A candidate HIV/AIDS vaccine (MVA-B) that enhances the magnitude and polyfunctionality of memory HIV-1-specific T-cell responses

CSIC

CNB

Juan García-Arriaza[1], José Luis Nájera[1], Carmen E. Gómez[1], Nolawit Tewabe[1], Carlos Oscar S. Sorzano[2], Thierry Calandra[3], Thierry Roger[3], Mariano Esteban[1]

1 Department of Molecular and Cellular Biology, Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain, 2 Biocomputing Unit, Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain,
3 Infectious Diseases Service, Department of Medicine, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Lausanne, Switzerland.

INTRODUCTION

The vaccinia virus (VACV) C6 protein has sequence similarities with the poxvirus family Pox_A46, involved in regulation of host immune responses, but its role is unknown. Here, we have characterized the C6 protein and its effects in virus replication, innate immune sensing and immunogenicity *in vivo*. C6 is a 18.2 kDa protein, which is expressed early during virus infection and localizes to the cytoplasm of infected cells. Deletion of the *C6L* gene from the poxvirus vector MVA-B expressing HIV-1 Env, Gag, Pol and Nef antigens from clade B (MVA-B ΔC6L) had no effect on virus growth kinetics; therefore C6 protein is not essential for virus replication. The innate immune signals elicited by MVA-B ΔC6L in human macrophages and monocyte-derived dendritic cells (moDCs) are characterized by the up-regulation of the expression of IFN-β and IFN-α/β-inducible genes. In a DNA prime/MVA boost immunization protocol in mice, flow cytometry analysis revealed that MVA-B ΔC6L enhanced the magnitude and polyfunctionality of the HIV-1-specific CD4⁺ and CD8⁺ T-cell memory immune responses, with most of the HIV-1 responses mediated by the CD8⁺ T-cell compartment with an effector phenotype. Significantly, while MVA-B induced preferentially Env- and Gag-specific CD8⁺ T-cell responses, MVA-B ΔC6L induced more Gag-Pol-Nef (GPN)-specific CD8⁺ T-cell responses. Furthermore, MVA-B ΔC6L enhanced the levels of antibodies against Env in comparison with MVA-B. These findings revealed that C6 can be considered as an immunomodulator and that deleting *C6L* gene in MVA-B confers an immunological benefit by enhancing IFN-β-dependent responses and increasing the magnitude and quality of the T-cell memory immune responses to HIV-1 antigens. Our observations are relevant for the improvement of MVA vectors as HIV-1 vaccines.

Figure 1 — Generation of MVA-B ΔC6L deletion mutant

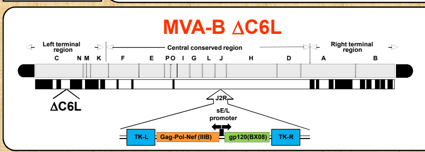
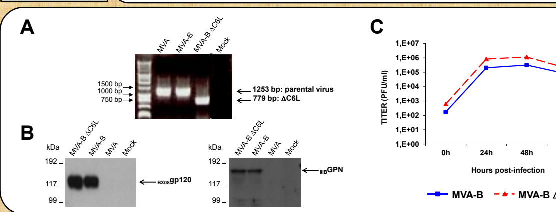


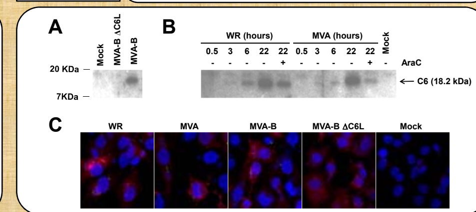
Diagram of the MVA-B ΔC6L deletion mutant. To improve the immunogenicity of MVA-based vaccine candidates against HIV/AIDS, we have constructed a novel poxvirus vector (termed MVA-B ΔC6L) by deleting vaccinia virus gene *C6L* (whose function was previously unknown) in the attenuated MVA-B recombinant which expresses four HIV-1 antigens from clade B (gp120, from isolate BX08; and Gag-Pol-Nef, from isolate IIIB). MVA-B ΔC6L was generated by screening for transient RED/GFP co-expression using a plasmid transfer vector that directs the deletion of *C6L* from MVA-B. In addition, MVA-B ΔC6L contains, as well as MVA-B, the same cassette of HIV-1 genes in the TK locus (*J2R*) and under regulation of a synthetic early/late promoter. The different regions are indicated by capital letters. The right and left terminal regions are shown. Below the map, the deleted or fragmented genes are depicted as black boxes. The deleted *C6L* gene is indicated.

