Enhancement of HIV-1 Env-Specific CD8 T Cell Responses Using Interferon-Stimulated Gene 15 as an Immune Adjuvant

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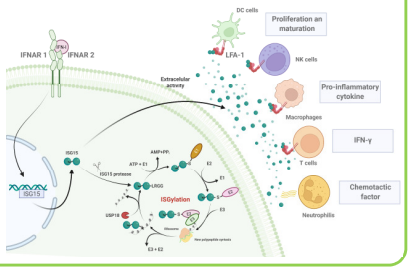
Abstract.

After a viral infection, one of the genes activated by Interferon-I (IFN-I) induction is the IFN-stimulated gene 15 (ISG15). ISG15 is a small ubiquitin-like protein which plays a central role in the antiviral response of the host organism but its role as an immunomodulator in the vaccine field remains to be defined. ISG15 exists in several forms: either intracellular, covalently and non-covalently conjugated to target proteins, or released as a cytokine. In this study we showed that ISG15 exerts an immunomodulatory role in Human Immunodeficiency virus (HIV) vaccines. Using a DNA prime/MVA boost immunization protocol, our results indicated an increase in the potency and the quality of the HIV-1 Env-specific CD8 T cell response when mice were primed with DNA vectors expressing either WT or mutant ISG15 (the non-covalently conjugable form). Moreover, the amount of DNA-gp120 vector used to immunize mice could be reduced 5-fold when combined with the DNA-ISG15 without affecting the potency and quality of the HIV-1 Env-specific immune responses. These results highlight the possibility to generate novel ISG15-based vaccines strategies that could elicit an improved viral antigen presentation to the immune cells resulting in the development of optimized HIV-1 immune responses.

Introduction.

After a viral infection, the host's innate immune system and Interferon induction are an essential first-line defense to prevent viral replication before a more specific protection induced by the adaptive immune system is elicited. Host pattern recognition receptors (PRRs) recognize viral components triggering several signaling pathways that ultimately lead to the production of type I IFN, responsible for the induction of genes called interferon-stimulated genes (ISGs), which are indispensable elements for host resistance to viral infections and for generation of the antiviral state. One of the most highly induced genes in the type I IFN signaling pathway is *ISG15*, a small ubiquitin-like (Ubl) protein involved in a reversible posttranslational modification process known as ISGylation. ISGylation is a reversible protein modification that involves a cascade of enzymatic reactions that finally bind ISG15 to a lysine residue of de novo-synthesized target proteins. In addition to the presence of conjugated ISG15, ISG15 protein can be found as a free molecule in two different states: intracellular and extracellular, acting as a cytokine.

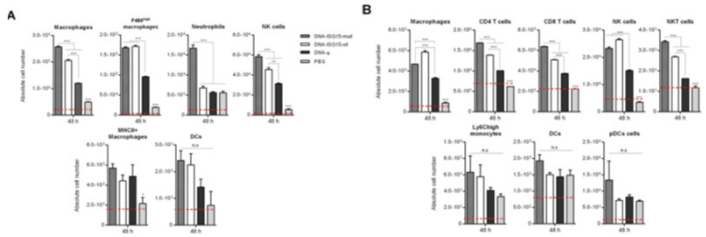


Objectives

The development of an effective vaccine against HIV/AIDS has proven to be one of the greatest complex scientific challenges: no vaccine candidate has improved the 31,2% efficacy demonstrate in the RV144 trial.

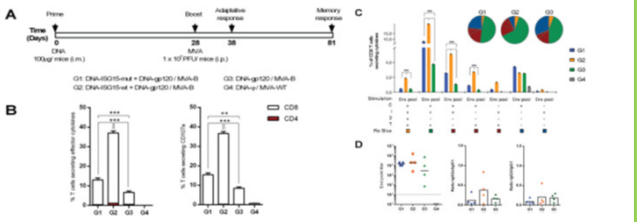
- Evaluate the effect of ISG15 expression on immune cell infiltration
- Evaluate the immunomodulatory role of ISG15 when expressed by DNA vector in the optimization of HIV/AIDS vaccine candidates

