Long-term consumption of Carnosine contributes to improving effector functions of NK cells

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Abstract

Carnosine can promote energy metabolism of cells as a kind of extremely natural antioxidant dipeptide, it has a very important role in maintaining body homeostasis. At present, the study of Carnosine in the immune system is still at a blank stage. Our study mainly explored the effect of Carnosine on the development and function of NK cells in vivo immune system in mice. ice started to drinking Carnosine-containing water at the early age，we observed an increase in the ability of NK cells to degranulate and secrete IFN-γ in adult mice. Moreover, it helps NK cells to conjugate with tumor cells for better killing function. These results further clarify the importance of Carnosine in the development and function of NK cells, providing theoretical and experimental basis for the basic research and clinical application of NK cell development and function.

Keywords

Carnosine; Natural killer cells（NK cells）; Function； Cytokine; Cytotoxicity.

1. Introduction

Carnosine is a natural dipeptide with strong antioxidant activity. It consists of β-alanine and L-histidine and is a natural antioxidant that is widely distributed in various tissues and organs of the human body[1]. Recent studies have found that Carnosine has a variety of biological functions, including antioxidants, anti-aging, anti-glycosylation, etc[2]. Carnosine has good water-solubility and easy absorption characteristics. Daily doses of Carnosine have a very important role in prolonging the lifespan of mice[3]. There were also reported in the related literature that Carnosine helps to inhibit the proliferation of cells induced by GAC and relieve diabetes. At the same time, Carnosine also played an important role in anti-cancer[4, 5]. However, the effect of Carnosine on the immune system is rarely reported. As an important part of the innate immune system, NK cells can effectively help fight the infection of viruses and tumor cells and have an indispensable role in maintaining the steady state of the body[6]. We fed mice with Carnosine-containing drinking water from childhood until the mice matured to analyze NK cells in immune tissues. The results showed that long-term consumption of Carnosine helps NK cells become younger and the killing ability of NK cells is further enhanced, which effectively prevents the infection of viruses and tumor cells.

2. Long-term consumption of Carnosine can not affect the NK cells differentiation and development

To investigate the effect of Carnosine on the occurrence and development of NK cells in mice, we started feeding mice with drinking water containing 2g/L of Carnosine, after 10 weeks, we analyzed NK cells in the spleen and bone marrow to study whether Carnosine affects the proportion and absolute number of NK cells in spleen and bone marrow. Experimental data showed that the effect of Carnosine was not significant during the differentiation of NK cells and there was no significant difference in the number of NK cells between the experimental and control groups (Fig. 1.A, B). Next, we analyzed whether the long-term consumption of Carnosine affects the development of NK cells. The experimental results showed that the development of NK cells in the Carnosine-treated

experimental group had changed, the $CD27^+CD11b^+$ double positive cells showed an upward trend, which further demonstrating that Carnosine can make NK cells younger (Fig. 1.C, D).

