Poster n°
VI-PO18-1

Macropinocytosis of human papillomaviruses in natural killer cells via CD16 induces cytotoxic granule exocytosis and cytokine secretion.

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Persistent infection with oncogenic human papillomavirus (HPV) genotypes is a necessary cause of anogenital cancer and HPV infections account for more than 50% of infection-linked cancers in women worldwide. The immune system controls, at least partially, viral infection and subsequent tumor development. Around 90% of HPV-infected women will clear the virus within two years. However, it remains unclear which immune cells are implicated in this process and no study has been performed evaluating the direct interaction between HPV and NK cells although these cells play a key role in host resistance to virus and tumor. Since HPV cannot grow in vitro, virus-like particles (VLP) were used as a model for studying the NK cell response against the virus. Interestingly, NK cells displayed a higher cytotoxic activity and cytokine production (TNF-α and IFN-γ) in the presence of VLP. Uptake of VLP by dendritic cells (DC) has been shown to induce their activation, therefore, we investigated whether the stimulation of NK cell activity is linked to VLP internalization. We observed a faster entry into these cells compared to DC. Furthermore, virus uptake by NK cells is mediated by macropinocytosis, whereas this entry is dependent of clathrin or caveolin endocytosis pathways in DC. Using NK cell lines expressing or not CD16 and blocking antibody, we demonstrated that CD16 is necessary for HPV-VLP internalization, but also for degranulation and cytokine production. Moreover, we observed a phosphorylation of Erk and p38, two MAP Kinases (MAPK) involved in NK cell cytotoxic activity and with specific inhibitors, we demonstrated that these MAPK are implicated in NK cell degranulation against VLP.

1. VLP induce cytotoxic activity and cytokine release by NK cells

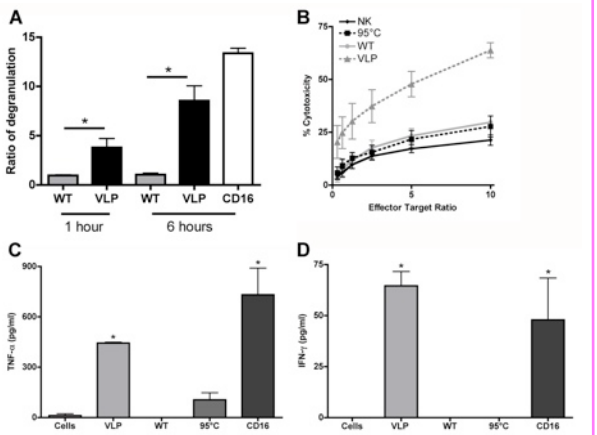

Fig 1: (A) Ratio of degranulation of NK cells in the presence of VLP, anti-CD16 mAb, a lysate of insect cells infected with WT baculovirus (WT) after 1h and 6 h of incubation (means ± SE, n>3). (B) NK cells cytotoxic activity against CasKi cell line in 10 h ^{51}Cr release assay (n=4), (C) TNF-α and (D) IFN-γ ELISA assays (means ± SE, n≥3, *p<0.05).

2. Rapid HPV-VLP internalization in CD16+ NK cells

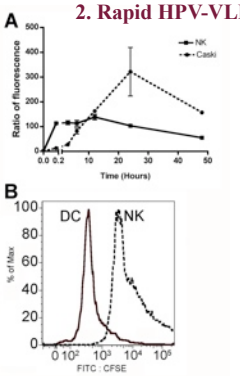

Fig 2.1: VLP were coupled with CFSE and the fluorescence is emitted after VLP entry by cleavage of the CFSE by intracellular serine esterases. (A) Entry kinetic in NK and CasKi cells. (B) Comparison of VLP entry after 10 min in NK and DC from the same donor.

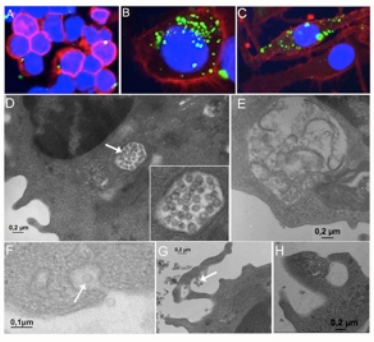

Fig 2.2: Confocal and electron microscopy of (A,D-E, G-H) NK cells (B) CasKi and (C, F) DC, arrow = VLP

