Gab3 Plays an Essential Role in CD8⁺ T-Cell Expansion through Regulating IL-2-Mediated Activation of PI3K/AKT/mTOR and Erk/FoxO Pathways

G. Fu[1], Y. Zheng[1], G. Xin[2], Z. Wang[3], K. D. Bunting[3], W. Cui[4], A. Salomon[5] and R. Wen[1,6]

[1]Versiti Blood Research Institute, Milwaukee, WI; [2]Ohio State University College of Medicine, Columbus; [3]Emory University School of Medicine, Atlanta, GA; [4]Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago; [5]Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence; [6]Department of Microbiology and Immunology, Medical College of Wisconsin, Milwaukee, WI

INTRODUCTION

Grb2-associated binding protein 3 (Gab3) is a member of the Gab family of scaffolding proteins that also includes Gab1 and Gab2 in mammals. Unlike its counterparts, Gab3 is predominantly expressed in hematopoietic tissues, particularly in the spleen and thymus, with a strong expression in splenic CD8⁺ T cells. While Gab3 deficient mice show normal hematopoiesis and T and B cell functions, the double deficiency of Gab2 and Gab3 makes mice more susceptible to colitis than wild-type mice. This susceptibility is partially attributed to the activation and proliferation of intestinal intraepithelial CD8⁺ cells, suggesting a potential role for Gab proteins in regulating CD8⁺ cells. It is unknown how Gab proteins regulate the function of CD8⁺ T cells. The current study aims to investigate the mechanism underlying the role of Gab3 in CD8 T cell function in vivo and ex vivo.

AIM

To investigate the mechanism underlying the role of Gab3 in CD8 T cell function in vivo and ex vivo.

METHOD

Bone marrow (BM) chimera mice and infection of the mice with LCMV Armstrong. Mixture of BM cells from CD8-deficient mice and either wild-type or Gab3$^{-/-}$ mice were transplanted into CD45.1 mice that had their CD8⁺ cells depleted and were then lethally irradiated. After 12-20 weeks, the mice were infected with LCMV Armstrong and analyzed on day 8 post-infection.

CD8 cell priming and expansion. Naïve CD8⁺ T cells were primed with plate-bound anti-CD3 and anti-CD28 for 3 days and then expanded with IL-2 for an additional 3 days. The expanded cells were then used for western blotting, seahorse, RNA-seq, and phosphoproteomics analysis as detailed in the legend.

RESULTS

Fig.1. Impaired CD8⁺ cell expansion and enhanced cell apoptosis in Gab3$^{-/-}$ mice upon LCMV Armstrong challenge. Spleen cells were analyzed from BM chimera mice, reconstituted for 12-20 weeks and subsequently infected with LCMV Armstrong for 8 days. **A**. Percentages of CD8⁺ cells gated in CD45.2⁺ population (*top left*) and percentages of CD44^{Hi}tetramer⁺ cells within CD45.2⁺CD8⁺ population (*top right and bottom*). **B**. Total number of CD45.2⁺CD8⁺ cells (*top left*) and *Tetramer-specific* (CD45.2⁺CD8⁺CD44^{Hi}Tetramer⁺) cells (*top right and bottom*). **C**. Total number of *Tetramer-specific* memory precursor (MP, CD127^{Hi}KLRG1^{Lo}), long lived effector (LLEC, CD127^{Hi}KLRG1^{Hi}), short lived effector (SLEC, CD127^{Lo}KLRG1^{Hi}), and early effector (EEC, CD127^{Lo}KLRG1^{Lo}) cells. **D**. Percentages of dead (AnnexinV⁺DAPI⁺), apoptotic (AnnexinV⁺DAPI⁻), and total AnnexinV⁺ cells in CD45.2⁺CD8⁺ (*top left*) and *Tetramer-specific* cells (*top right and bottom*). Unpaired t test for A, B. Multiple t test using Holm-Sidak method for C , D. ★, p<0.05. ★★, p<0.01. ★★★, p<0.001. ★★★★, p<0.0001.

