



HOKKAIDO UNIVERSITY

Title	Differential effects of interleukin-12 and interleukin-15 on expansion of NK cell receptor-expressing CD8+ T cells
Author(s)	Sugita, Junichi; Tanaka, Junji; Yasumoto, Atsushi et al.
Citation	Annals of Hematology, 89(2), 115-120 https://doi.org/10.1007/s00277-009-0780-0
Issue Date	2010-02
Doc URL	https://hdl.handle.net/2115/44772
Rights	The original publication is available at www.springerlink.com
Type	journal article
File Information	AH89-2_115-120.pdf



Differential effects of interleukin-12 and interleukin-15 on expansion of NK cell receptor-expressing CD8⁺ T cells.

Junichi Sugita¹⁾, Junji Tanaka¹⁾, Atsushi Yasumoto¹⁾, Souichi Shiratori¹⁾, Kentaro Wakasa¹⁾, Misato Kikuchi¹⁾, Akio Shigematsu¹⁾, Takeshi Kondo²⁾ Masahiro Asaka²⁾, Masahiro Imamura¹⁾

1) Department of Hematology and Oncology, Hokkaido University Graduate School of Medicine, Sapporo, Japan.

2) Department of Gastroenterology and Hematology, Hokkaido University Graduate School of Medicine, Sapporo, Japan.

Address correspondence to Dr. Junichi Sugita, Department of Hematology and Oncology, Hokkaido University Graduate School of Medicine, N15 W7, Kita-Ku, Sapporo 060-8638, Japan.

Tel: +81-11-706-7214, Fax: +81-11-706-7823

E-mail: junichi_s@pop21.odn.ne.jp

【running title】

Differential effects of IL-12 and IL-15 on T cells

Key words

CD94; NKG2A; NKG2D; cytolytic activity

Abstract

The cytolytic activity of cells expressing natural killer cell receptors(NKRs) depends on the balance between stimulatory and inhibitory signals. We investigated both inhibitory NK receptor (CD94/NKG2A) expression and stimulatory NKR (NKG2D) expression on T cells after stimulation with cytokines (IL-12 or IL-15).

Cytolytic NKR-expressing CD8⁺ T cells were expanded from normal adult peripheral blood mononuclear cells using anti-CD3 monoclonal antibody and cytokines (IL-12 or IL-15). The proportion and absolute number of CD94/NKG2A-expressing T cells expanded by IL-12 were significantly larger than those of the cells expanded by IL-15. On the other hand, the proportion and absolute number of NKG2D-expressing T cells expanded by IL-15 were significantly larger than those of the cells expanded by IL-12. The proportions of NKG2D and intracellular granzyme A expression in CD94-expressing cells were much more increased in PBMCs cultured with IL-15 than those of cells cultured with IL-12. A real-time polymerase chain reaction assay showed that there was a 1.68-fold increase in NKG2D mRNA expression level and a 1.37-fold increase in DAP10 mRNA expression level in CD94-expressing cells expanded by IL-15 compared with those of the cells expanded by IL-12. The cytolytic activity levels of purified CD94-expressing cells from 8-day culture with IL-15 tested against ⁵¹Cr-labeled K562 cells by standard 4-hour ⁵¹Cr release assays without prior sensitization were much higher than those of cells from 8-day culture with IL-12.

IL-15 appears to be able to enhance the cytolytic activity of CD94/NKG2A-expressing cells through induction of NKG2D and intracellular granzyme expression much more efficiently than does IL-12.

Introduction

Natural killer cell receptors (NKR) have been shown to be expressed on not only NK cells but also T cells [1-8]. These NKRs have a stimulatory or inhibitory function through their binding to major histocompatibility complex (MHC) class I molecules or MHC class I-like molecules. As one of the inhibitory receptors, CD94/NKG2A heterodimer recognizes an HLA-E that preferably binds to a peptide derived from the signal sequences of most HLA class I molecules [9-12]. On the other hand, the stimulatory NKG2D interacts with MHC class I chain-related proteins A and B (MICA and MICB) [13,14]. Although NKG2D has no identifiable signaling motifs, it attains signaling capability through association with DNAX-activating protein of 10 kDa (DAP10) [7].

It has been reported that IL-12 [12,15], IL-15 [16-18], prostaglandin E2 [19] and transforming growth factor- β [20,21] can induce the expression of CD94/NKG2A on T cells. On the other hand, it has been reported that IL-2 [22-24], IL-12 [25] and IL-15 [26] can induce NKG2D expression on T cells. Cytolytic activity of NKR-expressing cells depends on the balance between stimulatory and inhibitory signals. However, only the expression of either CD94/NKG2A or NKG2D was examined in those studies. In the present study, we investigated cytolytic activity of both inhibitory NK receptor (CD94/NKG2A)-expressing and stimulatory NK receptor (NKG2D)-expressing T cells after stimulation with immobilized anti-CD3 monoclonal antibody (mAb) and IL-12 or IL-15 in peripheral blood mononuclear cells (PBMCs) for 8 days.

Materials and methods

Peripheral blood mononuclear cells

Normal adult peripheral blood mononuclear cells (PBMCs) were obtained from healthy donors with informed consent from Hokkaido Red Cross Blood Center Sapporo.

Immunofluorescent staining for flow cytometric analysis and monoclonal antibodies

The phycoerythrin (PE) –conjugated monoclonal antibody (mAb) HP-3D9 (anti-CD94)

was obtained from Ancell (Bayport, MN, USA), and Z199 (anti-NKG2A) and ON72 (anti-NKG2D) were obtained from Immunotec (Marseilles, France). Fluorescein isothiocyanate (FITC)-conjugated anti-CD3 and anti-CD8 mAb were purchased from Pharmingen (San Diego, CA, USA). FITC-conjugated anti-NKG2D was obtained from Serotec (Oxford, England). Anti-CD56 mAb, anti-granzyme A mAb and HI111 (anti-LFA-1) were obtained from Becton Dickinson (BD, San Jose, CA, USA). Anti-CD3 mAb OKT3 was obtained from Ortho Biotech (Raritan, NJ, USA). Anti-NKG2D mAb was obtained from R&D Systems (Minneapolis, MN). Intracellular granzyme A was stained using cytofix/cytoperm reagent according to the instructions of the manufacturer (BD). The fluorescence intensity of the cells was analyzed using a FACS Calibur (BD). Statistical analysis was performed using Student's t-test.