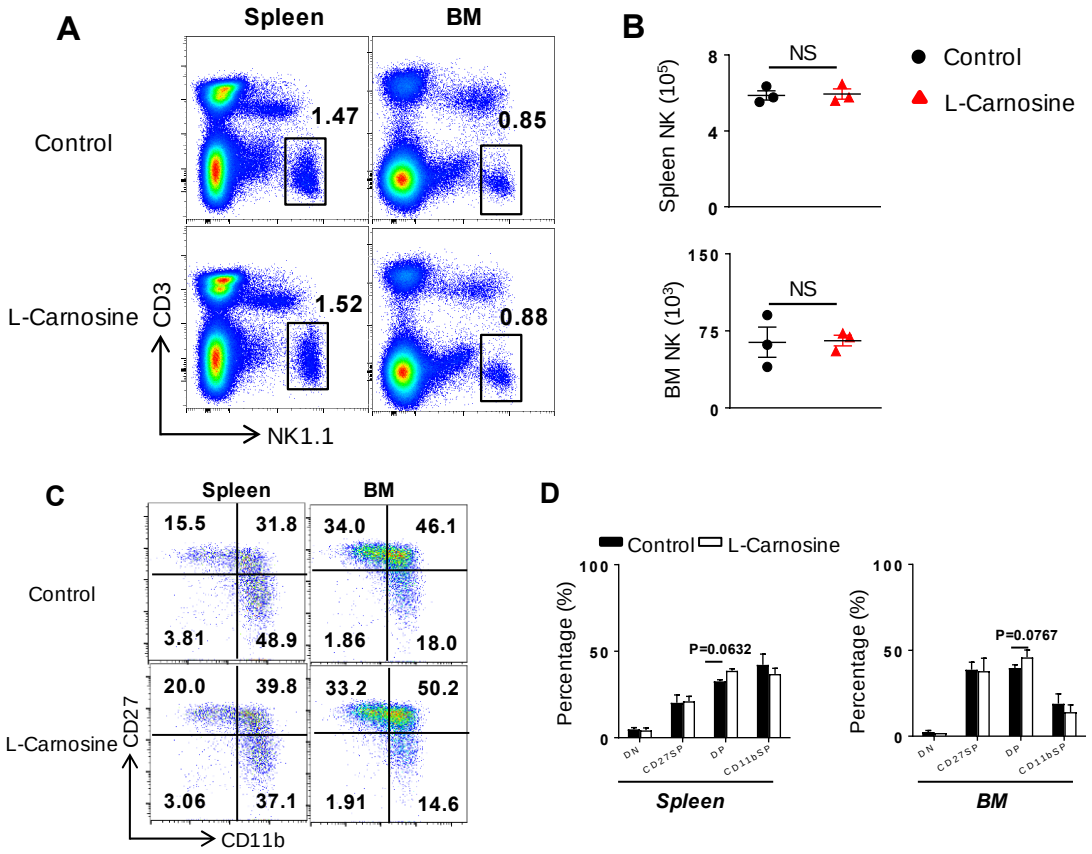

Figure 1. Long-term consumption of Carnosine can not affect the NK cell differentiation and development

(A). Representative flow cytometric profiles of NK cells ($CD3^-NK1.1^+$) in the spleens, bone marrow (BM) of Control and experimental groups. (B) The absolute number of NK cells in the indicated tissues and organs from the Control and experimental groups. Each symbol represents an individual mouse. Small horizontal lines indicate the average. (C) CD27 vs CD11b expression on $CD3^-NK1.1^+$ NK cells were detected by flow cytometry. The numbers indicate the percentages of cells in each quadrant. (D) Percentages of four-stage development: DN ($CD27^-CD11b^-$), CD27 SP ($CD27^+CD11b^-$), DP ($CD27^+CD11b^+$), and CD11b SP ($CD27^-CD11b^+$), in gated $CD3^-NK1.1^+$ NK cells in the spleen and BM of Control and experimental groups.

3. Carnosine can increase the ability of NK cells to secrete cytokines

Previous results showed that the treatment of Carnosine can increase the population of $CD27^+CD11b^+$ double positive cells in NK cells. Indirectly, Carnosine may affect the function of NK cells, such as the secretion of cytokines. NK cells secrete IFN-γ in response to two types of stimuli: target cell ligands or specific antibodies engaging activating receptors, and cytokines, including IL-12 and IL-18 secreted by DC and macrophages. First, we stimulated NK cells with hematopoietic RMA-S and YAC-1 cell lines *in vitro*. Conjugation of NK cells with tumor cells induced IFN-γ production. The results showed that the ability of NK cells to secrete IFN-γ can be effectively increased after treatment with Carnosine(Fig. 2.A, B). NK cells possess multiple activating receptors, including NK1.1, Ly49D and NKp46. To test whether metabolic signaling is required for their effector function, NK cells were stimulated using plate-coated antibodies against ITAM-containing receptors, NK1.1 or Ly49D. Consistent with the data obtained from tumor cell stimulation, it was demonstrated that Carnosine also enhances the ability of specific antibody stimuli to secrete IFN-γ of NK cells (Fig. 2.C, D). In conclusion, long-term consumption of drinking water containing Carnosine helps NK cells secrete IFN-γ, thereby enhancing their immunity.

