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Effect of HIV-1 envelope cytoplasmic tail on adenovirus primed virus encoded virus-like particle immunizations

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ABSTRACT

The low number of envelope (Env) spikes presented on native HIV-1 particles is a major impediment for HIV-1 prophylactic vaccine development. We designed virus-like particle encoding adenoviral vectors utilizing SIVmac239 Gag as an anchor for full length and truncated HIV-1 M consensus Env. Truncated Env overexpressed VRC01 and 17b binding antigen on the surface of transduced cells while the full length Env vaccine presented more and similar amounts of antigen binding to the trimer conformation sensitive antibodies PGT151 and PGT145, respectively. The adenoviral vectors were used to prime Balb/c mice followed by sequential boosting with chimpanzee type 63, and chimpanzee type 3 adenoviral vectors encoding SIVmac239 Gag and full length consensus Env. Both vaccine regimens induced increasing titers of binding antibody responses after each immunization, and significant differences in immune responses between the two groups were observed after the final immunization. Full length Env priming skewed antibody responses towards gp41, while truncated Env priming induced responses primarily targeting gp120 containing and derived antigens. Importantly, no differences in neutralizing antibody responses were found between the different priming regimens as both induced high titered tier 1 neutralizing antibodies, but no tier 2 antibodies, possibly reflecting the similar presentation of trimer specific antibody epitopes. The described vaccine regimens provide insight into the effects of the HIV-1 Env cytoplasmic tail on epitope presentation and subsequent immune responses, which is relevant for the interpretation of current clinical trials that are using truncated Env as an immunogen. The regimens described here provide similar neutralization titers, and thus are useful for investigating the importance of specificity in non-neutralizing antibody mediated protection against viral challenge.

1. Introduction

The envelope glycoprotein (Env) is the major focus of antibody-inducing prophylactic vaccine development against HIV, and has proven to be an exceptionally tough target [1]. Functional virion associated Env is folded as a compact trimer and contains heavily glycosylated surfaces that partially obstruct access to the trimer [2,3]. Epitopes that are exposed frequently display significant sequence variation and are termed variable loops (V1–V5). However, Env contains a number of structurally conserved and exposed regions that are vulnerable to broadly neutralizing antibodies [4,5], antibody dependent cellular cytotoxicity (ADCC) mediating antibodies, and have been described as correlates of risk in the RV144 vaccine trial [6].

We focused on virus-like particles (VLPs) encoded within viral vectors based on the antibody response elicited in the RV144 trial (canarypox based vaccine vector prime [7]), and the clinical development of VLP based vaccines delivered by modified vaccinia Ankara vectors (MVA) [8]. Viral vector encoded VLPs simultaneously provide the ability to present an antigen oligomerized in a VLP context in addition to the display of Env on the cell surface of vaccine transduced cells, and in this manner stimulate antibody responses that could exhibit ADCC activity. Early studies using encoded Env faced problems with genetic instability of the full

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length cytoplasmic tail in MVA vectors, but it was observed that truncation of the cytoplasmic tail improved poxviral replication and transgene expression [9]. Consequently, clinical development has progressed using an Env sequence with a truncated tail in the MVA vectors and a full length tail in an optional DNA prime [8], but testing the importance of the cytoplasmic tail in a side-by-side experiment has not been possible with MVA vectors. Adenoviral vectors with expression cassettes including a tetracycline operator in proximity to the antigen promoter have recently been developed [10], and now allow us to explore the unique features of the HIV-1 Env cytoplasmic tail. The HIV-1 Env cytoplasmic tail is not believed to be targeted by protective antibodies, but has been recognized as a determinant of the quantity of Env incorporated into HIV-1 VLPs [11]. Further interest in the cytoplasmic tail has been instigated by the recent publication by Chen et al. [12], which indicated the importance of the full length cytoplasmic tail for occluding induction of non-neutralizing antibody responses. These findings suggest a major role of the length of the tail in maintenance of a specific Env structure which could be important for virus-encoded Env priming of vaccine responses.

Here we show that a simple expression cassette encoding Gag and Env separated by a self-cleavable peptide efficiently directs the secretion of VLPs incorporating Env, with more total Env protein incorporated using the truncated tail. Surface staining of vaccine transduced cells with the gp120 monomer binding VRC01 antibody demonstrated that the tail truncation led to a major increased presentation of the CD4 binding site (bs) epitope, and an increase in the presentation of CD4 inducible epitopes (17b), whereas the gp120/gp41 interface sensitive and trimer specific antibodies showed less (PGT151) or similar (PGT145) cell surface expression using the truncated tail. Sequential administration of up to three heterologous vectors primed with either antigen gave rise to increasing antibody responses after each immunization. Whereas higher incorporation of Env in a virus-encoded VLP vaccine did not have any direct effect on the initial humoral response, there was a significant increase in the total Env trimer-specific humoral response following boosting with vectors encoding full length Env. These responses were also paralleled with significantly higher binding to CON-S gp140 in a binding antibody multiplex assay, and a tendency for higher V3 loop specific responses. Conversely, the full length Env primed group showed a tendency to have higher gp41 responses and a significant bias towards gp41 binding antibodies relatively to gp120 binding antibodies. Notably, the increase in CON-S binding antibodies obtained with the truncated Env priming did not translate into differences in neutralizing antibody responses.