Expansion of NK receptor-expressing cells

For coating with anti-CD3 mAb, 24-well flat-bottom plates or tissue culture flasks were preincubated with OKT3 ($1 \mu\text{g/mL}$) in 100 mM Tris [tris(hydroxymethyl)aminomethane]-HCl buffer (pH 9.5) for 16 hours at 4°C. PBMCs ($1 \times 10^6/\text{mL}$) were cultured on 24-well plates or tissue culture flasks in RPMI 1640 supplemented with 10% fetal calf serum with 2.5 ng/mL of recombinant human IL-12 or 5 ng/mL of recombinant human IL-15 (R&D Systems) at 37°C for 8 days as described previously [17,18]. Absolute numbers of CD94⁺/CD3⁺, CD94⁺/CD8⁺, NKG2A⁺/CD3⁺, NKG2A⁺/CD8⁺, NKG2D⁺/CD3⁺, NKG2D⁺/CD8⁺, CD94⁺/CD56⁺, and CD94⁺/NKG2D⁺ were calculated from multiplication of the total number of expanded cells and the proportion of these cells in expanded cells.

Immunomagnetic cell sorting

Purified CD94⁺ cells (> 90% CD94⁺, as determined by flow cytometric analysis) were obtained by magnetic cell sorting (MACS) using magnetic microbeads according to the instructions of the manufacturer (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany).

Real-time polymerase chain reaction

Total RNA was prepared using a cQIAamp RNA Blood Mini Kit (Qiagen, Valencia, CA, USA), and cDNA was synthesized from 1.2 mg total RNA using a TaKaRa RNA PCR Kit (AMV) Ver.3.0 following the instructions of the manufacturer (Takara, Shiga, Japan). Assays-on-Demand Gene Expression products for NKG2D and DAP10 were obtained from Applied Biosystems (Foster City, CA, USA). Glyceraldehyde-3-phosphate dehydrogenase was used as an endogenous control. Relative quantification assays for gene expression were performed using an Applied Biosystems 7300 real-time polymerase chain reaction system (Applied Biosystems).

Evaluation of cytolytic activity using 4-hour ⁵¹Cr release assay

After 8 days of stimulation by immobilized anti-CD3 mAb with IL-12 or IL-15 in a T25 flask, CD94-expressing cells were purified by MACS. The cytolytic activities of purified CD94-expressing cells were tested against ⁵¹Cr-labeled human erythroleukemic K562 cells (5×10^3) using a 4-hour ⁵¹Cr release assay. K562 cells were obtained from Riken (Tsukuba, Japan).

Results

Expansion of NK receptor-expressing cells

There was a very small number of NK receptor-expressing CD3⁺/CD8⁺ T cells in PBMCs before culture. However, the proportion and absolute number of NK receptor-expressing CD3⁺/CD8⁺ T cells in PBMCs were dramatically increased after stimulation with immobilized anti-CD3 mAb and cytokines (Figure 1). The proportion and absolute number of CD94/NKG2A-expressing T cells expanded by IL-12 were significantly larger than those of the cells expanded by IL-15 (CD94⁺CD3⁺/NKG2A⁺CD3⁺: median 39.8% (range 26.4 - 54.7) / 36.4% (22.3 - 47.9) by IL-12 vs 19.1% (11.0 - 21.9) / 12.3% (5.5 - 15.1) by IL-15, n=9, p < 0.01). On the other hand, the proportion and absolute number of NKG2D-expressing T cells expanded by IL-15 were significantly larger than those of the cells expanded by IL-12 (NKG2D⁺CD3⁺: median 22.0% (range 9.8 - 46.1) by IL-12 vs

34.9% (22.6 - 65.0) by IL-15, n=9, p < 0.05). Also, the ratio of stimulatory NKR (NKG2D) to inhibitory NKRs (CD94 or NKG2A) on CD8 T cells expanded by IL-15 was significantly larger than those of the cells expanded by IL-12 (NKG2D⁺/CD94⁺: median 0.35 (range 0.19 - 0.81) by IL-12 vs 1.62 (0.82 - 4.04) by IL-15, NKG2D⁺/NKG2A⁺: 0.39 (0.23 - 1.22) by IL-12 vs 3.17 (0.95 - 6.44) by IL-15, n=9, p < 0.01) (Figure 2).

Immunomagnetic cell sorting

Purified CD94⁺ cells (> 90% CD94, as determined by flow cytometric analysis) were obtained by MACS. The proportions of NKG2D and intracellular granzyme A expression in CD94-expressing cells were much more increased in PBMCs cultured with IL-15 than those of cells cultured with IL-12 (NKG2D⁺ / Granzyme A: median 56.3% (range 18.6 - 68.7) / 24.3% (7.7 - 36.6) by IL-15 vs 34.6% (12.9 - 50.5) / 0.9% (0 - 9.27) by IL-12, n=9, p < 0.01) (Figure 3). There was no difference in LFA-1 expression on CD94-expressing cells. Furthermore, a real-time polymerase chain reaction assay showed that there was a median 1.68-fold increase (range 1.03 - 2.39) in NKG2D mRNA expression level and a median 1.37-fold increase (range 1.10 - 2.36) in DAP10 mRNA expression level in CD94-expressing cells expanded by IL-15 compared with those of the cells expanded by IL-12 (Figure 4).

Cytolytic activity of CD94-expressing cells

The cytolytic activity levels of purified CD94-expressing cells from 8-day culture with IL-15 tested against ⁵¹Cr-labeled K562 cells by standard 4-hour ⁵¹Cr release assays without prior sensitization were much higher than those of cells from 8-day cultures with IL-12 (effector : target = 10 : 1, median 52.8% (range 41.2 - 70.5) by IL-15 vs 20.8% (18.1 - 30.7), n=15, p < 0.01) (Figure 5). Furthermore, anti-NKG2D mAb (60 μg/ml) significantly suppressed the cytolytic activity of CD94-expressing cells against K562 cells compared with the cytolytic activity of cells without anti-NKG2D (effector : target = 10 : 1, median 19.0% (range 15.6 - 20.4) vs 52.8% (41.2 - 70.5) by IL-15, 9.5% (8.7 - 10.9) vs 20.8% (18.1 - 30.7) by IL-12, n=6, p < 0.01) (Figure 5).