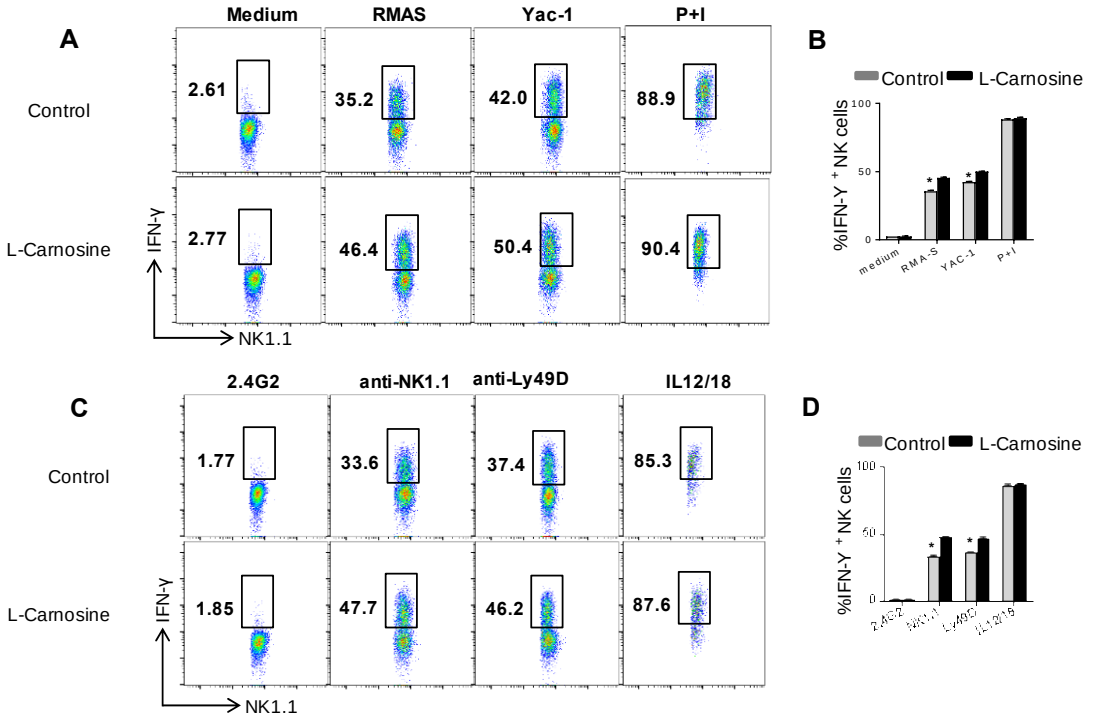

Figure 2. Carnosine helps NK cells secrete cytokines

(A). Splenic lymphocytes were prepared from poly I:C-treated mice from control and experimental groups were pretreated with the indicated inhibitor and then co-cultured with an equal number of tumor cell lines, RMA-S and YAC-1, for 4 hours in the presence of a Golgi blocker; DMSO alone served as a negative control. Intracellular staining was performed to assess the production of IFN-γ. The numbers are percentages of the IFN-γ positive cells among the gated $CD3^-$ $NKp46^+$ cells. (B). The percentage of the IFN-γ positive cells among the gated $CD3^-$ $NKp46^+$ cells under tumor cell stimulation. (C). Splenic lymphocytes that were pretreated with the indicated inhibitor were stimulated with plate-coated antibodies for 4 hours in the presence of a Golgi block. Intracellular staining was used for the detection of IFN-γ production. The numbers are percentages of the IFN-γ positive cells among the gated $CD3^-$ $NKp46^+$ cells. (D). The percentage of the IFN-γ positive cells among the gated $CD3^-$ $NKp46^+$ cells under antibodies stimulation.

