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Effects of Interleukin-17 on CD40 and CD40L Expression in Jurkat and Mino Cell Lines

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ABSTRACT

Interleukin-17 (IL-17, also known as IL-17A) plays a role in immune regulation and pathogenesis of various diseases. Cluster of differentiation 40 (CD40) and its ligand CD40L are critical for T and B cell communications and activation of immune responses. This study investigated the effects of IL-17A on CD40L expression in Jurkat T cells and CD40 expression in Mino B cells. The cells were treated with IL-17A (40 ng/mL for Jurkat cells and 20 ng/mL for Mino cells) for durations ranging from 0 to 24 hours. Protein expression levels were assessed using Western blotting combined with densitometric analysis. IL-17A treatment showed a trend toward increased CD40L expression in Jurkat cells, although this did not reach statistical significance ($p > 0.05$). In contrast, CD40 expression in Mino cells was significantly decreased at 24 h compared to the control group ($p < 0.05$). These findings indicate that IL-17A differentially regulates the expression of co-stimulatory molecules in T and B cells, potentially contributing to its modulatory effects on T-B cell interactions.

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1. Introduction

The interleukin-17 (IL-17) family consists of six pro-inflammatory cytokines: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25), and IL-17F, among which IL-17A and IL-17F have been most extensively investigated [1-2]. IL-17A is predominantly secreted by $CD4^+$ T helper 17 (Th17) cells and various subsets of innate immune cells, and it exerts its biological effects through heterodimeric receptor complexes that include IL-17 receptor A (IL-17RA) and IL-17 receptor C (IL-17RC) subunits [3-5]. Functionally, IL-17A has been implicated in a broad spectrum of inflammatory and immune-mediated diseases, including psoriasis, ankylosing spondylitis, rheumatoid arthritis, and inflammatory bowel disease, as well as in various cancers such as pancreatic, cervical, prostate, colorectal, lung, and digestive tract cancers [6-12]. Mechanistically, IL-17 initiates pro-inflammatory signaling cascades through its interaction with NF-κB activator 1 (Act1), a protein harboring a Similar Expression to Fibroblast growth factor genes and IL-17R (SEFIR) domain that binds to IL-17RA/IL-17RC via SEFIR-SEFIR domain interactions [13, 14]. This molecular engagement subsequently activates downstream signaling pathways, such as NF-κB and mitogen-activated protein kinase (MAPK) pathways, culminating in upregulation of chemokines and cytokines that facilitate immune cell activation and regulate expression of immune-related surface molecules, such as programmed death-ligand 1 (PD-L1)

[15-16]. In different cell types, this regulatory process may engage additional signaling mechanisms, such as activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) or AKT [15]. These signaling characteristics imply that IL-17 may modulate T-B cell interactions through regulation of cluster of differentiation 40 (CD4) and its ligand CD40L.

CD40 is a type I transmembrane protein with an approximate molecular mass of 45 to 50 kDa. It is a member of the tumor necrosis factor receptor (TNFR) superfamily. It is predominantly expressed on B cells and professional antigen-presenting cells (dendritic cells and macrophages), and it can also be induced on non-immune cells such as endothelial cells, smooth muscle cells, and fibroblasts [17-19]. CD40L (also known as CD154, gp39) is a type II transmembrane protein belonging to the tumor necrosis factor (TNF) superfamily and serves as the cognate ligand for CD40. Due to post-translational modifications, its apparent molecular mass ranges from approximately 32 to 39 kDa [20-22]. CD40L is predominantly expressed on activated $CD4^+$ T cells and platelets, and its expression can be induced under inflammatory conditions in monocytes/macrophages, dendritic cells (DCs), neutrophils, and non-hematopoietic cells such as endothelial and vascular smooth muscle cells. Activated B cells may express CD40L only rarely and in a context-dependent manner [23].

Dysregulation of CD40–CD40L signaling contributes to B-cell lymphomagenesis. Constitutive engagement of CD40 in B cells leads to sustained activation of the noncanonical NF-κB pathway, accompanied by persistent stimulation of MAPK signaling cascades, including JNK and ERK [24]. CD40-CD40L interactions play crucial roles not only in the development of autoimmune diseases across various animal models but also in the regulation of certain carcinoma growth [25]. Although IL-17A is known to regulate immune cell activation, its effect on the CD40L-CD40 axis in human T and B cells remains poorly understood. This study aimed to investigate IL-17A's effects on CD40L expression in Jurkat T cells and CD40 expression in Mino B cells, thereby exploring the potential role of IL-17A in regulating T-B cell interactions and adaptive immune responses.