Discussion

It has been reported that IL-12 [12,15,25] and IL-15 [16-18,26] can induce the expression of NK cell receptors on T cells. Derre et al. [12] showed that IL-12 induces the expression of CD94/NKG2A, and Mingari et al. [16] showed that IL-15 induces the expression of CD94/NKG2A. Ortaldo et al. [25] showed that IL-12 induces the expression of NKG2D, and Roberts et al. [26] showed that IL-15 induces the expression of NKG2D. However, the expression of both CD94/NKG2A and NKG2D was not assessed in those studies. We previously reported that CD94-expressing CD8⁺ T cells with high cytolytic activity against patients' leukemic cells could be expanded by stimulation with immobilized anti-CD3 monoclonal antibody (mAb) and IL-15 [17,18]. In the present study, we investigated both CD94/NKG2A expression and NKG2D expression on T cells after stimulation with different cytokines (IL-12 and IL-15) in order to assess the role of the balance between inhibitory and stimulatory NKRs in the cytolytic activity.

We found that the proportion and absolute number of NKR-expressing CD3⁺/CD8⁺ T cells in PBMCs were dramatically increased after stimulation with immobilized anti-CD3 mAb and a cytokine (IL-12 or IL-15).

We investigated for the first time both inhibitory NK receptor (CD94/NKG2A) expression and stimulatory NK receptor (NKG2D) expression on T cells after stimulation. Furthermore, we showed differential effects of IL-12 and IL-15 on the expansion of NK cell receptor-expressing CD8⁺ T cells. IL-12 induced significantly more CD94/NKG2A expression on T cells derived from PBMCs than did IL-15. On the other hand, IL-15 induced significantly more NKG2D expression on T cells derived from PBMCs than did IL-12. Also, IL-15 induced a significantly higher ratio of NKG2D to NKG2A. These expanded and purified CD94-expressing cells (>90% CD94⁺) had CD8 and stimulatory receptor NKG2D expression but not CD56 expression on their surfaces and contained granzyme A in the cytoplasm. The proportions of NKG2D and intracellular granzyme A expression in CD94-expressing cells were much more increased in PBMCs cultured with IL-15 than those of cells cultured with IL-12. Also, a real-time polymerase chain reaction assay showed that there were higher

NKG2D and DAP10 mRNA expression levels in CD94- expressing cells expanded by IL-15 than those of the cells expanded by IL-12.

We also compared the cytolytic activity levels in purified CD94-expressing cells from 8-day culture with IL-12 or IL-15 against ⁵¹Cr-labeled K562 cells by standard 4-hour ⁵¹Cr release assays without prior sensitization. Purified CD94-expressing cells after IL-15 stimulation exhibited higher cytolytic activity than did cells after IL-12 stimulation. Furthermore, these cytolytic activities were significantly suppressed by anti-NKG2D mAb. Therefore, the cytolytic activity of CD94-expressing cells depends at least partially on NKG2D.

In this study, we have shown differential effects of IL-12 and IL-15 on the expansion of NK cell receptor-expressing CD8⁺ T cells. IL-15 can induce a balance between expression of stimulatory NKRs and expression of inhibitory NKRs toward predominance of stimulatory signals. Therefore, IL-15 appears to be able to enhance cytolytic activity through induction of NKG2D and intracellular granzyme A expression much more efficiently than does IL-12.

Acknowledgements

We thank Ms. M. Yamane, Ms. M. Mayanagi and Ms. Y.Ishimaru for their technical assistance.

Reference

1. Ljunggren HG, Karre K (1990) In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol Today* 11:237-244
2. Phillips JH, Gumperz JE, Parham P, Lanier LL (1995) Superantigen-dependent, cell-mediated cytotoxicity inhibited by MHC class I receptors on T lymphocytes. *Science* 268:403-405
3. Mingari MC, Schiavetti F, Ponte M, Vitale C, Maggi E, Romagnani S, Demarest J, Pantaleo G, Fauci AS, Moretta L (1996) Human CD8⁺ T lymphocyte subsets that express HLA class I-specific inhibitory receptors represent oligoclonally or monoclonally expanded cell populations. *Proc Natl Acad Sci U S A* 93:12433-12438
4. Moretta A, Biassoni R, Bottino C, Pende D, Vitale M, Poggi A, Mingari MC, Moretta L (1997) Major histocompatibility complex class I-specific receptors on human natural killer and T lymphocytes. *Immunol Rev* 155:105-117
5. Bakker AB, Phillips JH, Figdor CG, Lanier LL (1998) Killer cell inhibitory receptors for MHC class I molecules regulate lysis of melanoma cells mediated by NK cells, gamma delta T cells, and antigen-specific CTL. *J Immunol* 160:5239-5245
6. Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, Spies T (1999) Activation of NK

- cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* 285:727-729
7. Wu J, Song Y, Bakker AB, Bauer S, Spies T, Lanier LL, Phillips JH (1999) An activating immunoreceptor complex formed by NKG2D and DAP10. *Science* 285:730-732
 8. Huard B, Karlsson L. (2000) KIR expression on self-reactive CD8+ T cells is controlled by T-cell receptor engagement. *Nature* 403:325-328
 9. Ulbrecht M, Honka T, Person S, Johnson JP, Weiss EH (1992) The HLA-E gene encodes two differentially regulated transcripts and a cell surface protein. *J Immunol* 149:2945-2953
 10. Borrego F, Ulbrecht M, Weiss EH, Coligan JE, Brooks AG (1998) Recognition of human histocompatibility leukocyte antigen (HLA)-E complexed with HLA class I signal sequence-derived peptides by CD94/NKG2 confers protection from natural killer cell-mediated lysis. *J Exp Med* 187:813-818
 11. Braud VM, Allan DS, O'Callaghan CA, Soderstrom K, D'Andrea A, Ogg GS, Lazetic S, Young NT, Bell JI, Phillips JH, Lanier LL, McMichael AJ (1998) HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* 391:795-799
 12. Derre L, Corvaisier M, Pandolfino MC, Diez E, Jotereau F, Gervois N (2002) Expression of CD94/NKG2-A on human T lymphocytes is induced by IL-12: implications for adoptive immunotherapy. *J Immunol* 168:4864-4870
 13. Vivier E, Tomasello E, Paul P (2002) Lymphocyte activation via NKG2D: towards a new paradigm in immune recognition? *Curr Opin Immunol* 14:306-311
 14. Raulet DH (2003) Roles of the NKG2D immunoreceptor and its ligands. *Nat Rev Immunol* 3:781-790
 15. Tanaka J, Toubai T, Miura Y, Tsutsumi Y, Kato N, Umehara S, Toyoshima N, Ohta S, Asaka M, Imamura M (2004) Differential expression of natural killer cell receptors (CD94/NKG2A) on T cells by the stimulation of G-CSF-mobilized peripheral blood mononuclear cells with anti-CD3 monoclonal antibody and cytokines: a study in stem cell donors. *Transplant Proc* 36:2511-2512
 16. Mingari MC, Ponte M, Bertone S, Schiavetti F, Vitale C, Bellomo R, Moretta A, Moretta L (1998) HLA class I-specific inhibitory receptors in human T lymphocytes: interleukin 15-induced expression of CD94/NKG2A in superantigen- or alloantigen-activated CD8+ T cells. *Proc Natl Acad Sci U S A* 95:1172-1177
 17. Tanaka J, Toubai T, Tsutsumi Y, Miura Y, Kato N, Umehara S, Kahata K, Mori A, Toyoshima N, Ota S, Kobayashi T, Kobayashi M, Kasai M, Asaka M, Imamura M (2004) Cytolytic activity and regulatory functions of inhibitory NK cell receptor-expressing T cells expanded from granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells. *Blood* 104:768-774
 18. Tanaka J, Iwao N, Toubai T, Tsutsumi Y, Miura Y, Kato N, Shigematsu A, Yamane M, Ota S, Kondo T, Kobayashi T, Takeda H, Kobayashi M, Asaka M, Imamura M (2005) Cytolytic activity against primary leukemic cells by inhibitory NK cell receptor (CD94/NKG2A)-expressing T cells expanded from various sources of blood mononuclear cells. *Leukemia* 19:486-489
 19. Zeddou M, Greimers R, de Valensart N, Nayjib B, Tasken K, Boniver J, Moutschen M, Rahmouni S (2005) Prostaglandin E2 induces the expression of functional inhibitory CD94/NKG2A receptors in human CD8+ T lymphocytes by a cAMP-dependent protein kinase A type I pathway. *Biochem Pharmacol* 70:714-724
 20. Bertone S, Schiavetti F, Bellomo R, Vitale C, Ponte M, Moretta L, Mingari MC (1999) Transforming growth factor-beta-induced expression of CD94/NKG2A inhibitory receptors in human T lymphocytes. *Eur J Immunol* 29:23-29
 21. Gunturi A, Berg RE, Crossley E, Murray S, Forman J (2005) The role of TCR stimulation and TGF-beta in controlling the expression of CD94/NKG2A receptors on CD8 T cells. *Eur J Immunol* 35:766-775
 22. Dhanji S, Teh HS (2003) IL-2-activated CD8+CD44high cells express both adaptive and innate immune system receptors and demonstrate specificity for syngeneic tumor cells. *J Immunol* 171:3442-3450
 23. Verneris MR, Karami M, Baker J, Jayaswal A, Negrin RS (2004) Role of NKG2D signaling

- in the cytotoxicity of activated and expanded CD8+ T cells. *Blood* 103:3065-3072
24. Karimi M, Cao TM, Baker JA, Verneris MR, Soares L, Negrin RS (2005) Silencing human NKG2D, DAP10, and DAP12 reduces cytotoxicity of activated CD8+ T cells and NK cells. *J Immunol* 175:7819-7828
 25. Ortaldo JR, Winkler-Pickett R, Wigginton J, Horner M, Bere EW, Mason AT, Bhat N, Cherry J, Sanford M, Hodge DL, Young HA (2006) Regulation of ITAM-positive receptors: role of IL-12 and IL-18. *Blood* 107:1468-1475
 26. Roberts AI, Lee L, Schwarz E, Groh V, Spies T, Ebert EC, Jabri B (2001) NKG2D receptors induced by IL-15 costimulate CD28-negative effector CTL in the tissue microenvironment. *J Immunol* 167:5527-5530

Titles and legends to figures

Figure 1.

Proportions of NKR-expressing cells before and after stimulation using different cytokines.

Each datapoint shows the proportion of NKR-expressing cells before (Pre) and after anti-CD3 stimulation in the presence of IL-12 or IL-15 (n=9). Bars indicate the median of the datapoints.

* p<0.01 ** p<0.05

Figure 2.

Balance between stimulatory NKR and inhibitory NKRs on CD8⁺T cells. Each datapoint shows the ratio of stimulatory NKR (NKG2D) to inhibitory NKRs (CD94 or NKG2A) on CD8-T cells (n=9). a) NKG2D⁺/CD94⁺, b) NKG2D⁺/NKG2A⁺. Bars indicate the median of the datapoints.

* p<0.01

Figure 3.

Proportions of NKG2D, LFA and intracellular granzyme A in CD94⁺ cells purified by magnetic cell sorting (MACS). Each datapoint shows the proportion of NKG2D, LFA and intracellular granzyme A in CD94⁺ cells purified by MACS (n=9). a) NKG2D⁺, b) LFA⁺, c) granzyme A. Bars indicate the median of the datapoints.

* p<0.01

Figure 4.

Quantitative analysis of NKG2D mRNA and DAP10 mRNA expression in CD94⁺ cells purified by magnetic cell sorting (MACS). Each datapoint shows the fold expression levels of NKG2D and DAP10 mRNA in CD94⁺ cells expanded by IL-15 compared with expression levels in CD94 cells expanded by IL-12 (n = 9). a) NKG2D, b) DAP10. Bars indicate the median of the datapoints.

