-1BB ligand (4-1BBL; CD137L)와 *trans*-interaction을 한다고 알려져 있다. 하지만 최근 4-1BBL는 APC 뿐만 아니라 T 세포 표면에서도 발현되는 것으로 보고되어, *cis*-interaction에 의한 T 세포 신호전달도 가능할 것으로 예상되었다.

이를 증명하기 위해 agonistic & antagonistic anti-4-1BB와 anti-4-1BBL 항체를 사용하여 실험을 수행하였다. Agonistic anti-4-1BBL 항체는 agonistic anti-4-1BB 항체의 항암효과를 강화하는 것으로 나타났으며, 4-1BBL 신호전달의 강화는 4-1BB로 자극된 CD8$^+$ T 세포가 활성화 후 림프구에서 빠져나가는 과정(egress)을 촉진하는 것으로 나타났다. 또한, 4-1BBL는 정상적인 T 세포 표면에서는 거의 검출되지 않으며, 4-1BB는 세포막 보다는 세포질내 더 많이 위치하고 있는 것으로 확인되어, 4-1BB-4-1BBL의 *cis*-interaction 발생 가능성을 조사하였다. 4-1BB$^{-/-}$ & 4-1BBL$^{-/-}$ pmel-1 Tg 생쥐로부터 분리한 CD8$^+$ T 세포의

adoptive transfer 실험을 통해, 4-1BBL가 결핍된 pmel-1 $CD8^+$ T 세포는 anti-4-1BB 항체의 효과가 절반으로 감소하며, anti-4-1BBL 항체의 효과는 완전히 사라짐을 확인하였다. 이러한 일련의 실험을 통해 4-1BB-4-1BBL의 *cis*-interaction이 *trans*-interaction 보다 4-1BB-4-1BBL에 의한 $CD8^+$ T 세포 증식 강화효과에 더 높은 비중을 차지하고 있음을 확인하였으며, 활성화된 $CD8^+$ T 세포에서는 4-1BB와 4-1BBL가 서로 *cis*-interaction하여 세포내에 대부분 위치함으로써 세포막에서는 극히 일부만이 검출되고 있는 것으로 예상되었다. 본 실험들을 통해 생체내 $4\text{-}1BB^+CD8^+$ T 세포의 비율이 극히 낮음에도 불구하고 agonistic anti-4-1BB 항체의 효과가 강력하게 나타나는 이유를 설명할 수 있었다.

그러나 여전히 agonistic anti-4-1BB 항체가 생체내에서 $CD8^+$ T 세포의 증식 및 분화를 지속적으로 강화하는 이유는 여전히 명확하지 않았다. 최근 'Warburg effect'라고 알려진 빠르게 증식하는 세포들이 호기성조건에서도 대량의 glucose를 흡수하여 젖산 (lactic acid)를 분비하는 현상에 대한 새로운 연구결과들이 발표되었다. 빠르게 증식하는 세포들이 분비하는 젖산은 그동안 단순 부산물로 알려져 왔지만, 최근 연구들에서는 젖산이 빠르게 증식하는 세포들이 필요로하는 에너지원이라고 주장하였다. 기존연구를 통해 이미 agonistic anti-4-1BB 항체는 $CD8^+$ T 세포의 glucose & fatty acid metabolism을 강화하며, 젖산의 대량분비를 촉진함으로 보고하였다. 따라서 최근 연구결과를 반영하면, 4-1BB 신호전달에 의한 증가된 젖산이 $CD8^+$ T 세포의 증식을 지속화 하는데 중요한 역할을 담당할 것으로 예상되었다.

이 가설을 증명하기 위해, agonistic anti-4-1BB 항체와 젖산을 MC38 종양을 투여한 마우스에 병용 투여하였다. 생쥐 암모델에서 적정 농도의 젖산 투여는 agonistic anti-4-1BB 항체의 항암효과를 더욱 증진시키는 것으로 확인되었다.

또한 이러한 병용투여에 의한 항암효과는 면역세포의 침윤비율이 높은 “hot tumor”에서만 나타났으며, 면역세포의 침윤이 적은 “cold tumor”에서는 나타나지 않았다. 따라서 생체내 활성화된 $CD8^+$ T 세포들은 agonistic anti-4-1BB 항체 투여에 의해 증식이 촉진되며, 이 과정에서 증가한 젖산은 $CD8^+$ T 세포의 증식을 다시 보조함으로써 agonistic anti-4-1BB 항체의 효과를 지속시키는 것으로 판단되었다.

핵심단어 : $CD8^+$ T 세포, 젖산, 대사과정, 4-1BB (CD137)

PUBLICATION LIST

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