2. Materials and Methods

2.1 Cell Culture

Jurkat cell line (human acute T-cell leukemia, cat# TIB-152) and Mino cell line (human mantle cell lymphoma B cells, cat# CRL-3000) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Jurkat and Mino cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (GenClone, cat# 25-506, Genesee Scientific, San Diego, CA, USA), supplemented with 10% fetal bovine serum (FBS; Peak Serum, Wellington, CO, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells were maintained at 37 °C in a humidified incubator containing 5% CO_2.

2.2 Reagents

Recombinant human IL-17 (cat# 7955-IL) was purchased from R&D Systems, Minneapolis, MN, USA. For protein detection, the following primary antibodies were used: rabbit monoclonal anti-CD40 (D8W3N) (cat# 40868T, Cell Signaling Technology, Danvers, MA, USA), rabbit polyclonal anti-CD40L (cat# A0327, ABclonal, Woburn, MA, USA), and mouse monoclonal anti- glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (cat# MAB374; MilliporeSigma, Burlington, MA, USA). Fluorescent secondary antibodies, IRDye® 680RD goat anti-rabbit IgG (cat# 926-68071, LI-COR Biosciences, Lincoln, NE, USA) and IRDye® 800CW goat anti-mouse IgG (cat# 926-32210, LI-COR Biosciences, Lincoln, NE, USA), were also utilized. Each antibody specifically recognized its corresponding target protein, producing a single band at the expected molecular weight with no observable cross-reactivities.

2.3 Western Blot Analysis

Jurkat and Mino cells were serum-starved for 12 hours to minimize baseline signaling activity. Jurkat cells were subsequently treated with 40 ng/mL recombinant human IL-17, while Mino cells were treated with 20 ng/mL IL-17 for 0.5, 3, 8, and 24 hours. Cells collected at the 0-hour time point (prior to IL-17 stimulation) were used as untreated controls. Following the treatment, cells were harvested and lysed in radioimmunoprecipitation assay (RIPA) buffer, which contained 50 mM sodium fluoride, 0.5% Igepal CA-630 [NP-40], 10 mM sodium phosphate, 150 mM sodium chloride, 25 mM Tris (pH 8.0), 1 mM phenylmethylsulfonyl fluoride, 2 mM EDTA, and 1.2 mM sodium vanadate, and was supplemented with 1% protease inhibitor cocktail (Sigma-Aldrich). Equal amounts of protein were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred onto polyvinylidene difluoride (PVDF) membranes using

a Bio-Rad semi-dry transfer system for 30 minutes. Membranes were blocked with 2.5% bovine serum albumin (BSA) in TBS-T buffer (25 mM Tris-HCl, 125 mM NaCl, 0.1% Tween-20) for 30 minutes, followed by overnight incubation at 4 °C with primary antibodies against CD40 (1:1000), CD40L (1:500), or GAPDH (1:5000). After three washes with TBS-T (15 minutes each), membranes were incubated for 1 hour at room temperature with IRDye® 680RD goat anti-rabbit IgG (1:5000) for CD40/CD40L detection or IRDye® 800CW goat anti-mouse IgG (1:5000) for GAPDH detection. Protein bands were detected using the Odyssey infrared imaging system (LI-COR Biosciences), and GAPDH served as the loading control.

2.4 Densitometric and Statistical Analyses

Band intensities of Western blots were quantified using ImageJ software (version 2.16.0, Fiji distribution), normalized to GAPDH, and presented as fold change relative to the 0-hour control. Data are presented as mean ± standard error of the mean (SEM) from three independent experiments. Quantitative data were analyzed using one-way analysis of variance (ANOVA) in GraphPad Prism (version 10.6.0, GraphPad Software, San Diego, CA, USA). Differences were considered statistically significant when $p < 0.05$.