* p<0.01

Figure 5.

Cytolytic activities of CD94-expressing cells after stimulation with IL-12 or IL-15 against K562 cells with and without anti-NKG2D. Each datapoint shows the specific % ^{51}Cr release of CD94-expressing cells after stimulation with IL-12 or IL-15 against K562 with (n=6) and without (n=15) anti-NKG2D(α -ND). Bars indicate the median of the datapoints. (Effector-to-target ratio is 10:1.).

* p<0.01

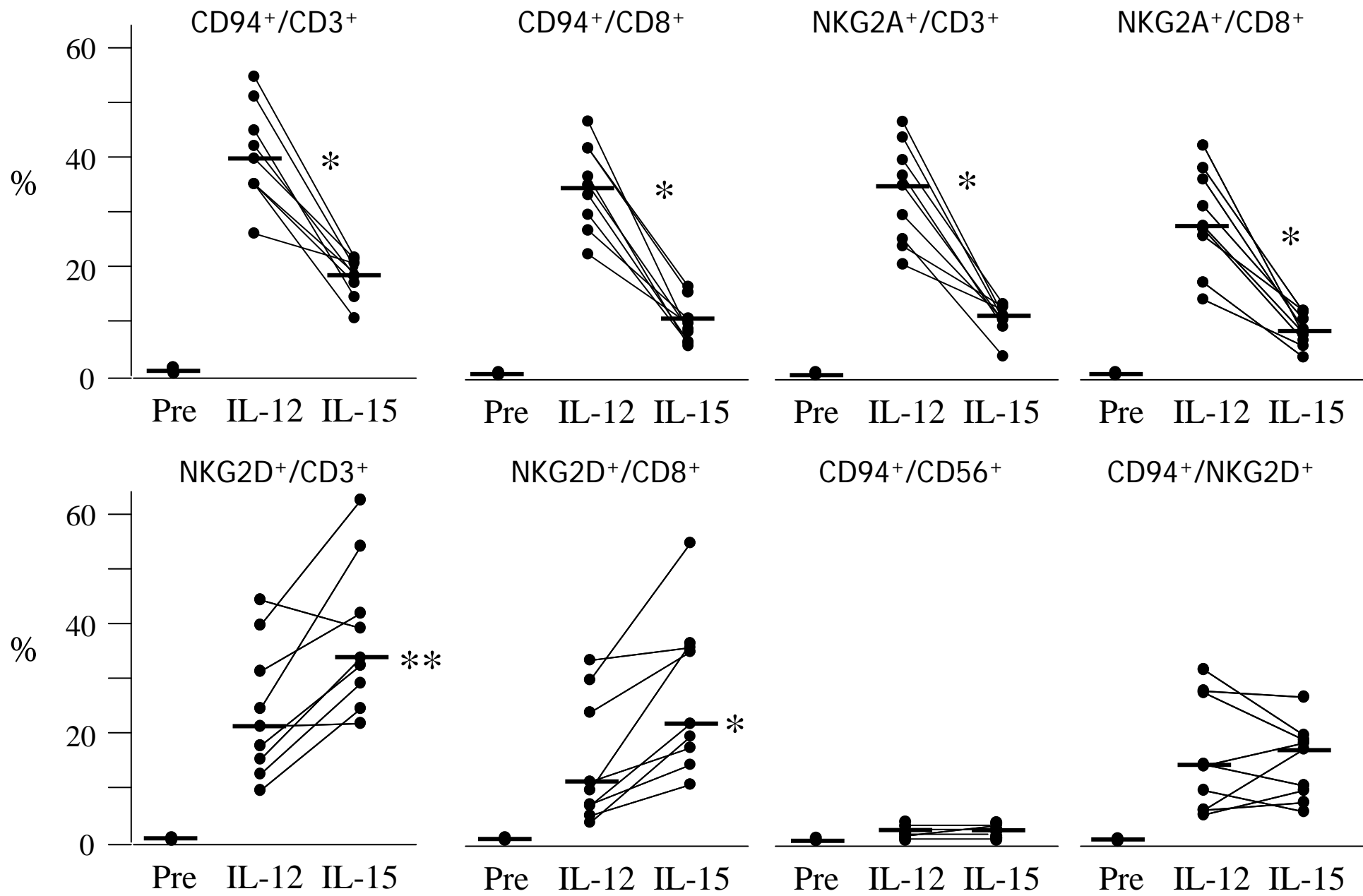


Figure 1.