4. Carnosine contributes to enhance the ability of NK cell degranulation

NK cells contain high concentrations of cytotoxic granules in their cytoplasm as they circulate in the periphery. As degranulation occurs, secretory lysosomes are released, and the lysosome-associated membrane protein-1 (LAMP-1, CD107a) is transported to the surface of NK cell. Therefore, the upregulated expression of CD107a on the surface of NK cells correlates with the lysis of target cell mediated by NK cell. We thus quantified the degranulation pathway by measuring CD107a expression in NK cells following multi-stimulations[7]. CD107a expression was analyzed by flow cytometry after incubation of NK cells using APC-anti-CD107a. We found enhanced degranulation as demonstrated by the reduced CD107a expression after NK cells stimulated with tumor cell lines RMA-s and YAC-1（Figure3.A,B）. We also analyzed CD107a expression when NK cells were stimulated by cross-linking their activating receptors, consistent with the data obtained from tumor cell stimulation（Figure3.C,D）. It is further illustrated that Carnosine can affect the degranulation ability of NK cells and enhance the effector function of NK cells.

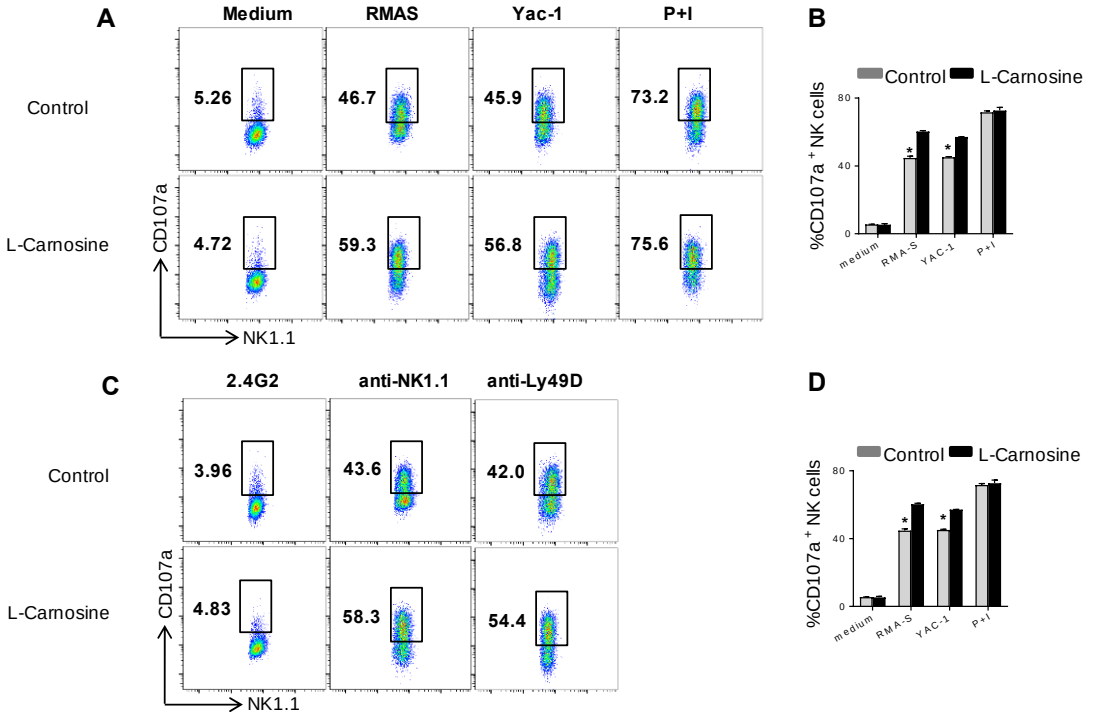

Figure 3. Carnosine contributes to effect cell degradation of NK cells.

