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-related 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, but also for degranulation and cytokine production. Moreover, we observed a phosphorylation of Erk and p38, two MAP Kinases (MAPK) involved in pathways (means ± SE, n=3; *p<0.05).

Fig 1: (A) Ratio of degranulation of NK cells in the presence of VLP, anti-CD16 mAb, a lysis 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 [51Cr] release assay (n=4), (C) TNF-α and (D) IFN-γ ELISA assays (means ± SE, n=3, *p<0.05).

Fig 2: Confocal and electron microscopy of (A-D, F, G) NK cells (B) Caski and (C, E) DC, arrow = VLP entry after 10 min in NK and DC from the same donor.

Fig 3: 1. VLP uptake in NK cells is mediated by macropinocytosis.

Fig 4: 1. VLP induce cytotoxic activity and cytokine release by NK cells

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 CD56high CD16+ NK cells compared to CD56low CD16- NK cells. (E) Cytotoxic activity of NK92 CD16+ cells (means ± SE of 10 h [51Cr] release assay (n=4), (F) 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 [51Cr] release assay, (D) TNF-α and (E) IFN-γ ELISA assays on supernatant of NK92 CD16+ and CD16- cells (means ± SE of 4 h, *p<0.05).

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