IL-12p35 and IL-12p40 functionally synergize to activate NK cells

Jinghua Wang, Christian Erickson, Mark Renelt, Vida Gavino, Jing Sun, Jun Li, Ming Bi, Anthony Person

Department of Protein Development, R&D Systems, Bio-Techne, Minneapolis, MN 55413

Abstract

Interleukin-12 (IL-12) is a member of a family of heterodimeric cytokines that play critical roles in regulating the immune response toward different effector pathways. The formation of functional IL-12 requires association of the IL-12p35 subunit with the IL-12p40 subunit, and the secretion of IL-12 relies on p40 and p35 being expressed and assembled within dendritic cells and macrophages. IL-12p40 can also be released from these hematopoietic cells as a monomer and is usually found in great excess over IL-12 in serum and cell culture supernatants. The precise biological significance of the secreted IL-12p40 monomer is not clear, although recent studies indicate that it has immunomodulatory properties. Previous studies have reported that p40 and p35 can assemble to form functionally active IL-12 in vitro. IL-12p40 associates with p35 extracellularly to generate IL-12–like activities on T cells, but the biological significance on NK cells remains unclear. In this study, we show that only high concentrations of IL-12p35 could activate NK cells to secrete IFN-gamma and induce cell proliferation and IL-12p40 had no effect on NK cell activation. Interestingly, IL-12p35 and p40 functionally synergized to activate NK cells by inducing IFN-gamma secretion and cell proliferation in vitro. Surprisingly, pretreatment of NK cells with IL-12p35 or p40 didn't affect IL-12 induced IFN-gamma secretion and cell proliferation in NK cells. Furthermore, a functional ELISA binding assay revealed that IL-12p35 binds to both IL-12R beta1 and IL-12R beta2 with a low affinity, while IL-12p40 only binds to IL-12R beta1 with a high affinity. Fluorescent conjugated human IL-12, IL-12p35 and IL-12p40 proteins all significantly bound to human NK cells and IL-12p35 and p40 didn't significantly interfere with IL-12 binding to these cells. Collectively, the data we show herein indicates that IL-12 activity can come from the collaboration between the IL-12p35 and p40 subunits and impact NK cell activation.

Introduction

Interleukin 12 (IL-12) is a heterodimeric cytokine composed of a p40 and a p35 subunit, each expressed by separate genes on different chromosomes (1). IL-12p35 is produced at lower levels and not secreted alone and requires binding to p40 for correct folding and secretion (2). IL-12p40 is produced and secreted in excess during activation. IL-12p40 as a monomer or homodimer (p40 or p80, respectively) can interact with its receptor IL-12Rβ1 to exert agonistic and/or antagonistic functions that are distinct from those of its other family members (3). The precise biological significance of secreted p40 monomer is not yet clear, although recent studies indicate immunomodulatory properties. The p40-p40 homodimer can inhibit the IFN gamma-promoting activity of IL-12, but the amounts of p40 monomer far exceeds this form (4).

IL-12 receptors are located mainly on T cells and NK cells. IL-12 is a central cytokine acting on T and natural killer (NK) cells. IL-12p40 monomer in combination with p35 released from necrotic cells can generate IL-12-like activities. IL-12p40 and p35 can assemble to form functionally active IL-12 (5). In this study, IL-12p35 and p40 proteins were produced by cloning human IL-12p35 (aa 23-219) and human IL-12p40 monomer (aa 23-328) in mammalian or Sf 21 (baculovirus)-expression vectors. Using various in vitro experimental systems, we demonstrated that IL-12p35 and IL-12p40 functionally synergize to activate NK cells.

