Human papillomavirus entry into NK cells requires CD16 expression and triggers cytotoxic activity 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) composed of L1 or L1L2 capsid proteins, were used as a model for studying the NK cell response against the virus. Interestingly, a fast entry is observed into NK cells compared to DC (Fig1). Furthermore, virus uptake by NK cells is mediated by macropinocytosis, whereas this entry is dependent of clathrin or caveolin endocytosis pathways in DC (Fig1-2). We investigated whether the internalization of VLP is linked to NK cell activity and a higher cytotoxic activity and cytokine production (TNF-α and IFN-γ) is observed in the presence of HPV-VLP (Fig3). Using NK cell lines expressing or not CD16 (generous gift of B. Clémenceau, France) and blocking antibody, we demonstrated that CD16 is necessary for HPV-VLP internalization, but also for degranulation and cytokine production (Fig4).

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

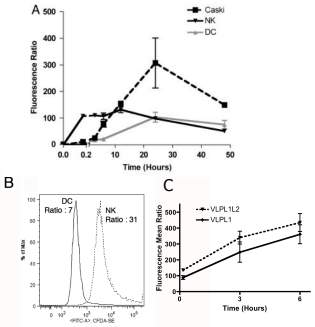

Fig 1.1: VLP were coupled with CFSE and the fluorescence is emitted after VLP entry by cleavage of the CFSE by intracellular serine esterases. (A) VLPL1 entry kinetic in NK and CasKi cells. (B) Comparison of VLP entry after 10 min in NK and DC from the same donor. (C) VLPL1 and L1L2 entry kinetic in NK cells

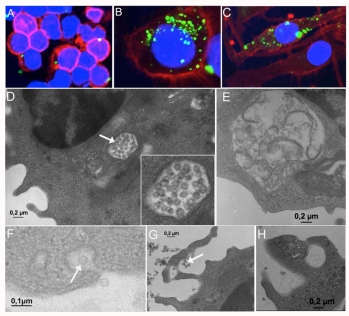

Fig 1.2: Confocal and electron microscopy of (A,D-E,G-H) NK cells (B) CasKi and (C, F) DC, arrows = HPV16-VLP

4. CD16 is required for rapid HPV16-VLP uptake and NK cell activation

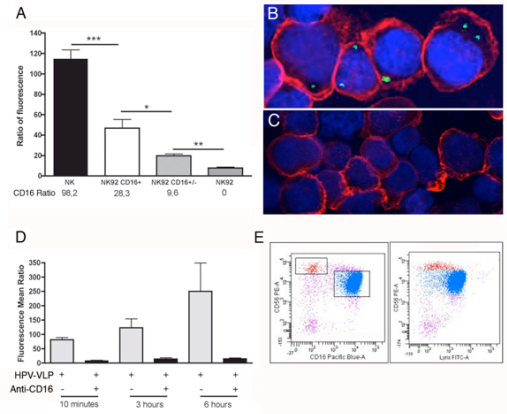

Fig 4.1: (A) Entry of CFSE HPV-VLP in NK92 CD16- (no CD16 expression), NK92 CD16+/-, NK92 CD16+ (means + SE, n ≤ 3). (B-C) Confocal microscopy of CFSE HPV-VLP entry after 10 min of incubation into (B) the NK92 CD16+ or into (C) the NK92 CD16- cell line. (D) Lynx HPV-VLP internalization into NK cells with or without pre-incubation with an anti-CD16 antibody (n ≤ 3). (E) Lynx HPV16-VLP internalization into $CD56^{+bright}$ $CD16^{-}$ NK cells compared to $CD56^{+dim}$ $CD16^{+}$ NK cells.

2. HPV-VLP uptake in NK cells is mediated by macropinocytosis

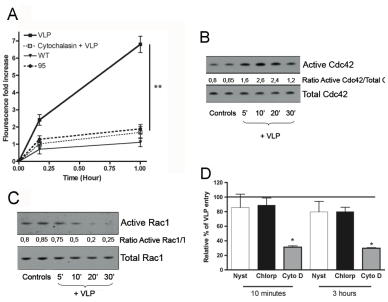

Fig 2: (A) FITC-Dextran uptake in NK cells (means ± SE of fluorescence fold increase over the control condition, n=3). (B-C) GTPase assays of (B) Cdc42 and of (C) Rac1 on NK cells (1 of 3 independent experiments) (D) The percentages of VLP entry inhibition in presence of chlorpromazine, nystatine and cytochalasin, respectively inhibitor of clathrin, caveolaor and macropinocytosis pathways (means + SE, n = 6-9; * p<0.05, ** p<0.005)

3. HPV-VLP induce cytotoxic activity and cytokine release by NK cells

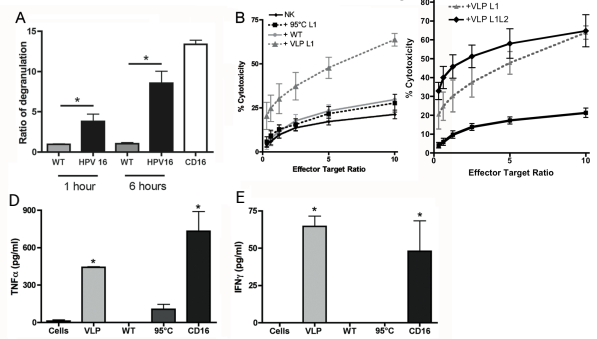

Fig 3: (A) Ratio of degranulation in NK cells in the presence of HPV16-VLP, anti-CD16 mAb, a lysate of insect cells infected with WT baculovirus (WT) after 1h or 6 h of incubation (means + SE, n>3). (B-C) NK cells cytotoxic against CasKi cell line in 10 h ^{51}Cr release assay (n = 2-4), (D) TNF-α and (E) IFN-γ ELISA assays (means + SE, n≥3, * p<0.05).

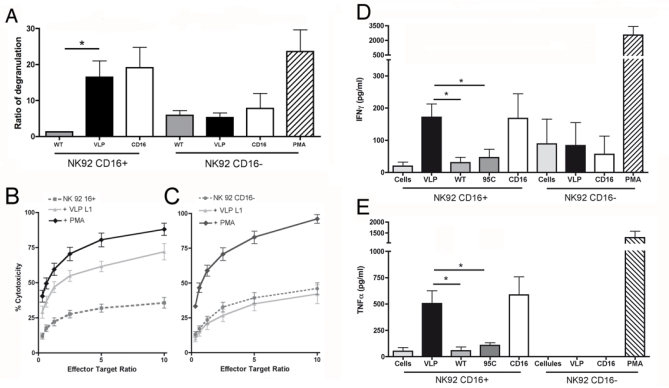

Fig 4.2: (A) Ratio of degranulation in NK92 CD16+ and NK92 CD16- cells (means + SE, n>3), (B-C) NK92 CD16+ cells cytotoxic activity against CasKi cell line in a 10 h ^{51}Cr release assay (n = 4), (D) TNF-α and (E) IFN-γ ELISA assays on supernant of NK92 CD16+ and CD16- cells (means + SE, n = 4; * p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

Conclusions

- NK cell infiltration in HPV-associated lesions (data not shown)
- HPV-VLP entry induces cytotoxic activity and cytokine secretion by CD16+ NK cells.
- CD16 is necessary for HPV-VLP entry by macropinocytosis and NK activation.

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

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