DC infected by the ANRS MVA_{HIV} vaccine candidate prime NK cells with anti-HIV specific activity through a mechanism involving NKG2D and NKp46 on NK cells and membrane-bound IL-15 on DC

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Introduction

Natural Killer (NK) cells are the major antiviral effector cell population of the innate immune system. It has been demonstrated that NK cell activity can be modulated by the interaction with dendritic cells (DC). The vaccine candidate Modified Vaccinia virus Ankara encoding an HIV polypeptide (MVA_{HIV}), developed by the French National Agency for Research on AIDS (ANRS), has the ability to infect DC and to prime T cells. However, whether or not MVA_{HIV}-infected DC are able to induce anti-HIV specific NK cell activity remains undetermined.

Objectifs

Analyze whether NK cells are able to acquire specific anti-HIV activity through the stimulation by MVA_{HIV}-infected DC. And if so, investigate the implication of NK cell receptors as well as mbIL-15 in the induction of the anti-HIV specific priming of NK cells.

Material and methods

DC were infected by MVA_{HIV} or MVA_{WT} vector as control and co-cultured with autologous NK cells for 4 days. Then, NK cells were transferred to a culture of HIV-1-infected autologous DC or $CD4^+$ T cells. The control of HIV-1 infection was assessed by intracellular staining of HIV-1 p24 at days 9 or 10 post-infection (p.i) and analysis was done by flow cytometry. The implication of NKG2D and NKp46 on NK cells, and membrane-bound IL-15 (mbIL-15) on DC, during the priming of NK cells was determined by using blocking mAbs.

Results

1 Control of HIV infection by NK cells is increased after MVA_{HIV} priming

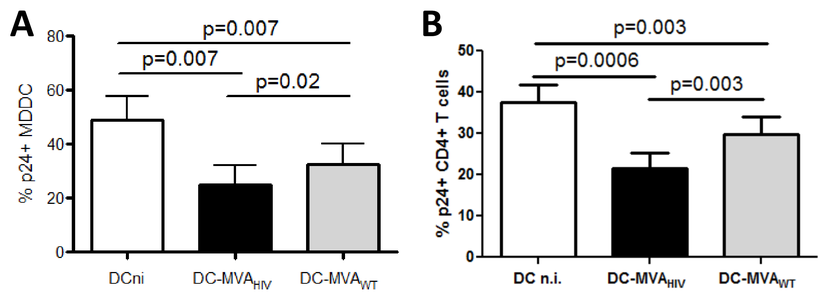

Figure 1. Analysis of the capacity of MVA-primed NK cells to control HIV-1 infection in autologous DC and $CD4^+$ T cells. NK cells were cultured with non-infected DC, MVA_{HIV}- or MVA_{WT}-infected DC during 4 days (white, black or grey bars, respectively). Then, NK cells were transferred to a culture of HIV-1-infected autologous DC or $CD4^+$ T cells. (A) Graph represents cumulative results from 8 independent experiments, showing the percentage of $p24^+$ DC in culture with primed NK cells at day 7 post-infection (p.i.). (B) Graph represents cumulative results from 10 independent experiments, showing the percentage of $p24^+$ $CD4^+$ T cells in culture with primed NK cells at day 10 p.i. Results are expressed as mean ± SE and p values are shown (Wilcoxon matched-pairs test).