Results

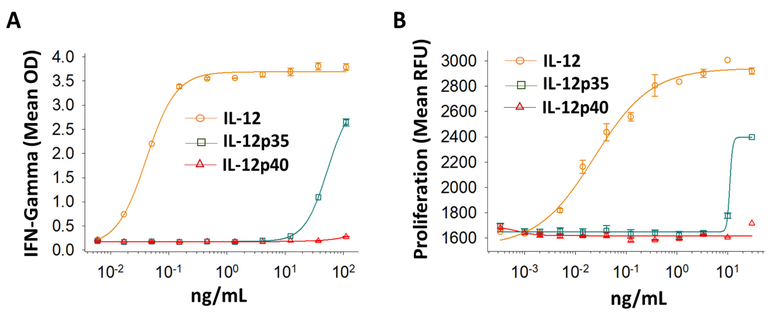

Figure 1. High concentrations of IL-12p35 could activate NK cells to secrete IFN-gamma and induce cell proliferation and IL-12p40 had no effect on NK cell activation. (A) NK92 cells were treated with the indicated concentrations of Recombinant Human IL-12/IL-23 p40 Monomer Protein (R&D Systems, Catalog # 309-IL), Recombinant Human IL-12 p35 (C96S) Protein (R&D Systems, Catalog # 11209-IL) or Recombinant Human IL-12 Protein (R&D Systems, Catalog # 219-IL) for 24h. The cytokine levels in the cell culture supernatants were measured using Human IFN-γ Quantikine® ELISA Kits (R&D Systems, Catalog # DIF50C). **(B)** NK92 cells were treated with the indicated concentrations of Recombinant Human IL-12/IL-23 p40 Monomer Protein (R&D Systems, Catalog # 309-IL), Recombinant Human IL-12 p35 (C96S) Protein (R&D Systems, Catalog # 11209-IL) or Recombinant Human IL-12 Protein (R&D Systems, Catalog # 219-IL) for 3 days. Cell proliferation was assessed by a fluorometric assay using the redox-sensitive dye Alamar Blue (Resazurin; R&D Systems, Catalog # AR002).

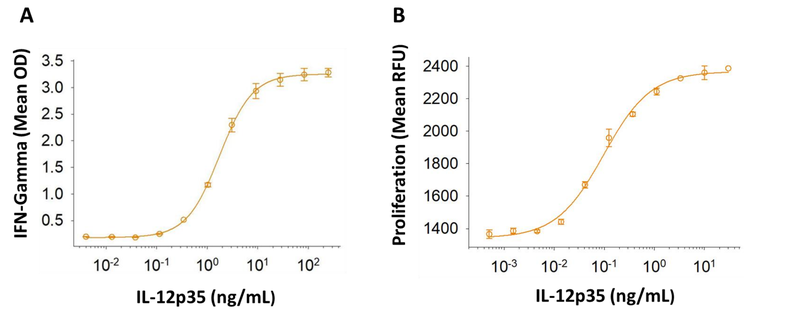

Figure 2. IL-12p35 and p40 functionally synergized to activate NK cells by inducing IFN-gamma secretion and cell proliferation. (A) NK92 cells were treated with 0.25 µg/mL of Recombinant Human IL-12/IL-23 p40 Monomer Protein (R&D Systems, Catalog # 309-IL) and the indicated concentrations of Recombinant Human IL-12 p35 (C96S) Protein (R&D Systems, Catalog # 11209-IL) for 24h. The cytokine levels in the cell culture supernatants were measured using Human IFN-γ Quantikine® ELISA Kits (R&D Systems, Catalog # DIF50C). **(B)** NK92 cells were treated with 30 ng/mL of Recombinant Human IL-12/IL-23 p40 Monomer Protein (R&D Systems, Catalog # 309-IL) and the indicated concentrations of Recombinant Human IL-12 p35 (C96S) Protein (R&D Systems, Catalog # 11209-IL) for 3 days. Cell proliferation was assessed by a fluorometric assay using the redox-sensitive dye Alamar Blue (Resazurin; R&D Systems, Catalog # AR002).

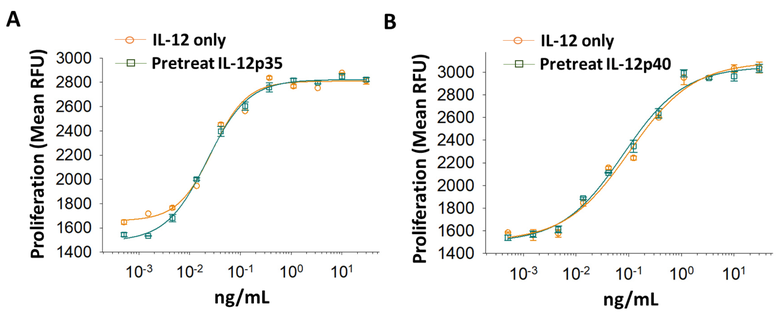

Figure 3. Pretreatment of NK cells with IL12p35 or p40 didn't affect IL-12 induced cell proliferation in NK cells. NK92 cells were pretreated with **(A)** 10 ng/mL of Recombinant Human IL-12/IL-23 p40 Monomer Protein (R&D Systems, Catalog # 309-IL) or **(B)** 10 ng/mL of Recombinant Human IL-12 p35 (C96S) Protein (R&D Systems, Catalog # 11209-IL) for 2h, then treated with the indicated concentrations of Recombinant Human IL-12 Protein (R&D Systems, Catalog # 219-IL) for 3 days. Cell proliferation was assessed by a fluorometric assay using the redox-sensitive dye Alamar Blue (Resazurin; R&D Systems, Catalog # AR002).