Immune cell infiltration



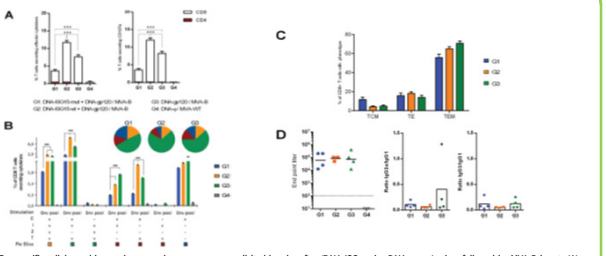
Effect of ISG15 expression on immune cell infiltration. Four groups of animals were inoculated with DNA-ISG15-wt, DNA-ISG15-mut, DNA-, or PBS by the intramuscular (i.m.) route. At 48 h postinoculation, total muscle from the site of inoculation and the proximal DLN were excised, and the different immune cell populations were determined by flow cytometry. **(A) Immune cell infiltration in muscle.** Large amounts of cell infiltration of macrophages, $F4/80^{high}$ macrophages, neutrophils, NK cells, and DCs were detected in muscle from all groups that received DNA vectors compared with the PBS group, in groups where ISG15-wt or ISG15-mut was expressed, the absolute numbers of these populations were significantly higher, the infiltration of immune cells being higher in the DNA-ISG15-mut group. **(B) Immune cell infiltration in the proximal draining lymph node (DLN).** A similar behavior in the proximal DLN was observed, with the absolute numbers of macrophages, CD_4 and CD8 T cells, and NK and NKT cells being higher in groups receiving DNA-ISG15-wt or DNA-ISG15-mut. The dotted red line indicates the absolute cell numbers of the different cell populations detected in naive mice. n.s., not statistically significant. *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$.

HIV-1 Env-specific adaptive immune response



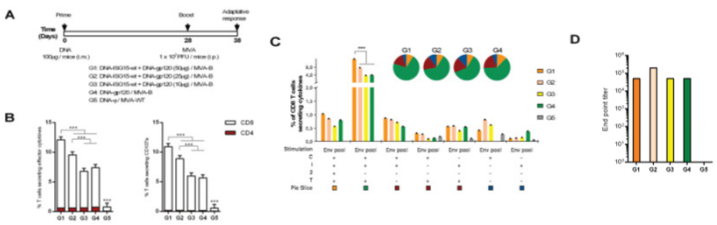
HIV-1 Env-specific cellular and humoral adaptive immune responses elicited in mice after (DNA-ISG15 plus DNA-gp120) prime followed by MVA-B boost. (A) Immunization schedule. Groups of female BALB/c mice (n=8) received the indicated doses of DNA-based vectors by the i.m. route, and 28 days later, animals were immunized with 10^7 PFU of MVA-WT or MVA-B by the intraperitoneal (i.p.) route. **(B) Magnitude of the HIV-1 Env-specific T cell responses.** 10 days postboost T cells responses were misured by ICS assay following *ex vivo* stimulation of splenocyte from immunized mice with HIV-1 clade B consensus peptide pools. The total value in each group represents the sum of the percentages of $CD4^+$ plus CD8+ T cells secreting IL-2, and/or IFN-γ, and/or TNF- α (left panel) or the percentages of $CD4^+$ plus CD8+ T cells expressing $CD107^+$ (right panel). **(C) Polyfunctional profile of the HIV-1 Env-specific CD8+ responses in the different immunization groups.** The seven positive combinations of the responses are indicated on the x axis, while the percentages of the functionally different cell populations within the total CD8 T cells are represented on the y axis. Specific responses are grouped, and color coded based on the number of functions. Abbreviations: C, CD107a; I, IFN-α; 2, IL-2; T, TNF-γ. **(D) Levels of HIV-1 gp120 Bx08-specific total IgG binding antibodies measured in the sera from individual immunized mice at 10 days postboost by ELISA.** (Middle and right panels) Levels of HIV-1 gp120 Bx08-specific IgG1, IgG2a, and IgG3 binding antibodies at a serum dilution of 1:25,600 by ELISA, and ratios of IgG2a to IgG1 (middle) and IgG3 to IgG1 (right) are represented. The different colored circles represent the antibody ratios for each mouse, and the open columns indicate the mean antibody ratios for each group.