2 MVA_{HIV} priming of NK cells seems to be anti-HIV specific

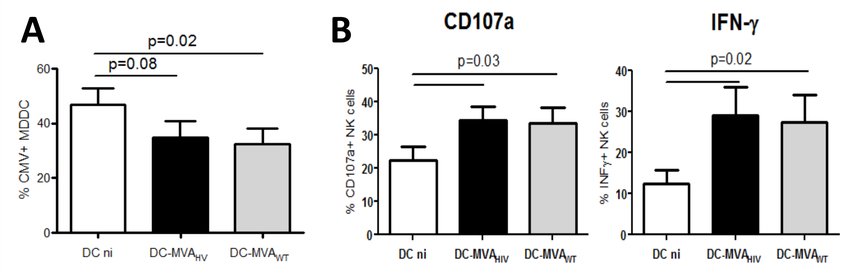

Figure 2. Anti-viral and anti-tumoral activity of MVA-primed NK cells. NK cells were cultured with non-infected DC, MVA_{HIV}- or MVA_{WT}-infected DC during 4 days (white, black or grey bars, respectively). Then, NK cells were transferred to a culture of CMV-infected autologous DC or tumoral K562 cell line. (A) Graph represents cumulative results from 10 independent experiments, showing the percentage of CMV^+ DC in culture with primed NK cells at day 6 post-infection (p.i.). (B) Graph represents cumulative results from 6 independent experiments, showing the percentage of degranulation (CD107a expression) and IFN-γ production by primed NK cells in culture with tumoral K562 cell line, after 4 hours of culture. Results are expressed as mean ± SE and p values are shown (Wilcoxon matched-pairs test).

3 Increased early NK cell response against MVA_{HIV}-infected DC

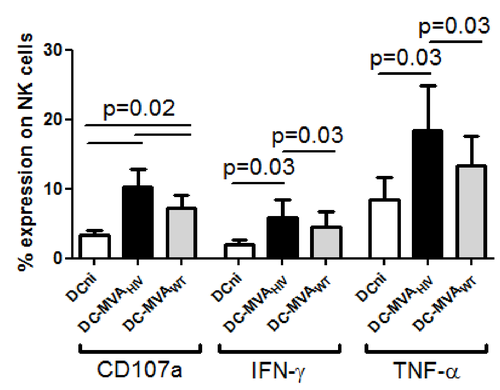

Figure 3. Early NK cell degranulation and cytokine production against MVA-infected DC. Resting NK cells were cultured with non-infected DC, MVA_{HIV}- or MVA_{WT}-infected DC for 4 hours (white, black or grey bars, respectively). Then, the surface expression of CD107a and the intracellular production of IFN-γ and TNF-α was assessed. Graph represents cumulative results from 7 independent experiments, showing the percentage of NK cells expressing CD107a, IFN-γ or TNF-α. Results are expressed as mean ± SE and p values are shown (Wilcoxon matched-pairs test).

4 Priming of NK cells by MVA_{HIV} is modulated by NKG2D and NKp46

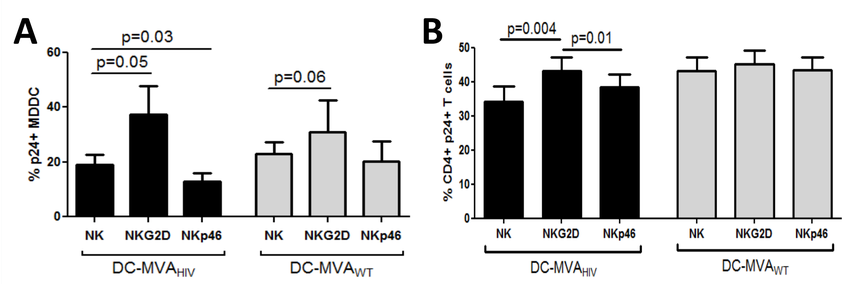

Figure 4. Analysis of the implication of NKG2D and NKp46 during the MVA-priming. NK cells were cultured with MVA_{HIV}- or MVA_{WT}-infected DC during 4 days (black or grey bars, respectively) in the presence or not of blocking mAbs against NKG2D or NKp46. Then, NK cells were transferred to a culture of HIV-1-infected autologous DC or $CD4^+$ T cells. (A) Graph shows the percentage of $p24^+$ DC cells in culture with primed NK cells, at day 7 p.i. Cumulative results from 8 independent experiments are shown. (B) Graph shows the percentage of $p24^+$ $CD4^+$ T cells in culture with primed NK cells, at day 9 p.i. Cumulative results from 10 independent experiments are shown. Results are expressed as mean ± SE and p values are shown (Wilcoxon matched-pairs test).