(A). The mice from control and experimental groups were pre-activated for 16 hours following poly I:C stimulation in vivo. Splenic lymphocytes were prepared and co-cultured with an equal number of tumor cell lines, RMA-s and YAC-1, for 4 hours; medium alone served as a negative control, and PMA plus ionomycin served as a positive control. The expression of CD107a was analyzed. The numbers are percentages of the CD107a positive cells among the gated $CD3^-$ $NKp46^+$ cells. (B). The percentage of the CD107a positive cells among the gated $CD3^-$ $NKp46^+$ cells was compared between the mice control and experimental groups under tumor cell stimulation. (C). Poly I:C-activated splenic lymphocytes were seeded on 24-well plates coated with the indicated antibodies or stimulated with cytokines for 4 hours. The expression of CD107a was analyzed. The numbers are percentages of the CD107a positive cells among the gated $CD3^-NKp46^+$ cells. (D). The percentage of the CD107a positive cells among the gated $CD3^-NKp46^+$ cells was compared between WT and PDK1 deficient mice under antibodies stimulation. Data represent the mean ± s.d of 3-4 mice and are representative of three independent experiments. *p < 0.01.

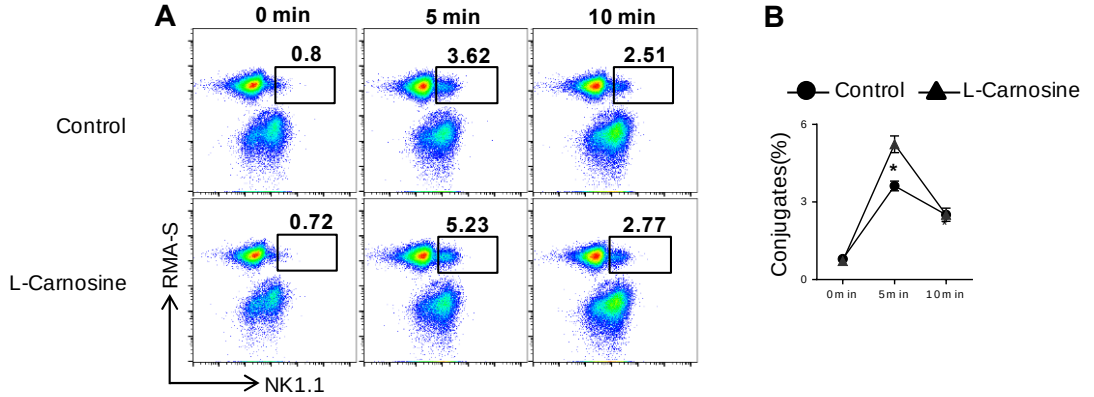

Figure 5. Carnosine contributes to regulate NK cells conjugate formation

(A). NK cell conjugate formation. NK cells from control and experimental mice were purified by MACS and expanded for 5 days in the presence of 1000 IU IL-2. Equal numbers of NK1.1-APC antibody-stained NK cells and GFP-expressing RMA-S cells were mixed and stimulated at 37℃ for the indicated times. Conjugate formation was monitored by FACS. Double-positive GFP^+APC^+

conjugate cells are presented as the percent of NK cells. The numbers are percentages of double positive cells among the gated cells. (B). The percentage of the double positive cells was compared between control and experimental mice in the indicated time.

5. Carnosine contributes to regulate NK cells conjugate formation

NK cell killing function highly relies on integrin-mediated signaling, which helps NK cells to form stable synaptic interfaces with target cells[8]. To determine whether Carnosine-treated NK cells could stably contact tumor target cells, NK cell conjugation with target cells was tested. Compared with the control, Carnosine-treated NK cells formed fewer conjugates with RMA-S cells at the 5 and 10 min time points (Figure 4.A, B). These results indicated that Carnosine-treated NK cells fail to form stable conjugates with targets tumor cells.

6. Conclusion

The results of this study strongly demonstrate that Carnosine helps to increase the function of NK cells in the body's innate immune system. The long-term consumption of Carnosine observed that Carnosine can promote the NK cells younger, we further confirmed that Carnosine can increase NK cell cytokine secretion and degranulation ability in the case of tumor stimulation and specific antibody stimulation. Finally, we further established that Carnosine can help NK cells bind to tumor cells, thereby exerting more effective effector functions. This study clarified the regulation of Carnosine on the development and function of NK cells. It can provide the theoretical guidance for practical treatment of clinical disease treatment and has a certain social value.

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