HIV-1 Env-specific memory immune response



HIV-1 Env-specific cellular and humoral memory immune responses elicited in mice after (DNA-ISG15 plus DNA-gp120) prime followed by MVA-B boost. (A) Magnitude of the HIV-1 Env-specific T cell responses. 53 days postboost T cells responses were misured by ICS assay following *ex vivo* stimulation of splenocyte from immunized mice with HIV-1 clade B consensus peptide pools. The total value in each group represents the sum of the percentages of $CD4^+$ plus $CD8^+$ T cells secreting IL-2, and/or IFN-γ, and/or TNF- α (left panel) or the percentages of $CD4^+$ plus $CD8^+$ T cells expressing $CD107^+$ (right panel). **(B) Polyfunctional profile of the HIV-1 Env-specific CD8+ responses in the different immunization groups.** The seven positive combinations of the responses are indicated on the x axis, while the percentages of the functionally different cell populations within the total CD8 T cells are represented on the y axis. Specific responses are grouped, and color coded based on the number of functions. Abbreviations: C, CD107a; I, IFN-α; 2, IL-2; T, TNF-γ. **(C) Phenotypic profile of the memory HIV-1 Env-specific CD8 T cells.** The phenotype of the vaccine-induced memory responses was determined based on expression of the $CD127$ and $CD62L$ surface markers on activated cells as follows: T central memory (TCM; $CD127^+$ $CD62L^+$), T effector memory (TEM; $CD127^-$ $CD62L^-$), and T effector (TE; $CD127^-$ $CD62L^+$). **(D, left panel) Levels of HIV-1 gp120 Bx08-specific total IgG binding antibodies measured in the sera from individual immunized mice at 53 days postboost by ELISA.** (Middle and right panels) Levels of HIV-1 gp120 Bx08-specific IgG1, IgG2a, and IgG3 binding antibodies were measured in individual sera from immunized mice at 53 days postboost as the OD450 at a serum dilution of 1:25,600 by ELISA, and ratios of IgG2a to IgG1 (middle) and IgG3 to IgG1 (right) are represented. cells.

Coadministration of ISG15-wt allows reducing 5-fold the dose of DNA-gp120 without affecting the HIV-1 Env-specific responses



HIV-1 Env-specific cellular and humoral adaptive immune responses elicited in mice after the administration of DNA-ISG15-wt with decreasing amounts of DNA-gp120 in the prime followed by the MVA-B boost. (A) Immunization schedule. Groups of female BALB/c mice (n4) received the indicated doses of DNA-based vectors by the i.m. route, and 28 days later, animals were immunized with $2x10^7$ PFU of MVA-WT or MVA-B by the i.p. route. **(B)) Magnitude of the HIV-1 Env-specific T cell responses.** 10 days postboost T cells responses were misured by ICS assay following *ex vivo* stimulation of splenocyte from immunized mice with HIV-1 clade B consensus peptide pools. The total value in each group represents the sum of the percentages of $CD4^+$ plus $CD8^+$ T cells secreting IL-2, and/or IFN-γ, and/or TNF- α (left panel) or the percentages of $CD4^+$ plus $CD8^+$ T cells expressing $CD107^+$ (right panel). **(C) Polyfunctional profile of the HIV-1 Env-specific CD8+ responses in the different immunization groups.** The seven positive combinations of the responses are indicated on the x axis, while the percentages of the functionally different cell populations within the total CD8 T cells are represented on the y axis. Specific responses are grouped, and color coded based on the number of functions. Abbreviations: C, CD107a; I, IFN-α; 2, IL-2; T, TNF-γ. **(D) Levels of HIV-1 gp120 Bx08-specific total IgG binding antibodies measured in pooled sera from immunized mice at 10 days postboost by ELISA.** The endpoint titer is defined as the last serum dilution that gave three times the mean optical density at OD_{450} of the control group.

Discussion and conclusions

We observed that the intramuscular delivery of DNA vectors expressing ISG15-wt or ISG15-mut proteins in mice induced enhanced immune cell infiltration in muscle and proximal DLN. This innate activation at the inoculation site seemed to impact the HIV-1 Env-specific adaptive and memory CD8 T cell responses when DNA-ISG15-wt or DNA-ISG15-mut was coadministered with DNA-gp120 in the prime, since enhanced magnitude of effector $CD8^+$ T cells with cytotoxic capacity and polyfunctional profile were detected in these groups. Regarding the migratory capacity of macrophages stimulated after intramuscular plasmid inoculation, our results do not evidence any relevance of ISGylation. We observed that ISG15-wt exhibits better immunostimulatory activity than ISG15-mut, indicating that in addition to the free ISG15 or intracellular conjugation-independent functions, the intracellular ISGylation also plays an important role in the connection between innate and adaptive immunities. **Overall, this study reveals the immunomodulatory properties of either ISG15-wt or ISG15-mut proteins when expressed by a DNA vector, conferring immune potency to an HIV-1 vaccine candidate, with a possible role for ISGylation in the connection between innate and adaptive immunities. Hence, the DNA-ISG15 vector could be used as a promising CD8 T cell-driven vaccine adjuvant for the modulation of specific immune responses to HIV-1 or other vaccine candidates to enhance control of infectious diseases.**