3. Results

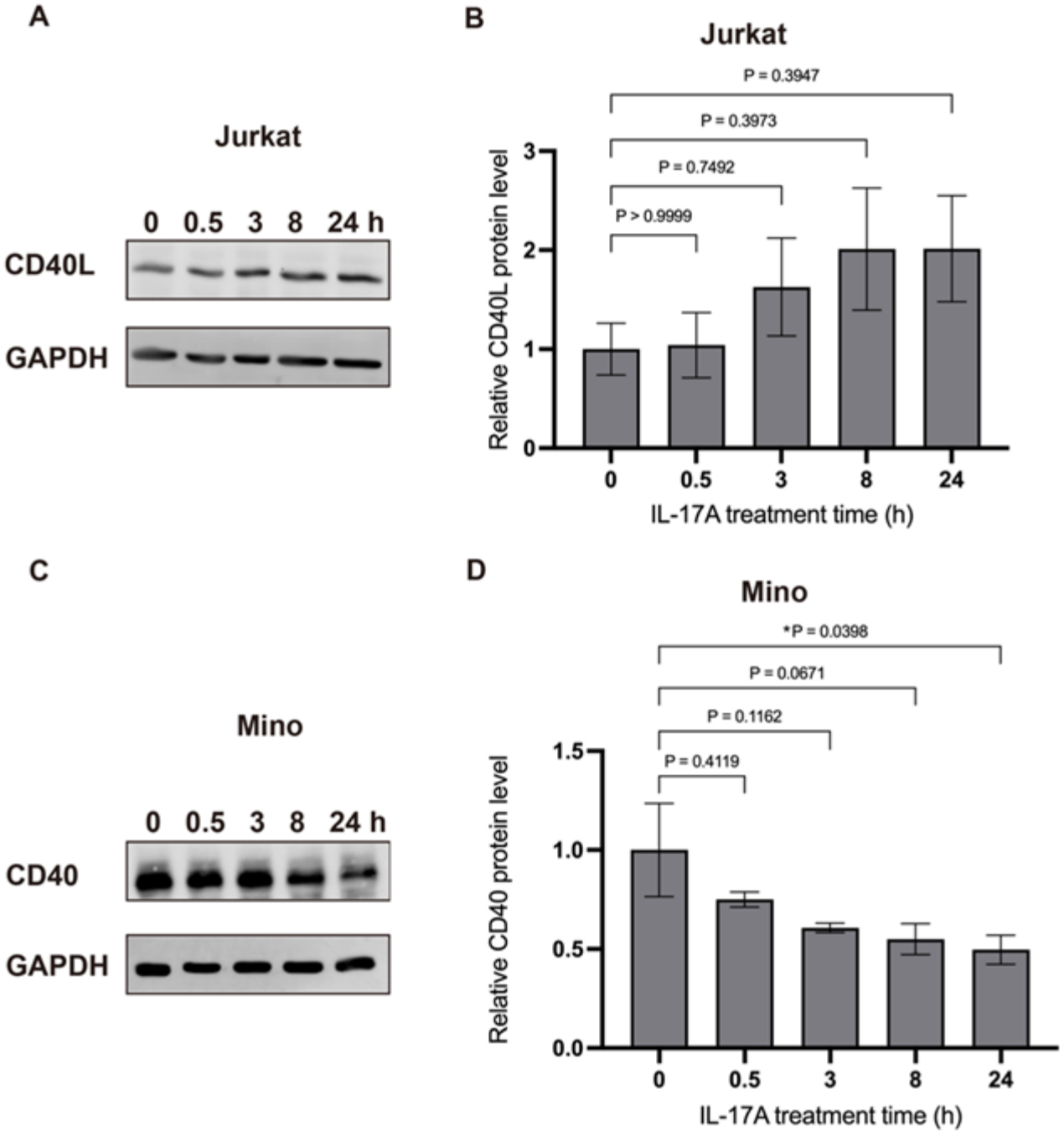
Western blot analysis demonstrated that IL-17A treatment induced an increasing trend in CD40L expression in Jurkat cells (Figure 1A), with protein levels at 24 h reaching approximately 2-fold that of the control group. However, this trend did not achieve statistical significance (Figure 1B). In Mino cells, IL-17A treatment resulted in a decrease in CD40 expression (Figure 1C), with protein levels at 24 hours reduced to approximately half of the control group, which was statistically significant (*$p < 0.05$; Figure 1D).

