

EX VIVO ANALYSIS OF SPECIFIC MEMORY CD4⁺ T CELL RESPONSES AFTER HPV VACCINATION WITH GARDASIL[®]

Ramseger AK¹, Glastetter E¹, Seipel M¹, Kastner U¹, Waterboer T², Pawlita M², Schneider A¹, Kaufmann AM¹

¹Klinik für Gynäkologie, Charité – Universitätsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany

²German Cancer Research Center (DKFZ), Research Program Infection and Cancer, Heidelberg, Germany

Background

The virus-like particle (VLP)-based prophylactic vaccine Gardasil[®] provides protection against infection with the human papillomavirus types 6, 11, 16 & 18.

After i.m. injection at day 0, month 2 & month 6 it leads to a 100% protection against HPV 16 & 18 induced CIN2+ and to a 98% protection against HPV 6 & 11 associated genital warts (1).

The vaccine is highly immunogenic. At month 7 antibody titres are around 100 times higher compared to titres after natural HPV infections, though antibody concentrations decrease in the first years after vaccination (2).

Until now no direct monitoring of cellular immune responses has been described. T helper cell responses in particular are important for B cell activation, antibody class switch and for memory, recall and anamnestic immune responses (Fig. 1).

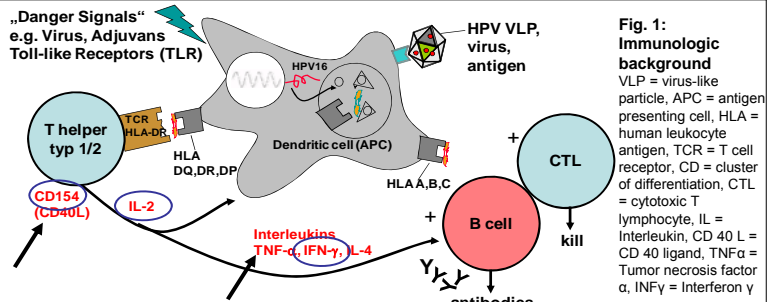


Fig. 1: Immunologic background
VLP = virus-like particle, APC = antigen presenting cell, HLA = human leukocyte antigen, TCR = T cell receptor, CD = cluster of differentiation, CTL = cytotoxic T lymphocyte, IL = Interleukin, CD 40 L = CD 40 ligand, TNF α = Tumor necrosis factor α , IFN γ = Interferon γ

1. Is it possible to detect HPV L1-specific CD4⁺ T cells *ex vivo*?
2. Is there an increase of specific T cells during the course of vaccination?
3. Are there differences between the 4 HPV types?

1. Is it possible to detect HPV L1-specific CD4⁺ T cells *ex vivo*?
2. Is there an increase of specific T cells during the course of vaccination?
3. Are there correlations between specific antibodies and T cells?

Methods

In a cross sectional study 63 subjects were included according to their vaccination status (Fig. 2). In addition, 11 subjects of the once vaccinated group were followed up during the course of vaccination as a confirmatory longitudinal study. Whole blood was stimulated *ex vivo* with different HPV L1-peptide pools for 14 to 20h. Antigen-specific T helper cells were identified by intracellular staining for CD4, CD154, IL-2 and IFN γ and analysed by flow cytometry (Fig. 3). Plasma was analysed for the presence of HPV L1-specific antibodies by multiplexed human papillomavirus serology based on *in situ*-purified glutathione S-transferase fusion proteins.