Figure 2 — *In vitro* characterization of MVA-B ΔC6L deletion mutant



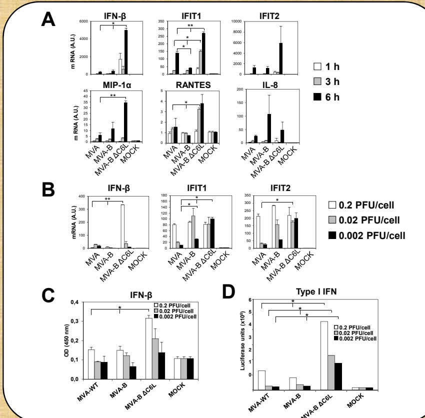
***In vitro* characterization of MVA-B ΔC6L deletion mutant. (A)** PCR analysis of *C6L* locus. DNA extracted from DF-1 cells infected at 2 PFU/cell with MVA-WT, MVA-B or MVA-B ΔC6L was used for PCR analysis. The DNA products corresponding to the parental virus or to the deletion are indicated by an arrow on the right, with the expected size in base pairs. Molecular size marker (1 Kb ladder) with the corresponding sizes (base pairs) is indicated on the left. Lane Mock, cells not infected. **(B)** Expression of HIV-1 $_{BX08}$gp120 and $_{IIIB}$GPN proteins in DF-1 cells infected at 2 PFU/cell with MVA-B and MVA-B ΔC6L, and detected by Western blot at 24 h post-infection. Arrows on the right indicate the position of HIV-1 $_{BX08}$gp120 and $_{IIIB}$GPN proteins. **(C)** Viral growth kinetics of MVA-B and MVA-B ΔC6L in infected (0.01 PFU/cell) DF-1 cells at different times and titrated by plaque immunostaining assay with anti-WR antibodies. The mean of three independent experiments are shown.

Figure 3 — Characterization of C6 expression and localization



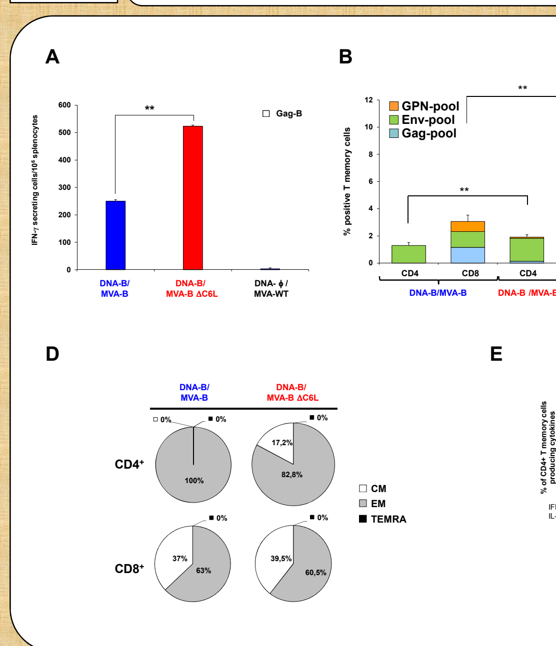
Characterization of C6 expression and localization. DF-1 cells were infected with 5 PFU/cell of MVA-B and MVA-B ΔC6L **(A)** or WR and MVA **(B)** in the presence or absence of AraC **(B)**. Cells extracts collected 24 hours post-infection **(A)** or at the indicated time **(B)** were analyzed by SDS-PAGE. VACV C6 protein was detected by Western blot using rabbit polyclonal sera against C6. **(C)** DF-1 cells were infected with WR, MVA, MVA-B or MVA-B ΔC6L or mock-infected for 18 hours. The localization of C6 was analyzed by immunofluorescence. Cells were staining with DAPI (blue, staining cellular nucleus), purified rabbit polyclonal anti-C6 (green) and anti-14K (red).

Figure 4 — MVA-B ΔC6L up-regulates IFN-β expression in human macrophages and dendritic cells



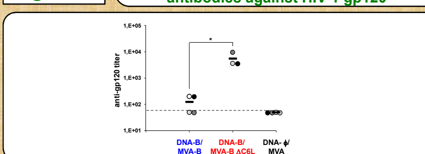
MVA-B ΔC6L induces the production of IFN-β and type I IFN inducible genes in macrophages and dendritic cells. Human THP-1 macrophages **(A)** and moDCs **(B, C, D)** were infected with MVA, MVA-B and MVA-B ΔC6L (5 PFU/cell in A, and 0.2, 0.02 and 0.002 PFU/cell in B, C and D). At different time post-infection (1 h, 3 h and 6 h in A, 6 h in B), RNA was extracted and the mRNA levels of IFN-β, type I IFN inducible genes (IFIT1 and IFIT2), chemokines and HPRT were analyzed by RT-PCR. Results were expressed as the ratio of gene to HPRT mRNA levels. A.U: arbitrary units. Data are means ± SD of duplicate samples. * $p<0.05$, ** $p<0.005$. **(C, D)** Human moDCs were infected with 0.2, 0.02 and 0.002 PFU/cell of MVA, MVA-B and MVA-B ΔC6L. Six hours later, cell-free supernatants were collected to quantify the concentration of IFN-β by ELISA **(C)** and the concentration of type I IFN using the HL116 reporter cell line **(D).** Results were expressed in absorbance values at 450 nm **(C),** and in luciferase units **(D).** Data are means ± SD of duplicates and are representative of two independent experiments. * $p<0.05$.