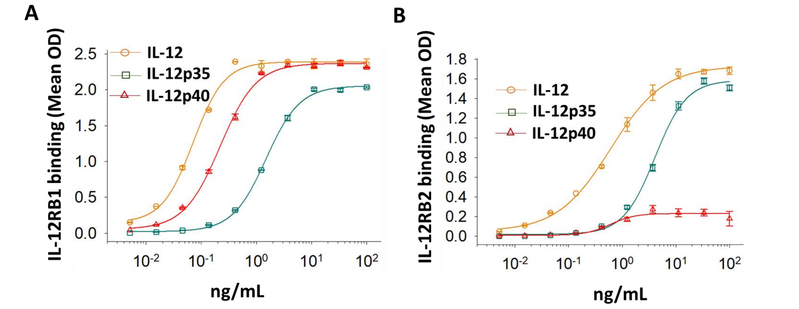

Figure 4. IL-12p35 binds to both IL-12R beta1 and IL-12R beta2 with a low affinity, while IL-12p40 only binds to IL-12R beta1 with a high affinity on a functional ELISA binding assay. (A) IL-12p35 and IL-12p40 bind to IL-12R beta1 in a functional ELISA binding assay. When IL-12R beta1 was immobilized, the concentrations of IL-12p40 and IL-12p35 that produces 50 % of the optimal binding response was approximately 20 ng/mL and 1.5 µg/mL. **(B)** IL-12p35 and IL-12p40 bind to IL-12R beta2 in a functional ELISA binding assay. When IL-12R beta2 was immobilized, the concentrations of IL-12p35 that produces 50 % of the optimal binding response was approximately 4.00 µg/mL. IL-12p40 didn't bind to IL-12R beta2.

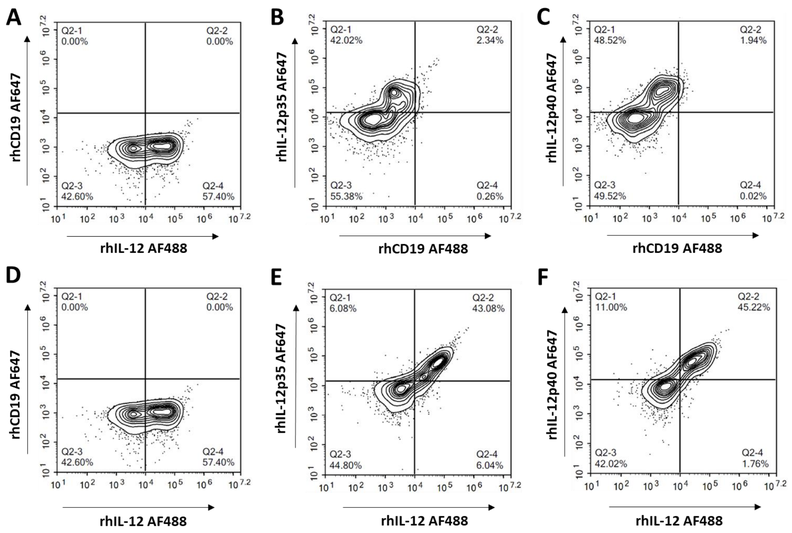

Figure 5. Fluorescent conjugated human IL-12, IL-12p35 and IL-12p40 proteins significantly bound to human NK cells. IL-12p35 and p40 didn't significantly interfere with IL-12 binding to these cells. NK92 cells were incubated with fluorescent conjugated human **(A, D)** IL-12, **(B)** IL-12p35 and **(C)** IL-12p40 proteins for 1 hour at 2-8◦C. NK92 cells were preincubated with fluorescent conjugated **(E)** IL-12p35 or **(F)** L-12p40 proteins for 0.5 hour, then incubated with fluorescent conjugated human IL-12 for 1 h at 2-8◦C. Protein binding to the cell surface was analyzed by flow cytometry.

Summary

- High concentrations of IL-12p35 could activate NK cells and IL-12p40 had no effect on NK cell activation.
- In vitro IL-12p35 and p40 functionally synergized to activate NK cells by inducing IFN-gamma secretion and cell proliferation.
- IL-12p35 binds to both IL-12R beta1 and IL-12R beta2 with a low affinity, while IL-12p40 only binds to IL-12R beta1 with a high affinity.
- IL-12p35 and p40 didn't significantly interfere the interaction of IL-12 with IL-12R beta1 and IL-12R beta2.
- In conclusion, IL-12 activity can come from the collaboration between two p40 and p35 subunits and impact NK cell activation.

References

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