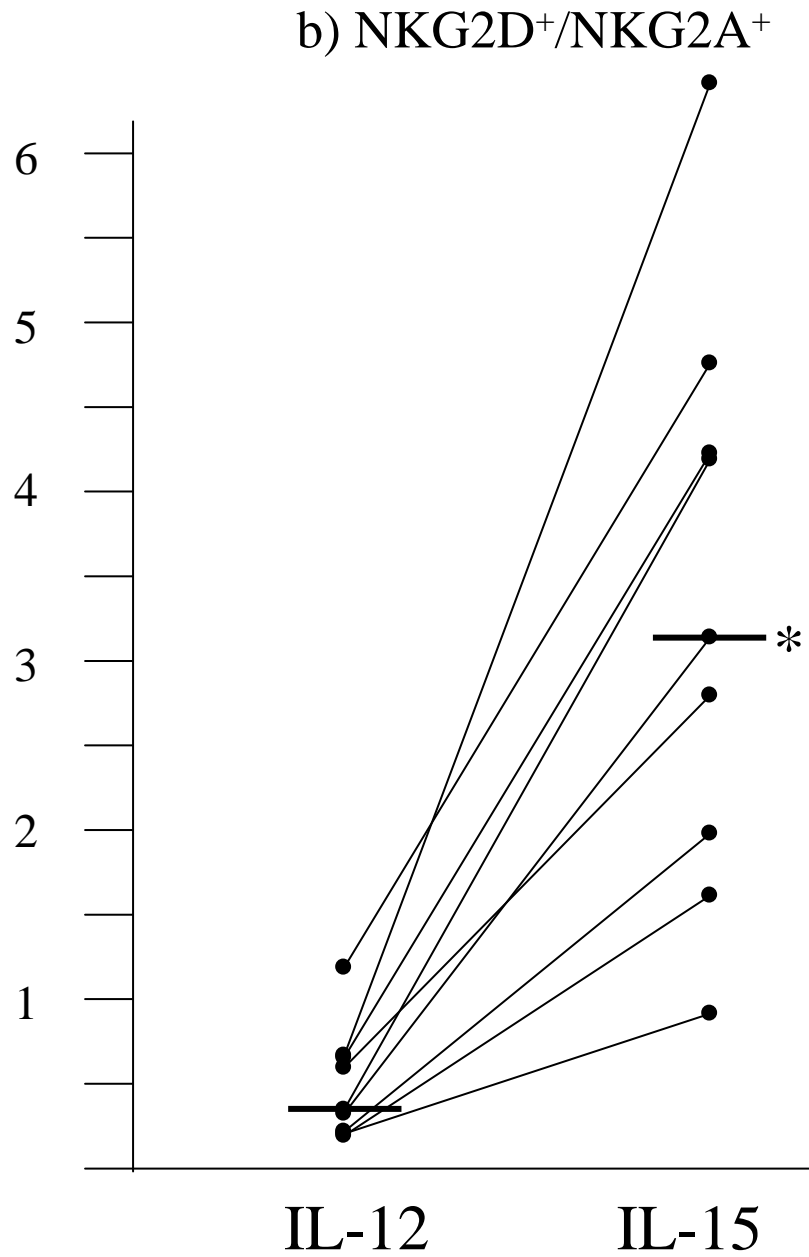
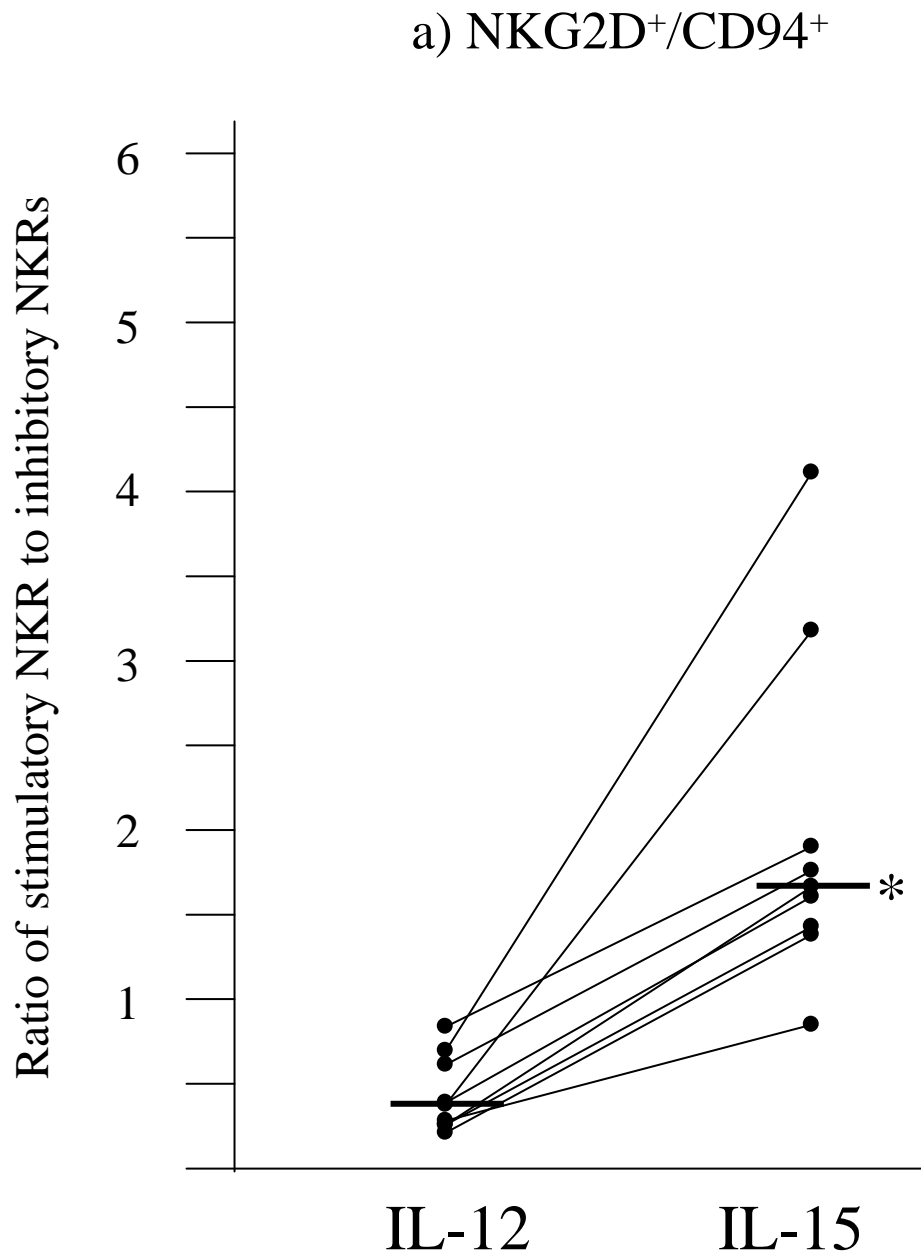


Figure 2.