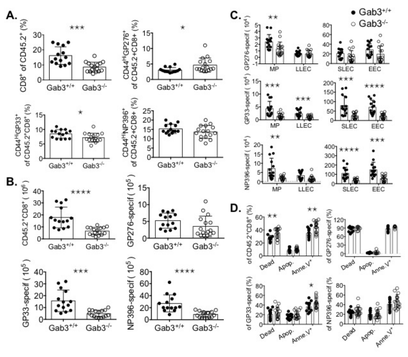


Fig. 2. Impaired Gab3$^{-/-}$ CD8⁺ T cell expansion induced by IL-2 due to increased cell apoptosis and reduced cell proliferation. Naïve CD8⁺ T cells were initially activated with anti-CD3 and anti-CD28 for 3 days, followed by expansion in IL-2 for an additional 3 days. **A.** Fold change in the number of CD8⁺ T cells. Data were means ± SD of eight independent experiments. Multiple unpaired t test. ★★★, p<0.001. **B.** *Left:* Representative dot plot showing dead (AnnexinV⁺DAPI⁺) and apoptotic (AnnexinV⁺DAPI⁻) CD8⁺ T cells. *Right*: Statistical data of of dead and apoptotic CD8⁺ cells (% of singlets). Paired t test. **C.** *Left:* Representative histogram of Propidium Iodide (PI) staining indicating cell cycle phases. *Right*: Statistical data of cells in S+G2M phase representing proliferated cells. Paired t test.

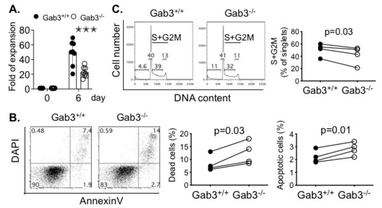


Fig. 3. Impaired IL-2 mediated metabolism in Gab3$^{-/-}$ CD8⁺ T cells. After a 6-day culture, the cells were rested and then re-stimulated with IL-2 for 4 hr. Subsequently, extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were measured. **A.** *Left:* Representative ECAR curve comparing non-stimulated versus IL2-stimulated cells. *Right:* Statistical data of key ECAR parameters calculated from tabulated results. Data were means ± SD of two independent experiments, including 3 pairs of mice. Unpaired t test. **B.** *Left:* Representative OCR curve comparing non-stimulated versus IL2-stimulated cells. *Right:* Normalized results of key OCR parameters, presented as the fold change of IL-2 stimulated relative to non-stimulated cells. Data were means ± SD of five independent experiments, including 6 pairs of mice. Unpaired t test.

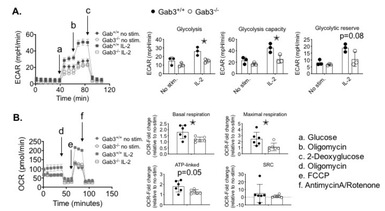


Fig. 4. Gab3 deficiency alters IL-2 induced gene expression in CD8⁺ cells. RNA-seq analysis of IL-2-blasted CD8⁺ cells that underwent a 16-hour resting without IL-2, followed by an 8hr re-stimulation with IL-2. **A.** Volcano plots, Principal Component Analysis (PCA), and heatmaps showing contrasting gene expression in unstimulated (*left panel*) and IL-2 stimulated (*right panel*) CD8⁺ cells, comparing Gab3$^{-/-}$ to Gab3$^{+/+}$ cells. In the volcano plots, blue dots signify genes with significantly altered expression (DN, down-regulated; Up, up-regulated) of at least 2-fold in Gab3$^{-/-}$ versus Gab3$^{+/+}$ CD8⁺ cells, with an adjusted p value < 0.05. **B-F.** Comparative Gene Set Enrichment Analysis (GSEA) of IL-2 induced genes related to mTOR signaling and metabolism (**B**), cell apoptosis and proliferation (**C**), Erk-MAPK targets (**D**), FoxO3 targets (**E**), and Stat5 targets (**F**) in Gab3$^{-/-}$ relative to Gab3$^{+/+}$ CD8⁺ cells. False Discovery Rate (FDR) and Normalized Enrichment Score (NES) were shown on Enrichment plot for each gene set. **G.** Pathways predominantly affected by IL-2 were determined via DAVID analysis (GO_BP_FAT) using top 200 genes exhibiting the most significant down-regulation (top panel) or up-regulation (lower panel) induced by IL-2 in Gab3$^{-/-}$ CD8⁺ cells.