2. Materials and methods

2.1. Mice

Female Balb/c mice aged 6–8 weeks were obtained from Taconic M&B (Ry, Denmark). The mice were allowed to acclimatize for one week prior to the initiation of an experiment. All experiments were approved by the national animal experiments inspectorate (Dyreforsøgstilsynet) and performed according to national guidelines.

2.2. Adenoviral vaccine production

Replication-deficient E1 deleted human adenovirus type 5 (huAd5) [13] and chimpanzee adenovirus type 3 (chAd3) and 63 (chAd63) [14] vectors with deleted E1 and E3 genes were used in this study. The adenoviral vectors expressed SIVmac239 Gag and HIV-1 M CON-S gp160 (CON-S gp160) [15], linked to Gag via a Glycine/Serine/Glycine (GSG) linker followed by a self-cleaving porcine teschovirus-1 2A peptide (P2A) [16], and were produced in Procell 92 cells [17]. A truncated CON-S gp160 version (CON-S gp160Δ139) in a huAd5 vector was produced expressing SIVmac239 Gag linked via P2A to CON-S gp160 truncated after amino acid (aa) 721 (HXB2 numbering, GenBank accession number K03455). The huAd5 vectors and chAd vectors were produced by homologous recombination in Procell 92 cells [18] and BJ5183 [19], respectively. The antigens were inserted in the E1 region for all constructs as described for huAd5 [18] and the chAd vectors [19] and all vectors encoded tetracycline operator (TetO) sequences [10] downstream of the huCMV promoter. After amplification of clonal adenoviral genomes, the particles were purified using a caesium chloride gradient as described [18].

2.3. Immunizations

Mice were injected intramuscularly in the quadriceps muscle (i.m.) (10 mice per group) with 3×10^9 adenoviral particles in a total volume of 50 μl PBS changing leg at each vaccination time-point.

2.4. Humoral responses

CON-S gp160 responses were measured by enzyme linked immunosorbent assay (ELISA) as adapted from [20]. CON-S gp160 was produced in 293FT cells using a plasmid encoding CON-S gp160 and the pSG3$^{\Delta env}$ plasmid [21] (NARRP) by transient transfection. ELISA was performed coating MaxiSorp plates (NUNC) with concanavalin A (ConA) (Sigma-Aldrich) captured CON-S gp160 cell supernatant. Serum samples were added in a series of 2-fold dilutions and a standard anti-Env serum sample was included on all plates in all runs. Binding was detected with HRP labelled polyclonal rabbit anti-mouse immunoglobulins (Dako) used for detection of bound antibody. The anti-Env responses at the final time-point were also measured against CON-S gp140 CFI avi protein (2 μg/ml in carbonate buffer). Gag specific responses were determined using p27 SIVmac239 Gag (Immune Technology) as a coating antigen (2 μg/ml in PBS). Samples were added in 3-fold dilutions, and the mAb 55-2F12 [22] (NARRP) served as a positive control. Binding was detected as above. Area under the curve (AUC) and end-point dilution titers were used to assess the humoral responses. AUC and titers were calculated from either 3-fold (Gag responses) or 2-fold (Env responses) titration series of the samples.

2.5. Binding antibody multiplex assay (BAMA) for determination of the specificity of antibody responses

The HIV-1 binding antibody multiplex assay was performed as previously described [23–26]. Briefly, the HIV-1 antigens, gp41 (clade B HIV-1_{MN} recombinant gp41 protein, ImmunoDiagnostics), Con6 gp120 (Consensus gp120 Env), CON-S gp140 CFI (Group M consensus gp140 CFI Env), gp41 ID epitope (Biotin – CRVLAVER YLRDQQLLGIWGCSGKLICTTAV) [23], murine leukemia virus (MuLV) gp70 B.CaseA2 V1–V2, MuLV gp70, and clade B V3 (Biotin – KKKNNTRKSIHIGPGRAFYATGDIIGDIRQAHC, JPT Peptide Technologies) were conjugated to polystyrene beads (Bio-Rad) and binding was detected using goat anti-mouse IgG-PE or mouse anti-human IgG-PE (Southern Biotech). MuLV gp70 coupled beads were used to account for MuLV gp70 background binding in the V1–V2 antigen coupled to gp70 (gp70 B.CaseA2 V1–V2). Antigen specific binding was measured as mean fluorescent intensity (MFI). Positive controls included HIV-1 immunoglobulin (HIVIG) (NARRP), and the 7B2 and 3B3 IgG monoclonal antibodies (mAbs). Negative controls were blank beads, normal human serum (Sigma), and pre-immune pooled mouse serum. Responses were considered positive if they met antigen-specific positivity criteria of the mean