ELSEVIER

Blood 142 (2023) 3931–3932

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

203.LYMPHOCYTES AND ACQUIRED OR CONGENITAL IMMUNODEFICIENCY DISORDERS

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

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Grb2-associated binding protein 3 (Gab3) is a member of the Gab family of scaffolding proteins. Its role in T cells is largely unknown. In the current study, we investigated its in vivo function in CD8 $^{+}$ T cells using the lymphocytic choriomeningitis virus (LCMV) Armstrong infection model. We created BM chimera mice with a specific deficiency of Gab3 in CD8 $^{+}$ T cells. This was achieved by transplanting a mixture of BM cells obtained from CD8-deficient mice and Gab3-deficient (Gab3 $^{-/-}$) mice [(1)] into lethally irradiated CD45.1 mice. The control chimera mice were lethally irradiated CD45.1 mice transplanted with a mixture of BM cells from CD8-deficient mice and WT mice. Twelve weeks after the BM transplantation, the Gab3 $^{-/-}$ and control BM chimera mice were infected with Armstrong. On day eight following infection, Gab3 $^{-/-}$ CD8 $^{+}$ T cells exhibited reduced expansion compared to WT CD8 $^{+}$ T cells. Importantly, Gab3 $^{-/-}$ LCMV antigen specific CD8 $^{+}$ T cells, GP33 $^{+}$ and NP396 $^{+}$ CD8 $^{+}$ T cells, were significantly reduced compared to their WT counterparts. Furthermore, CD8 $^{+}$ T cells in Gab3 $^{-/-}$ BM chimera mice showed significant increase of apoptosis compared to that in WT BM chimera mice following LCMV infection.

To explore the mechanism underlying the observed phenotype, we used a primary CD8 $^{+}$ T-cell culture system, in which we stimulated CD8 $^{+}$ T cells with anti-CD3 and anti-CD28 followed by expanding the cells in IL-2. We observed that Gab3 deficiency severely impaired IL-2-induced CD8 $^{+}$ T cell expansion. Gab3 $^{-/-}$ CD8 $^{+}$ T cells in this culture system displayed increased apoptosis and reduced proliferation compared to wild-type (WT) controls, which may account for the reduced CD8 $^{+}$ T cell expansion. We then performed Seahorse analysis of the cultured CD8 $^{+}$ T cells after IL-2 stimulation. We found that IL-2-mediated extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were largely impaired in Gab3 $^{-/-}$ CD8 $^{+}$ T cells compared to WT CD8 $^{+}$ T cells. This data indicates a crucial role of Gab3 in both glycolysis and mitochondrial respiration.

We further investigated how Gab3 regulates the IL-2 signaling pathway by conducting transcriptomic and phosphoproteomic analysis. RNA-seq analysis revealed that genes associated with mTOR signaling, glucose metabolism, cell cycle, and Erk/MAPK targets were highly enriched in WT CD8 $^{+}$ T cells, whereas genes related to apoptosis, autophagy, and FoxO3 targets were highly enriched in Gab3 $^{-/-}$ CD8 $^{+}$ T cells. In contrast, the gene set enrichment analysis (GSEA) showed that IL-2-induced Stat5 target gene expression was comparable between WT and Gab3 $^{-/-}$ CD8 $^{+}$ T cells, indicating an important role of Gab3 specifically in the activation of the mTOR and Erk/MAPK pathways downstream of IL-2 signaling. Consistent with that observed in the transcriptomic analysis, phosphoproteomics analysis revealed a significant reduction in IL-2-induced phosphorylation of key components in ERK/FOXO signaling pathway, including RafA, MAPK1, FoxO3, AMPK, and mTOR downstream molecule Eif4B in Gab3 $^{-/-}$ CD8 $^{+}$ T cells compared to WT CD8 $^{+}$ cells. Furthermore, phosphorylation of Stat5 at different sites was comparable between Gab3 $^{-/-}$ and WT CD8 $^{+}$ T cells, further supporting an important role of Gab3 specifically in IL-2 mediated mTOR and Erk/MAPK activation. Finally, we validated the results obtained from the transcriptomic and phosphoproteomic analyses using western blot analysis. Our findings revealed that in Gab3 $^{-/-}$ CD8 $^{+}$ T cells, the IL-2-induced phosphorylation of signaling molecules in the mTOR pathway (S6K, S6, 4EBP1, AKT) and the Erk pathway (Erk) was impaired. Notably, the phosphorylation of Stat5 remained intact despite the absence of Gab3.

In summary, our studies 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.

(1) We gratefully acknowledge that Gab3-deficient mice were originally generated by Dr. Larry Rohrschneider at Fred Hutchinson Cancer Research Center (*Mol Cell Biol* 2003 Apr;23(7):2415-24. doi: 10.1128/MCB.23.7.2415-2424.2003.). The mice were subsequently backcrossed to C57BL/6.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-185077