Characterization of ANRS HIV-LIPO-5 vaccine in healthy volunteers combining cytokine multiplex and transcriptomic analyses

Sophie Hue1, Laura Richert2, Hakim Hocini3, Mathieu Surenaud1, Pascaline Tisserand1, Christine Lacabaratz1, Dominique Salmon2, Mathieu Raimbault2, Jean-Daniel Lelièvre1, Benoit Lique2, Yves Lévy1 and Rodolphe Thibaut4, on behalf of the ANRS HIV Vaccine Network/Vaccine Research Institute

1 Université Paris Est, Créteil - Hôpital Henri Mondor, Créteil, France; 2 INSERM U989, Bordeaux, France; and 3 Université Paris Descartes - Hôpital Cochin, Paris, France

Revised abstract

**Background**

The ANRS HIV-LIPO-5 vaccine is a lipopeptide vaccine including 5 HIV-1 peptides (Gag17-35, Gag355-364, Pol152-159, Pol636-647 and Nef113-124) coupled at a palmitoyl tail administered at weeks 0, 1, 2 and 12 (2.0 mL/vaccination). 84-93% of vaccinated developed HIV-specific CDR-3 (Conserved Domain Region-3) of PBMC cultured IFN-γ-stimulated peptide. Here, we measured cytokines and gene expression profiles associated with vaccine response.

**Methods**

PBMC from 12-15 volunteers were stimulated with either LIPO-5 vaccine, or a pool of 15-mers flag peptides. Transcriptomic (Affymetrix and cytokine analysis) (Milliplex) profiles were performed at 0, 1, 2, and 12 weeks, respectively. Statistical analyses (Wilcoxon for paired samples) were performed on cytokines and the transcriptome, using selected transcripts, and signature validation was performed using Fisher’s exact test.

**Results**

The ANRS HIV-LIPO-5 vaccine is a lipopeptide vaccine including 5 HIV-1 peptides (Gag17-35, Gag355-364, Pol152-159, Pol636-647 and Nef113-124) coupled at a palmitoyl tail.

The ANRS VAC38 trial was a phase IIa randomised multicenter trial conducted in France to evaluate the safety and immunogenicity of this vaccine in healthy volunteers. 160 healthy volunteers were randomised to receive either four injections of different doses of LIPO-5 vaccine (10 μg or placebo) at weeks 0, 1, 2, and 12. Depending on the dose of HIV-LIPO-5 vaccine, sustained (at least at 2 time points) HIV-specific CD8+ response frequencies was observed in HIV-1 Esopitox assays in 69-69% of participants, and persisted at week 48 in 52% of participants. Cumulative (up to week 26) HIV-specific CD4+ lymphoproliferation was detected in 44-55% of participants [1].

Luminex cytokine staining in a randomly selected subset of participants (n=80) showed that HIV-LIPO-5 vaccine elicited both ex vivo HIV-specific CD4+ and CD8+ T cell responses producing mainly IL-2 in 30-50% of participants. The majority of responses were directed against Gag sequences [2].

The aim of the present study was to assess cytokine secretion and gene expression profiles after PBMC stimulation with either pools of 15-mers overlapping Gag peptides or HIV-LIPO-5 itself.

**Conclusion**

Luminex analyses showed significant changes in the secretion of IFNγ, IL-10, IL-13 and TNFα after vaccination with HIV-LIPO-5 (Figure 1). No differences in cytokine secretion at any time point were detected for IL-12, IL-17, and IL-21. An unexpected response to HIV-LIPO-5 stimulation was detected in PBMC before vaccination compared to unstimulated PBMC (IFNγ and IL-10, see Table 1). As expected, no responses were observed after stimulation with gag peptides not included in the HIV-LIPO-5 vaccine.

Analysis of cytokine concentration (expressed in pg/ml) instead of fluorescence intensity resulted in the same trends (Figure 2 and Table 1).

In transcriptomic analyses after vaccination, expression of 2785 probes (2858 genes) and 1335 probes (1226 genes) was significantly increased after 6 and 24 hours of HIV-LIPO-5 stimulation, respectively, compared to unstimulated controls (Tables 2 and 3). Among these genes, expression of IFNγ, CXCL9, CXCL10, IL-12a, TNFαP6, CCL3L1, and IL-6 increased considerably (false change <2). In accordance with the Luminex results, a significant variation of gene transcripts (n=106 probes, 102 genes) was observed after 6-hour HIV-LIPO-5 stimulation before vaccination.

**Conclusions**

In healthy volunteers vaccinated with HIV-LIPO-5, stimulation of PBMC by HIV-LIPO-5 itself or by a pool of 15-mers Gag peptides belonging to the HIV-LIPO-5 sequence resulted in an increased secretion of Th1 (IFNγ, TNFα) and Th2 (IL-5, IL-13) -related cytokines. An unspecific cytokine response was detected in PBMC stimulated by HIV-LIPO-5 before vaccination possibly due to the adjuvant effect of the lipid tail of the vaccine.

HIV-LIPO-5 stimulation of PBMC led also to an expansion of gene transcription after 6 and 24 hours, to a small extent before vaccination and at a much higher level after vaccination in particular, expression of IFNγ, CXCL9, CXCL10, IL-12a, TNFalpha, CCL3L1, and IL-6 genes was enhanced after stimulation by HIV-LIPO-5.

The combined approach of transcriptomics and multiplex analyses might help to identify new signatures associated with HIV vaccine responses.

**References**

1. Adamson CH et al. AIDS 2020; 34:12:2311-23
2. Lacabaratz et al. AIDS 2003; 17:1314-21

Acknowledgements

The authors wish to thank all the volunteers included in the trial and all investigators and members of the ANRS VAC38 Trial Management Team, Ancestry Committee and Independent Data Monitoring Committee.

Participants: Participants: Paris (Cochon-D Villeneuve, O Lionetti), Rennes (Gue Curi), Toulouse (D-Bonnaire), Tumé (P. Pichard), Montpellier (M. Lefèvre), Marseille (S. Portman Marre)

Supported by: LIPO-5: GAG+ and Nef+ vaccine

NIH/Fogarty: provided HIV-LIPO-5 vaccine

This study was supported in part by ANRS. The HIV-LIPO-5 vaccine was provided by Small Pharma L. Hubert receives a PhD grant financed by Sidaction.