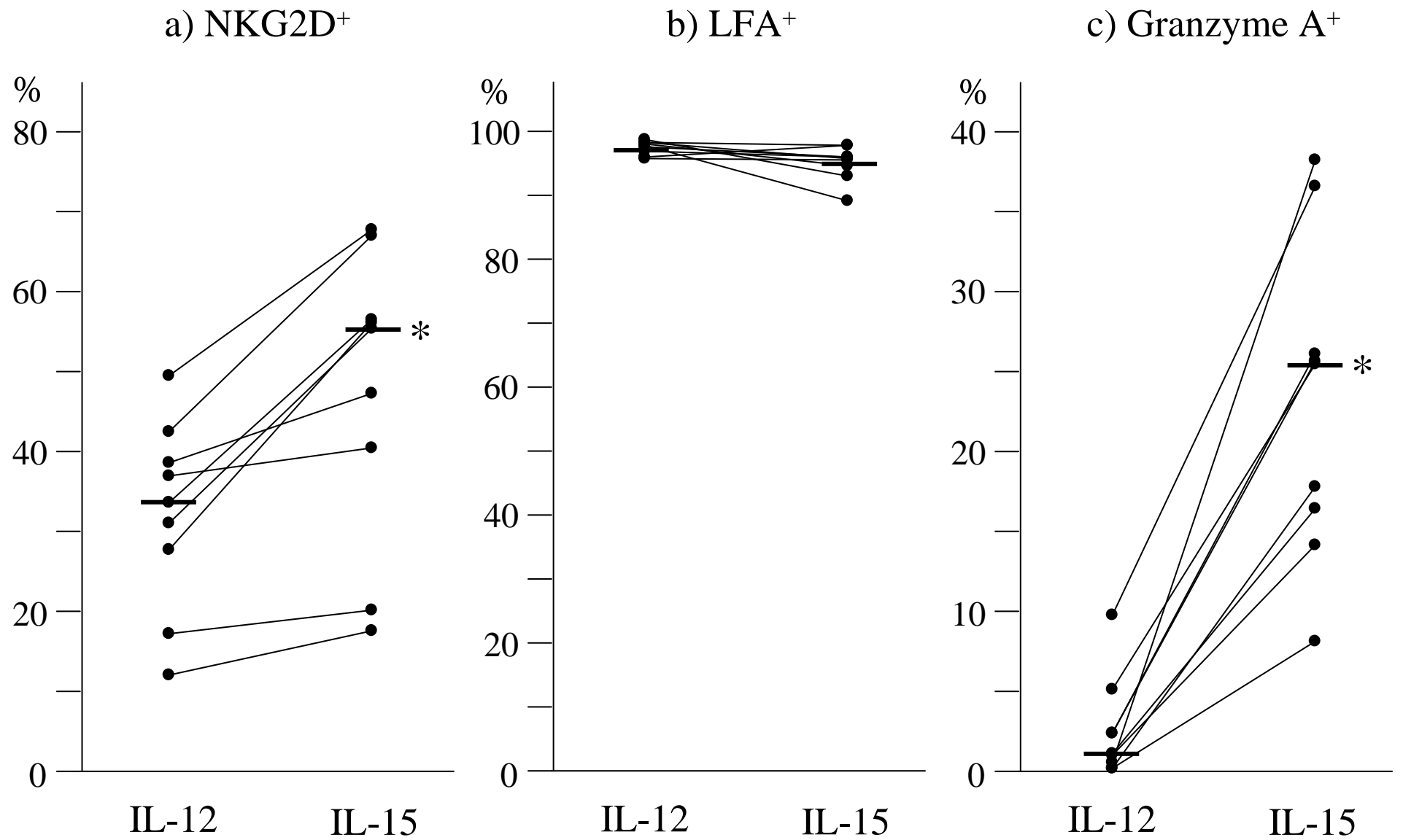
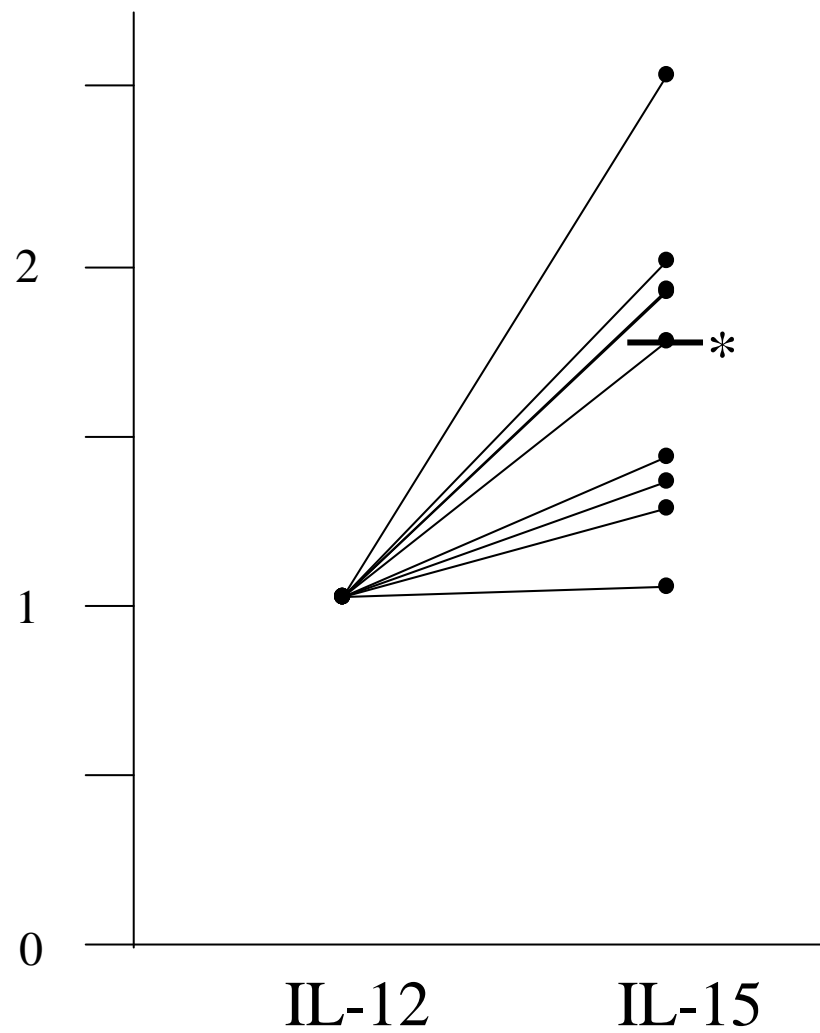


Figure 3.