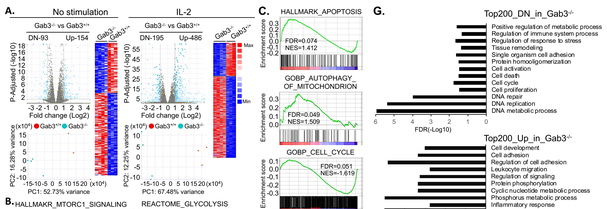


Fig. 5. Modulation of FoxO signaling in Gab3$^{-/-}$ CD8⁺ cells by IL-2. Following a 16-hour resting period, the cells were re-stimulated with IL-2 for 15min and immediately lysed. Phosphoproteomics analysis was performed on the resulting cell lysates. Proteins showing phosphorylation induced by IL-2 in Gab3$^{-/-}$ versus Gab3$^{+/+}$ cells, with a log2 fold change greater than 0.5 or less than -0.5, were selected. Subsequent DAVID analysis using the genes encoding these proteins revealed a significant impact of IL-2 on protein phosphorylation in the FoxO signaling pathway, with an FDR of 0.014.

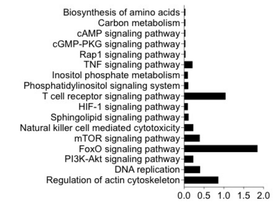
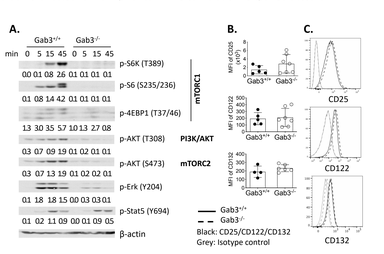


Fig. 6. Impaired mTOR/PI3K/AKT and unaffected JAK/STAT5 signaling in Gab3$^{-/-}$ CD8⁺ T cells . Following a 6-day culture period, the cells were rested and then re-stimulated with IL-2 for the indicated time. **A.** Cell lysates analyzed by western blot using different antibodies. The presented data were representative of three independent experiments. **B-C.** Mean Fluorescence Intensity (MFI) (**B**) and representative histogram (**C**) of CD25, CD122, and CD132 on the surface of cells that were rested for 16hr prior to IL-2 re-stimulation. Unpaired t test for **B**.



CONCLUSIONS

Our study reveals the important roles of Gab3 in CD8⁺ T cell expansion and survival following LCMV Armstrong infection and in T-cell culture.

Mechanistically, Gab3 exerts its influence on CD8 T cell metabolism, impacting both glycolysis and mitochondrial respiration. Gab3 deficiency resulted in impaired IL-2-mediated ECAR and OCR in CD8⁺ T cells. Our comprehensive analyses, encompassing RNA-seq, phosphoproteomics, and western blot, unveiled a pivotal role of Gab3 in IL-2 mediated mTOR and Erk/MAPK pathway activation in CD8⁺ T cells. Notably, Gab3 showed no significant influence on the Stat5 pathway.

In summary, our findings have demonstrated that Gab3 plays an essential role in CD8⁺ T-cell expansion by promoting cell proliferation and prohibiting cell apoptosis through regulating IL-2-mediated activation of PI3K/AKT/mTOR and Erk-FoxO3 pathways.

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CONTACT INFORMATION

Renren Wen, PhD. Versiti Blood Research Institute.
RWen@Versiti.org.

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