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HIV envelop proteins variability analysis and vaccine components tailoring

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Background: The great genetic variability of HIV particle-coating proteins was an overwhelming obstacle in the anti-HIV vaccine development. Library approach in isolation and mass spectrometry quantitative HIV envelop proteins analysis and primers mapping, a tailoring of an immunogenic composition gp120-gp160 infection-active envelop protein complexes and it's in vivo Hu-SCID-mice HIV-specific immune response are presented.

Methods: PEG-precipitation (stage 1), ultracentrifugation 160000g during 45 minutes through sucrose gradient with density 1,16-1,18 g/sm^3(2), HIV-1-gp160-specific phage-presented libraries and affine chromatography with native human polyclonal antibodies(3), SDS-PAGE, tryptic protein's cleavage, consequently, were used for viral proteins concentration. One-demensional LC-MS-MS Esquire-6000Plus (Bruker Daltonics) peptide's identification and PEAKS (Canada) software complexes sequences analysis were performed for envelop mapping-arranged primers that were assembled comprising V1-V5 loops beginning variability as forward and C5 end as reverse. The pools of original PCR products supplied with 6His insertions were tailored into pQE-80E, pcDNA4/HisMaxC and pLEXSY_I-neo2 vectors and transformed into transient IPTG-inducible TG1 e.coli host, Chinese hamster CHO-K1 culture host and secretory tet-inducible expression Leishmania tarentolae T7-TR host (400mV electroporation, BTX-830, Harward Instruments). Env proteins were isolated with ultrafiltration through 30 kDa membrane (Pelicon BIOMAX) and LC with 20ml HisTrap (GE Healthcare) or immobilized Ni-NTA agarose (Superose12, GE Healthcare). Phase contrast light microscopy (Nikon Eclipse2000U) and SPM contact mode (NanoWizard, JPK instruments) were run for Leishmania's visualization, SPM spectroscopy mode – for glycosylation-molecular weight analysis. Immunogenicity of recombinant gp120-gp160 cocktails was evaluated in Hu-SCID mice engrafted with single donor PBMC (peripheral blood mononuclear cells) and with DC (dendrite cells) immunocompetent cells.

Results and Conclusions: gp120 and gp160 isolation in sufficient for mass spectrometry identification amount was possible only using all three (1-3) stages in envelop proteins concentration. SDS-PAGE bands for trypsinolysis brought significantly more clear results for MS-MS env identifications than total LC concentration products. PEAKS complex was successfully used for env mapping and primers creation. The original PCR products were obtained using V1-V5-C5 primers sets and identified between for-rev ends loop, from V1 to V5, respectively. HIV env PCR products tailored into TG1 host could not provide most of envelop proteins expression due to prokaryotic codon deficiency for HIV env aminoacid sequence. PCR products transfected into CHO-K1 cells provided gp120 expression sufficient for Western blot analysis. Gp120-gp160 expressed in and extracted in/from suitable eukaryotic host LEXSY T7-TR provided normal for HIV proteins glycosylation level and HIV-specific immune response in Hu-SCID-PBMC mice. The immunogenic composition consisted of a number of recombinant HIV-1 envelop peptides/proteins in variability with length for gp120 representatives from 1323 to 1623 bp (441-541 aminoacids) and glycosylated protein molecular weight 90-115kDa and with length for gp160 representatives from 2448 to 2769 bp (816-923 aminoacids, respectively) and glycosylated protein molecular weight 145-175kDa. Three times subcutaneously immunized Hu-SCID-PBMC mice showed stable for 2 weeks p120-p160-specific immune response comparable in value to healthy BalbC mice immunogenicity to the same compositions immunizing. Hu-SCID-DC animals p120-p160-specific immune response was 40-70 percent lower in value than Hu-SCID-PBMC mice immunogenicity to the same compositions.