3. VLP uptake in NK cells is mediated by macropinocytosis

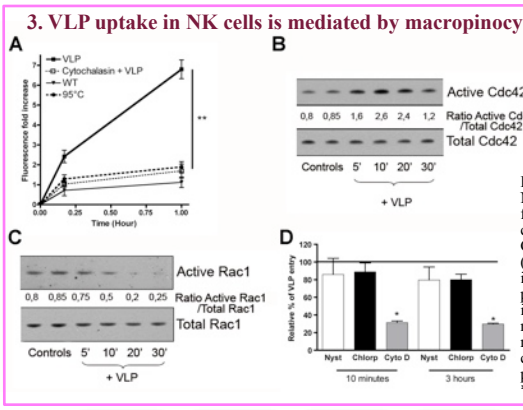

Fig 3.1: (A) FITC-Dextran uptake in NK cells (means ± SE of fluorescence fold increase over the control condition, n=3). (B-C) GTPase assays of (B) Cdc42 and of (C) Rac1 on NK cells (1 of 3 independent experiments) (D) The percentages of VLP entry inhibition in presence of chlorpromazine, nystatine and cytochalasin, respectively inhibitor of clathrine, caveola or macropinocytosis pathways (means±SE, n=6-9; *p<0.05, **p<0.005)

4. CD16 is required for rapid VLP uptake and NK cell activation

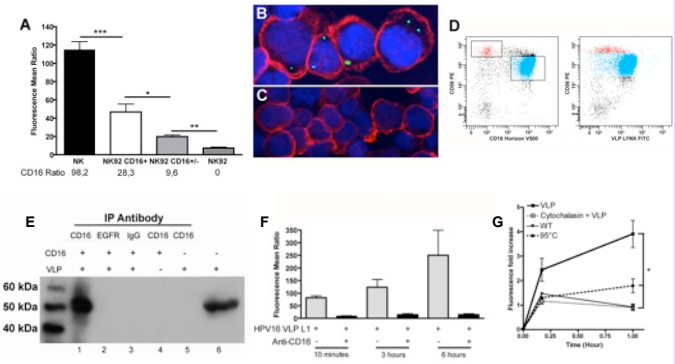

Fig 4.1: (A) Entry of CFSE-VLP in NK92 expressing or not CD16 (means ± SE, n≤3). (B-C) Confocal microscopy of CFSE-VLP entry after 10 min of incubation into (B) the NK92 CD16+ or into (C) the NK92 CD16- cell line. (D) Lynx-VLP internalization into CD56$^{+bright}$ CD16^{-} NK cells compared to CD56^{+dim} CD16^{+} NK cells. (E) Co-immunoprecipitation between CD16 and VLP. (F) Lynx-VLP internalization into NK cells with or without pre-incubation with an anti-CD16 antibody (n=4). (G) FITC-Dextran uptake in NK92 CD16+ cells (means ± SE of fluorescence fold increase over the control condition, n=3).

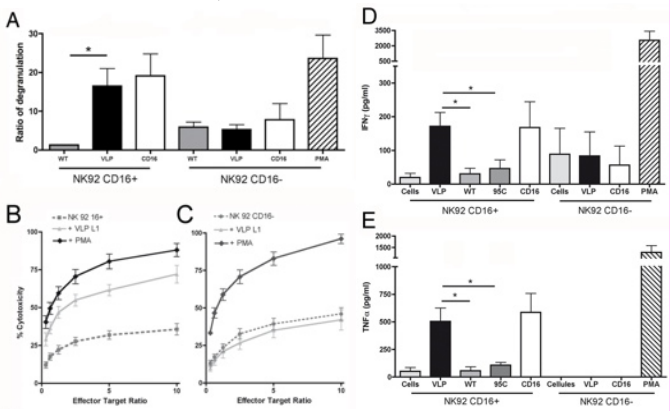

Fig 4.2: (A) Ratio of degranulation in NK92 CD16+ and NK92 CD16- cells (means±SE, n>3), (B-C) NK92 CD16+ cells cytotoxic activity against CasKi cell line in a 10 h ^{51}Cr release assay (n=4), (D) TNF-α and (E) IFN-γ ELISA assays on supernatant of NK92 CD16+ and CD16- cells (means ± SE, n=4; *p<0.05).

5. Phosphorylation of MAP Kinases in the presence of VLP

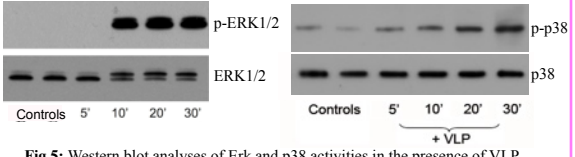

Fig 5: Western blot analyses of Erk and p38 activities in the presence of VLP

Conclusions

- NK cell infiltration in HPV-associated lesions (data not shown)
- VLP induce cytotoxic activity and cytokine secretion by CD16+ NK cells.
- CD16 is necessary for VLP entry by macropinocytosis and NK activation.
- VLP induce MAP kinase phosphorylation in NK cells which is involved in NK cell degranulation (data not shown)

→ NK cells interact with HPV and could participate in the immune response against HPV-induced tumors.