4. Discussion

The link of IL-17A to the CD40L-CD40 signaling axis has been implicated in various pathological contexts, with its effects differing in a cell-type-specific manner. For example, $CD4^+$ T cells co-cultured with fibrocytes exhibit increased production of IL-17A in a CD40-CD40L-dependent manner, indicating that cooperative signaling between fibrocytes and Th17 cells through the CD40 and IL-17A/F pathways enhances collagen synthesis and production of pro-angiogenic factors [26]. Similarly, within the tumor microenvironment, direct interaction between CD40 on breast tumor cells and CD40L on activated T cells enhances transforming growth factor (TGF)-β secretion and promotes Th17 differentiation, whereas IL-17 produced by Th17 cells activates signal transducer and activator of transcription 3 (STAT3) signaling to drive tumor cell proliferation [27]. In thyroid-associated ophthalmopathy, IL-17A, in combination with CD40L, induces regulated on activation, normal T-cell expressed and secreted (RANTES) production in orbital fibroblasts in a time- and dose-dependent manner, whereas IL-17A alone is insufficient to trigger RANTES release. This effect is mediated through MAPK signaling and can be inhibited by IL-17RA neutralizing antibodies or MAPK inhibitors, underscoring the essential role of IL-17A and CD40-CD40L interactions in amplifying local inflammation [28]. IL-17 and CD40L synergistically enhanced the production of IL-6, monocyte chemoattractant protein-1 (MCP-1), RANTES, and TGF-β1, as well as NF-κB activation in podocytes, thereby amplifying inflammation and promoting renal fibrosis. Moreover, inhibition of IL-17 reduced CD40 and TGF-β1 expression and improved podocyte viability, highlighting the pathogenic synergy between IL-17 and CD40–CD40L signaling in sustaining chronic tissue injury and remodeling [29]. More recently, the focus has shifted to the translational potential of this axis. Agonistic CD40 antibodies are being developed as immunotherapeutic agents in cancer, capable of licensing dendritic cells, enhancing $CD8^+$ T-cell priming, and synergizing with checkpoint blockade therapy [30]. In sepsis-induced lung injury, dendritic cells activated by CD40L promote Th17 differentiation while suppressing Th2 differentiation via the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) signaling pathway, thereby exacerbating inflammatory damage [31].

In this study, we investigated the regulatory effects of IL-17A on the CD40L-CD40 axis in two distinct human lymphoid cell lines: Jurkat T cells and Mino B cells. Our findings revealed a bidirectional modulation, with IL-17A treatment showing a trend toward increased CD40L expression, although this did not reach statistical significance, while significantly decreasing CD40 expression in Mino cells. This differential regulation suggests that IL-17A may induce a functional imbalance in T–B cell interactions, whereby T cells gain an enhanced capacity to

Figure 1. Effects of IL-17A on CD40L expression in Jurkat cells and CD40 expression in Mino cells at various time points. (A and B) Jurkat cells and (C and D) Mino cells were exposed to IL-17A (40 ng/mL for Jurkat cells and 20 ng/mL for Mino cells) for 0, 0.5, 3, 8, and 24 hours. The protein expression levels of CD40L (in Jurkat cells) and CD40 (in Mino cells) were assessed using Western blot analysis. GAPDH served as a loading control (A and C). Relative protein expression levels were determined by normalizing the intensity of the target protein bands to that of GAPDH and then presented relative to the control group (0 h), which was assigned an arbitrary value of "1" (B and D). Data are presented as mean ± SEM from three independent experiments. *p < 0.05 versus the 0-hour control group.



provide co-stimulatory signals, whereas B cells lose responsiveness due to reduced CD40 expression. Several mechanisms could account for this divergence. The induction of CD40L in Jurkat cells may be mediated via IL-17A-driven NF-κB or MAPK activation pathways, which are well established in T cells. Conversely, the downregulation of CD40 in Mino cells may reflect an inhibitory effect of IL-17A on transcription factors that regulate CD40 expression, or feedback signaling aimed at dampening excessive B-cell activation. Alternatively, the tumor-derived origin of these cell lines could contribute to their differential responses, as oncogenic mutations may alter downstream signaling thresholds. We examined only one T-cell and one B-cell line; therefore, the generalizability of these findings to other lymphoid subsets or primary cells remains to be validated. Despite these limitations, our findings provide novel insights into how IL-17A differentially modulates T and B cells. The observed cell-type–specific regulation of the CD40L-CD40 axis may contribute to dysregulated T-B cell collaboration, a hallmark of autoimmune disease, chronic inflammation, and tumor immunity. This raises the possibility that targeting IL-17A signaling in lymphocytes could represent a therapeutic strategy to restore immune homeostasis. Future studies exploring the downstream consequences of altered CD40L or CD40 expression will be crucial to further elucidate the biological significance of this pathway.

5. Conclusion

Our findings demonstrate that IL-17A modulates the CD40L–CD40 co-stimulatory axis in a cell type-specific and opposing manner, showing a trend toward enhancing CD40L expression in T cells while suppressing CD40 expression in B cells. Our findings demonstrate that IL-17A modulates the CD40L-CD40 co-stimulatory axis in a cell type-specific and opposing manner, showing a trend toward enhancing CD40L expression in T cells while suppressing CD40 expression in B cells. This differential regulation may represent a mechanism by which IL-17A contributes to both the regulation and dysregulation of adaptive immune responses.

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Conflicts of interest: The authors have no conflicts of interest to declare.

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