Figure 5 — MVA-B ΔC6L enhances the magnitude and polyfunctionality of long-lived memory HIV-1 specific T-cell responses



Immunization with MVA-B ΔC6L enhances the magnitude and polyfunctionality of HIV-1-specific CD4⁺ and CD8⁺ T-cell memory immune responses. Splenocytes were collected from mice (n=4 per group) immunized with DNA-B/MVA-B, DNA-B/MVA-B ΔC6L or DNA-ϕ/MVA, 53 days after the last immunization. **(A)** Gag-B-specific IFN-γ secreting splenocytes were quantified by ELISPOT assay. Data are means ± SD of triplicate cultures. ** $p<0.005$. **(B-D)** Flow cytometry phenotypic analysis of Env, Gag and GPN HIV-1 specific CD4⁺ and CD8⁺ T-cells. CD44 and CD62L expression was used to identify central memory (CM: CD44⁺/CD62L⁺), effector memory (EM: CD44⁺/CD62L⁻) and effector memory terminally differentiated (TEMRA: CD44⁻/CD62L⁻) sub-populations. IFN-y and IL-2 production was analyzed by ICS. **(B)** A representative flow cytometry is shown. The T-cell memory sub-populations are depicted as density plots. Blue and green dots represent T-cells producing IFN-y and IL-2, respectively. **(C)** Percentage of splenic Env, Gag and GPN HIV-1-specific CD4⁺ and CD8⁺ memory T-cells. Frequencies were calculated by reporting the number of memory T-cells producing IFN-γ and/or IL-2 to the total number of CD4⁺ and CD8⁺ splenocytes. Values from unstimulated controls were subtracted in all cases. ** $p<0.005$. **(D)** Pie charts representing the proportion of CM, EM and TEMRA within the Env, Gag and GPN HIV-1-specific CD4⁺ and CD8⁺ memory T-cells. (A-D) Data are from one experiment representative of two experiments. **(E)** The polyfunctionality of Env + Gag + GPN HIV-1-specific CD4⁺ (left part) and CD8⁺ (right part) memory T cells was defined based on IFN-γ and/or IL-2 production. All the possible combinations of the responses are shown on the X axis. The percentages of IFN-γ and/or IL-2 producing memory T-cells among total CD4⁺ and CD8⁺ T cells are shown on the Y axis. ** $p<0.005$. The pie charts summarize the data. Each slice corresponds to the proportion of CD4⁺ or CD8⁺ T cells producing single (IFN+ or IL-2+) or double (IFN+/IL-2+) responses within the total HIV-1-specific CD4⁺ or CD8⁺ T-cell memory populations. The size of the pie chart represents the magnitude of the specific HIV-1 memory immune response induced.

Figure 6 — MVA-B ΔC6L enhances the levels of antibodies against HIV-1 gp120



Immunization with MVA-B ΔC6L enhances the humoral immune responses elicited against HIV-1 gp160 protein. Serum was collected from individual mouse immunized with DNA-B/MVA-B, DNA-B/MVA-B ΔC6L or DNA-ϕ/MVA (n=4 in DNA-B/MVA-B and DNA-ϕ/MVA; n=3 in DNA-B/MVA-B ΔC6L), 53 days after the last immunization. Anti-gp120 antibody titers were determined by ELISA as described in *Materials and Methods*. Titers represent the last dilution of the serum that signals 3-fold higher than signals obtained with the serum of naïve mice. The dotted line represents the limit of detection of the ELISA. The horizontal bar represents the mean value. Each dot represents one mouse. * $p<0.05$.

CONCLUSIONS

- MVA-B ΔC6L deletion mutant was generated, contains deletion in *C6L* vaccinia viral gene, and express HIV-1 antigens $_{BX08}$gp120 and $_{IIIB}$GPN at the same level as their parental virus MVA-B. Deletion of *C6L* gene in the MVA-B genome does not affect virus replication and hence, this gene is not essential for virus propagation in cultured cells.
- C6 is expressed early in cells infected with VACV virus, and localizes to the cytoplasm of infected cells.
- MVA-B ΔC6L up-regulated the expression of IFN-β and IFN-α/β-inducible genes (IFIT1 and IFIT2) in human THP-1 cells and moDCs.
- MVA-B ΔC6L enhanced the magnitude and polyfunctionality of the HIV-1-specific T-cell memory immune responses.
- MVA-B ΔC6L induced more GPN-specific responses, while MVA-B induced preferentially Env-specific responses.
- MVA-B ΔC6L triggered higher levels of antibodies against HIV-1 Env.
- These findings revealed that both vectors induced robust, polyfunctional and durable T-cell responses to HIV-1 antigens, but MVA-B ΔC6L deletion mutant showed enhanced memory and quality of HIV-1 responses. Our observations are relevant in the improvement of MVA vectors as HIV-1 vaccines.