Fold expression level of NKG2D mRNA

a) NKG2D



b) DAP10

Fold expression level of DAP10 mRNA

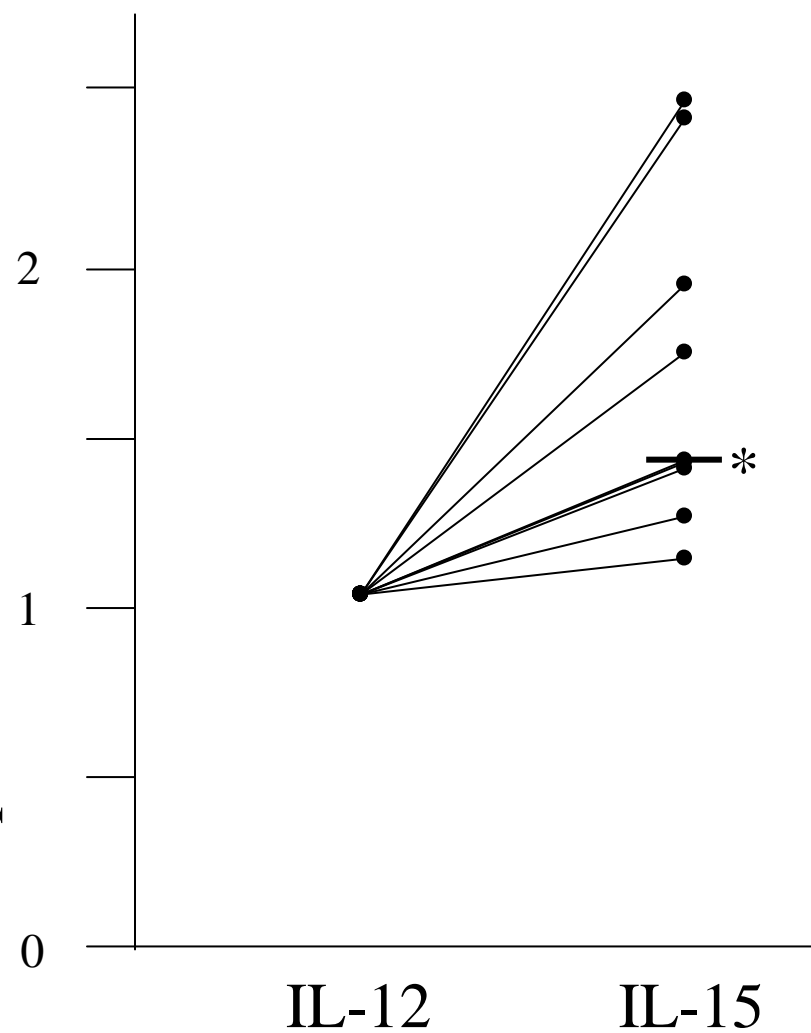


Figure 4.

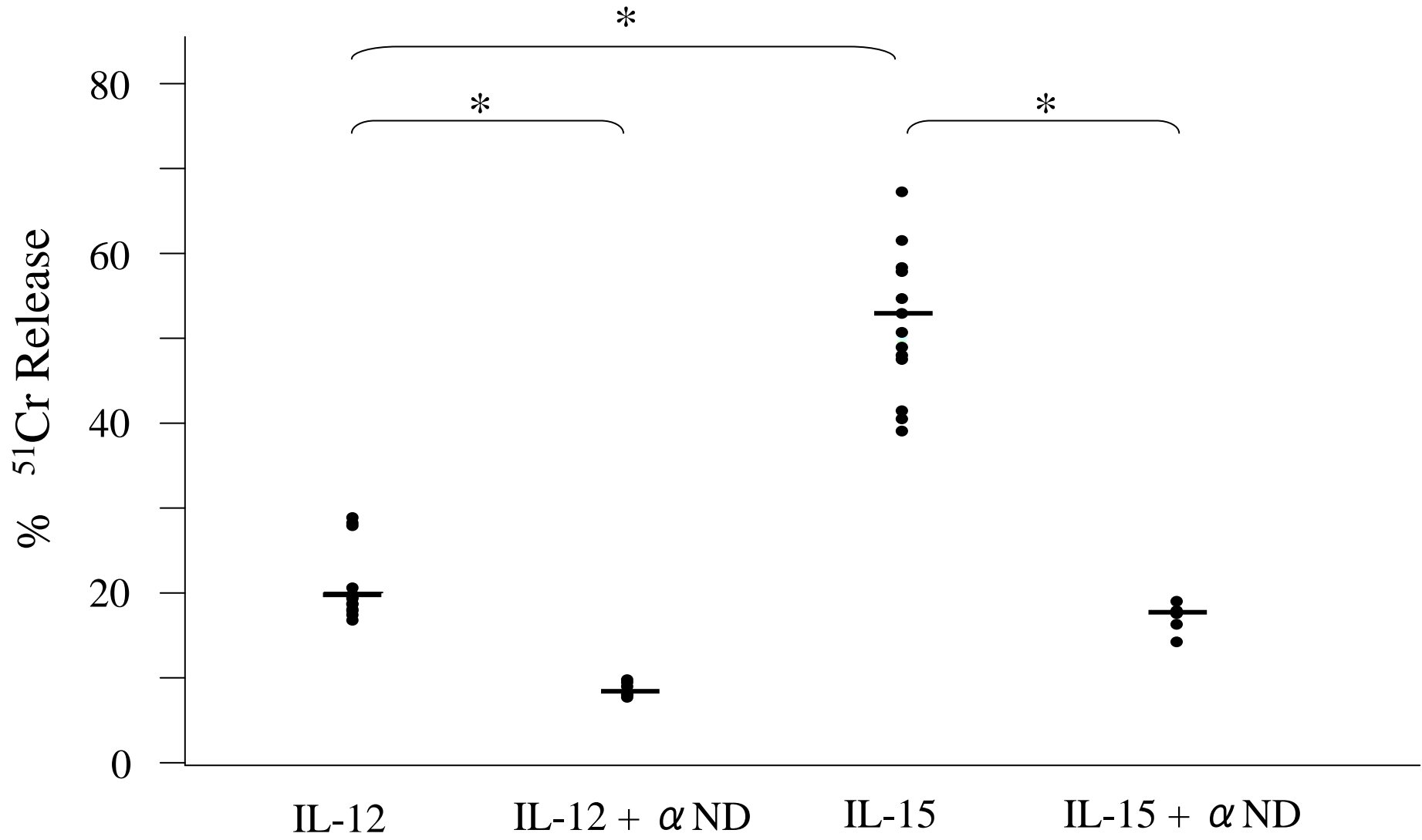


Figure 5.