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Scaffold protein JLP mediates TCR-initiated CD4$^+$T cell activation and CD154 expression

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ABSTRACT

CD4$^+$ T-cell activation and its subsequent induction of CD154 (CD40 ligand, CD40L) expression are pivotal in shaping both the humoral and cellular immune responses. Scaffold protein JLP regulates signal transduction pathways and molecular trafficking inside cells, thus represents a critical component in maintaining cellular functions.

Its role in regulating CD4$^+$ T-cell activation and CD154 expression, however, is unclear. Here, we demonstrated expression of JLP in mouse tissues of lymph nodes, thymus, spleen, and also CD4$^+$ T cells. Using CD4+ T cells from jlp-deficient and jlp-wild-type mice, we demonstrated that JLP-deficiency impaired T-cell proliferation, IL-2 production, and CD154 induction upon TCR stimulations, but had no impacts on the expression of other surface molecules such as CD25, CD69, and TCR. These observed impaired T-cell functions in the jlp-/- CD4$^+$ T cells were associated with defective NF-AT activation and Ca^{2+} influx, but not the MAPK, NF-κB, as well as AP-1 signaling pathways. Our findings indicated that, for the first time, JLP plays a critical role in regulating CD4$^+$ T cells response to TCR stimulation partly by mediating the activation of TCR-initiated Ca^{2+}/NF-AT.

1. Introduction

CD4$^+$ T helper cell subset is important in orchestrating innate and adaptive immune responses, through cytokines production and cell-to-cell interactions (Pepper and Jenkins, 2011). In addition to secreting cytokines to polarize an immune response (Basu et al., 2013; Chen et al., 2012; Mahon et al., 1995; Mosmann et al., 1986), these cells regulate expressions of specific surface proteins upon activations (Badovinac et al., 2006; Janssen et al., 2005; Lederman et al., 1992; Yates et al., 2013). The costimulatory molecule CD154, a type II membrane protein of the TNF superfamily (Armitage et al., 1992; Hollenbaugh et al., 1992), is considered as an early activation marker on CD4$^+$ T cells (Lederman et al., 1992). The interactions of CD154 with CD40 molecules are essential in generating optimal immune response (Quezada et al., 2004).

Previous studies have demonstrated that induction of the CD154 on the surface of the CD4$^+$ T cells depends on the initial increase of the free intracytoplasmic Ca^{2+}([Ca^{2+}]i) concentration, followed by calmodulin/calcineurin activation (Fuleihan et al., 1994; Roy et al., 1993). The latter can be initiated by the ligations of T-cell antigen receptor (TCR-CD3 complex) (Crabtree, 2001). Activated calcineurin leads to dephosphorylation of the cytoplasmic transcription factor NF-AT. Subsequently, the active NF-AT translocates to the nucleus and mediates transcription of target genes such as CD154 and IL-2 (Cron, 2003; Rao et al., 1997; Vavassori and Covey, 2009). Immunosuppressive drugs cyclosporine A (CsA) and FK506 inhibit calcineurin, decrease translocations of cytosolic NF-AT to the nucleus, and block the expression of CD154 and IL-2 (Rao et al., 1997). In addition to NF-AT, several other transcriptional factors such as AP-1, NF-κB, AKNA are involved in coordinating the transcriptional regulation of CD154 gene expression (Cron, 2003).

Aberrant expression of CD154 on CD4$^+$ T cells has been observed in many autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis (MS) and experimental allergic encephalomyelitis (EAE) (Alaaeddine et al., 2012; Gerritse et al., 1996). In SLE patients, antigen receptor mediates

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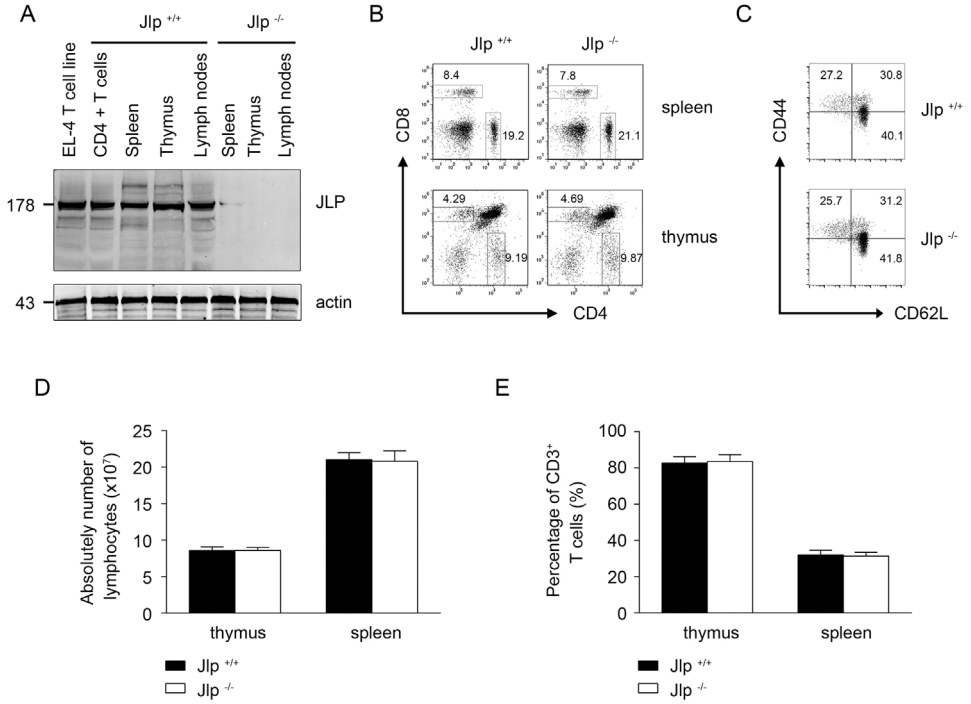