5 NKG2D blockade during MVA priming decreases mbIL-15 expression on DC

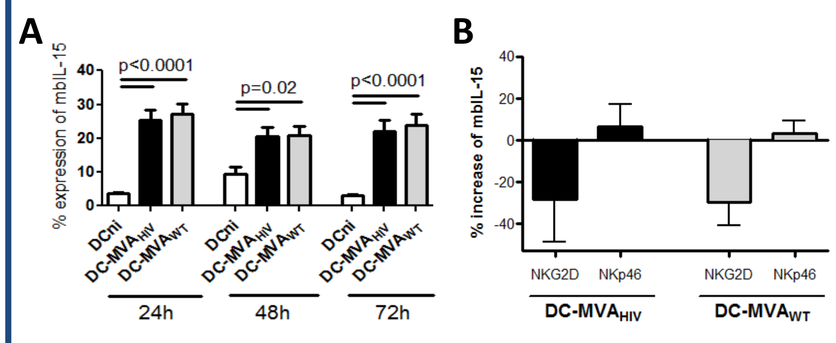

Figure 5. Expression of membrane bound IL-15 (mbIL-15) on MVA-infected DC and its modulation by NKG2D or NKp46 during the priming of NK cells. (A) Graph shows the percentage expression of mbIL-15 on non-infected, MVA_{HIV}- or MVA_{WT}-infected DC at 24, 48 and 72 hours of the co-culture. Cumulative results from 10 independent experiments are shown. Results are expressed as mean ± SE and p values are shown (Wilcoxon matched-pairs test). (B) Graph shows the percentage increase in the expression of mbIL-15 on MVA_{HIV}- or MVA_{WT}-infected DC at 72 hours of the co-culture in the presence or not of blocking mAbs against NKG2D or NKp46. Cumulative results from 5 independent experiments are shown.

6 IL-15 is important for the MVA_{HIV} priming

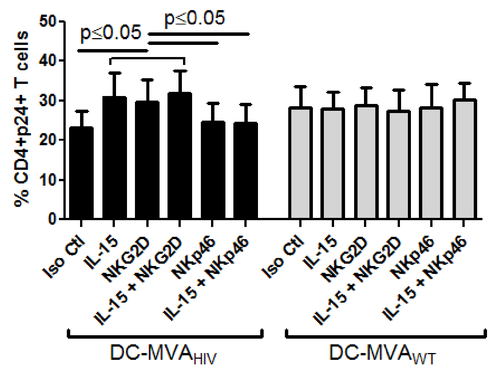

Figure 6. Implication of IL-15, NKG2D and NKp46 in the MVA_{HIV}-priming of NK cells. NK cells were cultured with MVA_{HIV}- or MVA_{WT}-infected DC during 4 days (black or grey bars, respectively) in the presence or not of blocking mAbs against IL-15, NKG2D, NKG2D/IL-15, NKp46 or NKp46/IL-15. Then, NK cells were transferred to a culture of HIV-1-infected autologous $CD4^+$ T cells. Graph shows the percentage of $p24^+$ $CD4^+$ T cells in culture with primed NK cells, at day 9 p.i. Cumulative results from 8 independent experiments are shown. Results are expressed as mean ± SE and p values are shown (Wilcoxon matched-pairs test).

Conclusions

NK cells primed by MVA_{HIV} have are able to better control of HIV infection in autologous DC and $CD4^+$ T cells. It is possible that NK cells select correctly HIV antigen loaded DC during the MVA_{HIV}-priming allowing for a specific stimulation. NKp46 blockade during MVA_{HIV} priming increases NK cell control of HIV infection in DC, whereas blockade of NKG2D decreases NK cell control of HIV infection in DC and $CD4^+$ T cells. NKG2D blockade decreases the expression of mbIL-15, and as IL-15 is important only for MVA_{HIV}-priming, the decreased expression of mbIL-15 after blockade of NKG2D might be responsible, at least in part, for the reduced HIV control.

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