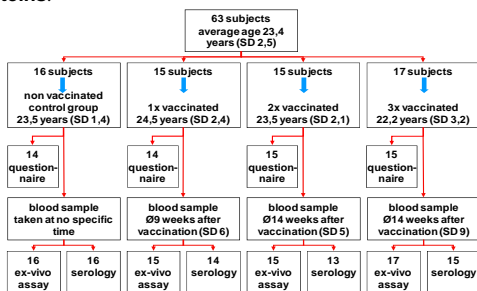


Fig. 2: Study design. SD = standard deviation

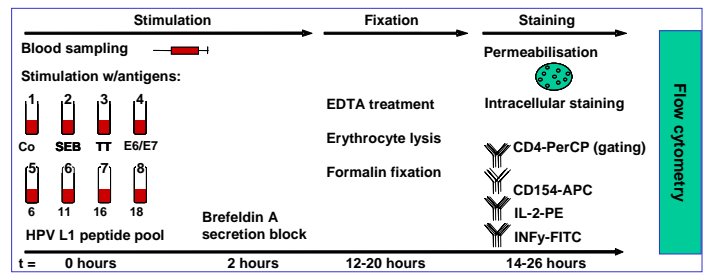


Fig. 3: Ex vivo T cell assay (based on Frensch et al. 2005 Nat Med 11:1118). Co = control, SEB = staphylococcal enterotoxin B, TT = Tetanustoxoid, E = early protein, L = late protein, EDTA = ethylenediaminetetraacetic acid, CD = cluster of differentiation, IL = Interleukin, INF γ = Interferon γ , PerCP = Peridinin Chlorophyll Protein, APC = Allophycocyanin, PE = Phycoerythrin, FITC = Fluoresceinisothiocyanate

Results

After the first vaccination a significant number of specific memory and T_H1 cells can be detected *ex vivo*. The second dose leads to a highly significant increase in the specific T cell response. After the third vaccination T cell frequencies do not increase significantly (Fig. 4). Low-risk HPV VLP induce higher specific T cell frequencies than high-risk HPV 16 and, in particular, HPV 18 VLP. HPV L1-specific antibodies continuously increase during the course of vaccination (Fig. 5). The antibody titres of HPV 6, 11 & 16 are comparable, HPV 18 VLP induce lower antibody concentrations. There is no direct correlation between Gardasil[®]-specific CD4⁺ T cells and antibodies (not shown). In general both the cross sectional and the longitudinal study show consistent results. During follow-up we observed a highly individualised development of the HPV-L1 specific T cell response (Fig. 6).

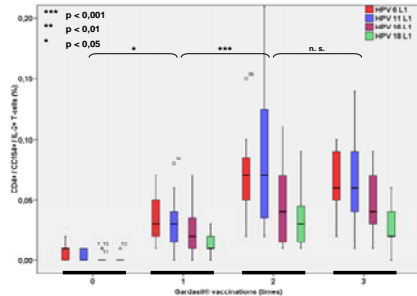


Fig. 4: HPV L1-specific, IL-2⁺ CD4⁺ T cells according to vaccination status and HPV type. CD = cluster of differentiation, IL = Interleukin, n. s. = non significant, HPV = Human Papillomavirus, L = late protein

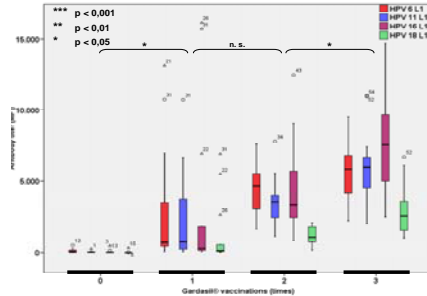


Fig. 5: Anti-HPV L1 antibody titers according to vaccination status and HPV type. MFI = median fluorescence intensity, n. s. = non significant, HPV = Human Papillomavirus, L = late protein

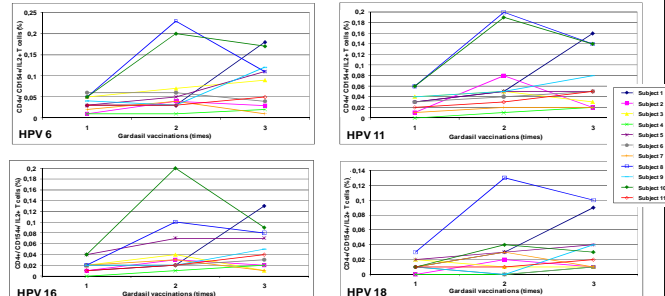


Fig. 6: Individual T cell response of 11 subjects during the course of vaccination. CD = cluster of differentiation, IL = Interleukin, HPV = Human Papillomavirus, L = late protein

Conclusions

We established successfully a feasible *ex vivo* method to monitor HPV antigen-specific CD4⁺ T cells from whole blood after prophylactic HPV vaccination.

The detection of IL-2⁺ memory T cells could be a hint for a possible long term effect of the vaccine Gardasil[®].

Follow-up projects should investigate vaccine-specific T cell responses several years after the last immunisation and vaccine-specific T_H2 response.

References

1. The FUTURE II study group. 2007, N Engl J Med 356(19):1915-27.
2. Villa LL et al. 2006, Vaccine 24(27-28):5571-83.