Fig. 1. JLP expression profiles in mouse immune tissues, and T-cell subsets frequency in the jlp-deficient mouse. (A) Spleen, thymus, lymph nodes, and purified $CD4^+$ T cells were collected from jlp-wild-type or jlp-deficient mice, and subjected to western blotting to detect the JLP expression. EL–4 T cells line and actin was set as positive and loading controls, respectively. (B) Splenocytes and thymocytes isolated from spleen and thymus from $jlp^{-/-}$ or $jlp^{+/+}$ mice were stained with anti-CD4 and anti-CD8 and subjected to flow cytometer to analyze the frequencies of CD4/CD8 subpopulations. (C) CD44/CD62L subpopulations of $CD4^+$ T lymphocyte from spleen of jlp-/- or $jlp^{+/+}$ mice were also analyzed. One representative result from three independent experiments is shown. (D) Splenocytes and thymocytes were obtained from spleen and thymus of $jlp^{-/-}$ or $jlp^{+/+}$ mice and the absolutely number was analyzed by flow cytometer. (E) Splenocytes and thymocytes were stained with CD3 mAb and performed by flow cytometer to calculate the percentage of $CD3^+$ T cells in given groups. SDs and mean values were calculated from at least three independent experiments. SDs were marked by error bars.

excessive [Ca^{2+}] i responses and CD154 overexpression in T cells (Katsiari et al., 2002; Vassilopoulos et al., 1995). The latter is thought to be a key mechanism underlying the immune disorder observed in the disease. Thus, targeting CD40-CD154 interactions represents a promising therapeutic target for these autoimmune diseases (Law and Grewal, 2009). Of interest, Ca^{2+}/calcineurin inhibitor CsA could not reverse such abnormality (Katsiari et al., 2002), necessitating the search for alternative strategies or therapeutic targets to inhibit the CD40-CD154 interactions in these diseases.

The JNK-associated leucine zipper protein (JLP) is a scaffold protein that belongs to the family of JNK-interacting proteins (JIPs). JIPs possess pleiotropic functions, including controlling signaling pathway transduction (Zeke et al., 2009), regulation of molecules trafficking (Ikonomov et al., 2009; Roberts et al., 2013; Verhey and Hammond, 2009; Wang et al., 2015; Wang et al., 2013), and organizing certain signaling modules structurally to coordinate functions. Compared with other JIPs, JLP interacts with a broader range of partner molecules (Dhanasekaran et al., 2007; Lee et al., 2002; Ramkumar et al., 2015; Sato et al., 2015; Takaesu et al., 2006; Wang et al., 2015). Our previous studies have demonstrated that JLP played a critical role in regulating CD40 translocation across cell membranes in both dendritic cells and B lymphocytes (Wang et al., 2015; Wang et al., 2013). However, to our knowledge, whether JLP is involved in $CD4^+$ T cell function regulation remains elusive. In this study, we examined the JLP expression in primary T cells and further investigated the role(s) of JLP in regulating $CD4^+$ T cell phenotype and functions.

2. Materials and methods

2.1. Animals

Heterozygous ($jlp^{+/-}$) mice (C57BL/6 background) were kindly provided by Prof. Katsuji Yoshioka (Kanazawa University, Japan). Wild type ($jlp^{+/+}$) and homozygous ($jlp^{-/-}$) mice were generated by in-breeding $jlp^{+/-}$ mice and they were genotyped with PCR and Western blot as previously described (Iwanaga et al., 2008). $Jlp^{+/+}$ and $jlp^{-/-}$ 8–10 weeks-of-age weight- and sex-matched littermates were used in the experiments. Mice were housed in specific pathogen-free conditions at the Center for Animal Experiments at Wuhan University. All animal experiments were approved by the Animal Ethics Review Board of Wuhan University and performed in accordance with the guidelines of the National Health and Medical Research Council of China.

2.2. Reagents

FITC-conjugated anti-CD4 (clone: GK1.5, 100406), PE-conjugated anti-CD8 (clone: 53-6.7, 100710), FITC-conjugated anti-CD62L (clone: MEL-14, 104406), PE-conjugated anti-CD44 (clone: IM7, 103008), PE-conjugated anti-CD154 (clone: MR1, 106506), FITC-conjugated anti-CD25 (clone: 3C7, 101908), PE-conjugated anti-CD69 (clone: H1.2F3, 104508) and PE- conjugated anti-IL-2 (clone: JES6-5H4, 503808) antibodies were purchased from BioLegend (San Diego, CA). Anti-mouse CD3 (clone: 145-2C11, 100331) and CD28 (clone: 37.51, 102112) antibodies (CD3 + CD28) were also from BioLegend. Phorbol myristate acetate and ionomycin (P + I) were from Sigma-Aldrich (St. Louis, MO). Carboxyfluorescein diacetate succinimidyl ester (CFSE) and fluo-4 AM were from Invitrogen (Life Technologies, Grand Island, NY). Anti-p38 (clone: D13E1, #8690), anti-p-p38 (clone: D3F9, #4511), anti-JNK (1:500, #9252), anti-p-JNK (clone: 81E11, #4668), anti-ERK (clone: 137F5, #4695), anti-p-ERK (clone: D13.14.4E, #4370), anti-IkBα (clone: 44D4, #4812), anti-p-IkBα (clone: 14D4, #2859) and anti-p-c-Jun (clone: D47G9, #8752) antibodies were purchased from Cell Signaling Technology (Danvers, MA). The rabbit anti-mouse JLP antibodies (ab12331) were from Abcam (Cambridge, MA) and anti-NFAT (clone: 4G6-G5, SC-7296) antibodies were from Santa Cruz Biotechnology (Dallas, TX).