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Nef modulates the immunogenicity of Gag encoded in a non-infectious HIV DNA vaccine

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ABSTRACT

Gag-CD8$^+$ T cell responses are associated with immune control of HIV infection. Since during HIV infection Nef impairs T cell responses, we evaluated whether deletion of *nef* from a non-infectious HIV DNA vaccine (Δ4 Nef(+)), creating Δ5 Nef(−), would affect its immunogenicity. When compared with Δ4, mice injected with Δ5 developed significantly lower CD8$^+$ T cell responses to Gag, but no significant change in the responses to Env was observed. *In vitro*, deletion of Nef abrogated the induced cell death, production of virus-like particles and release of Gag from transfected cells. Thus, the effect of Nef in causing extrusion of Gag might adjuvant the CD8$^+$ T cell responses to Gag in DNA vaccine.

1. Introduction

It is now well established that control of HIV in the early stages of the infection is associated with the development of cell-mediated immune (CMI) responses against multiple antigens of the virus. The most direct evidence for importance of CMI responses has come from SIV-infected macaques which, following treatment with anti-CD8 antibodies, failed to control the infection. These infected animals developed massive virus burdens and rapidly succumbed to disease [1–3]. However, the specific viral proteins needed to induce the protective CMI responses are still unknown. More recent studies on the nature of the protective CMI responses against HIV and SIV showed that the most frequent epitopes recognized by cytotoxic T lymphocytes (CTLs) are located in the Gag and Nef proteins. Surprisingly, less are located in the Env which houses moieties responsible for antigenic variation of the viruses, as well as docking sites for viral binding to cellular receptors [4]. The Gag and Nef contain the highest epitope density of the virus [5–12]. Clinically, the magnitude and breadth of the CD8$^+$ T cell responses to Gag and Nef in HIV-infected persons correlated inversely with plasma virus loads [13–15], with the lower virus load indicating control of the virus [16,17].

Examination of the immune responses induced by various vaccine strategies showed different results. Inactivated virus or individual viral proteins usually induced antibody rather than CMI responses. On the other hand, plasmid DNAs encoding viral proteins have been recognized as potent inducers of T cell responses. Most of these studies have been conducted in mice. In an earlier report, we had shown that an HIV DNA vaccine derived from the highly pathogenic SHIV$_{KU2}$, following deletion of the *int*, *rt*, and *vif* genes and substitution of the 3′LTR with polyA sequences of SV40, was non-infectious and capable of only half of a single cycle of replication of virus [18,19]. In a subsequent report, we showed that following exchange of the SIV *gag* and *nef* with those of HIV-1, the DNA now expressed the Gag, Env, and Nef of HIV. Immunization of mice with this DNA (Δ4 SHIV$_{KU2}$, Δ4 Nef(+)) induced CMI responses dominated by anti-HIV Gag CD8$^+$ T cells [20,21]. During natural infection, the viral protein Nef is well known to interfere with host immune response mechanisms. For example, in infected CD4$^+$ T cells, Nef favors the retention of T cell receptors and other

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adaptor molecules in the recycling endosomes thereby limiting the formation of the immunological synapse. Nef also acts to modify the status of antigen-presenting cells by triggering the secretion of multiple cytokines [22]. In addition, Nef modulates the apoptotic death of infected and bystander cells [23–26]. Consequently, the benefit of inclusion of a *nef* gene encoding a functional Nef in an HIV-based vaccine has been questioned. To minimize the undesirable effects of Nef, genetically engineered *nef* genes, in which the function was altered but the immunogenic epitopes were conserved, were used in HIV vaccine constructs [27,28]. In this report, we revisited the importance of a functional Nef in the induction of immune T cell responses by an HIV DNA vaccine. We examined whether deletion of *nef* from the Δ4 Nef(+) DNA creating Δ5 Nef(−) would affect the immunogenicity of this vaccine in immunized mice. Results of this study showed that the Δ4 Nef(+) DNA induced strong $CD8^+$ T cell responses to Gag, whereas Δ5 Nef(−) DNA induced Gag-specific $CD8^+$ T cell responses that were significantly reduced. Data from experiments in cell cultures pointed out some mechanisms that might explain the intriguing difference in the induced immune responses obtained with the two constructs. Since anti-Gag $CD8^+$ T cell responses are important for protection, our results suggest that inclusion of *nef* with *gag* coding sequences in HIV DNA vaccine constructs may be of great importance in the future design of an efficacious HIV vaccine.

2. Materials and methods

2.1. DNA constructs

Details of construction of the Δ4 plasmid DNA have been described in an earlier report [19,20]. The inserted sequences were derived from $SHIV_{KU2}$ (GenBank data base accession # AY751799) and $HIV\text{-}1_{SF2}$. The DNA is comprised of *vpx* and *vpr* genes from SIVmac239, and *gag, pro*, *vpu, tat, rev, env, nef* and a portion of *rt* genes from HIV-1, all under the transcriptional control of the SIV 5′LTR promoter and the polyA sequences of SV40. The *pol* gene was truncated to remove 80% of *rt*, and the complete *integrase* and *vif* coding sequences together with the 3′LTR from $SHIV_{KU2}$. The Δ5 plasmid was derived from Δ4 DNA by deleting 562 bp of the 633 bp *nef* coding sequences following digestion with Bpu 1102I, removal of the fragment of 562 bp, and ligation of 9 kb (Fig. 1). Both viral constructs are carried by the pET9 plasmid (Novagen, EMD Chemicals, San Diego, CA).

2.2. Animals

BALB/c mice were purchased from Harlan Laboratories and housed in the Laboratory Animal Resources Building of KUMC. All mice were used in accordance with National Institute of Health and the KUMC Institutional Animal Care and Use Committee guidelines.

2.3. Inoculation of mice and splenocyte preparations

Two groups of six-week-old BALB/c mice were inoculated intramuscularly with a single dose of 200 μg of either the Δ4 or the Δ5 DNA. All DNAs used for inoculation were more than 85% in supercoiled form. DNA solutions were prepared in phosphate buffered saline (pH 7.4) and contained 2 μg/μl DNA. Each mouse was injected with a total of 100 μl of DNA solution, 50 μl in each gastrocnemius muscle. Four animals of each group were killed at 2 and 4 weeks, respectively, and spleens collected for CMI studies. Splenocytes were collected in Hanks solution, treated with BD lysing solution to remove the erythrocytes, and mononuclear cells counted as previously described [20]. A portion of the splenocytes from each mouse was used for ELISPOT assay, and a second portion was used as part of a pool comprised of cells from the four mice for flow cytometry as described below.

2.4. HIV peptides

Overlapping 15 mer peptides, with 11 amino acid overlaps, spanning the entire molecule of HIV Gag, Env, Tat, Rev, and Nef proteins were obtained from the NIH AIDS Research and Reference Reagent Program (Catalog # 8117, 6451, 5138, 6445 and 5189, respectively).

2.5. Detection of HIV-specific CMI responses in splenocytes of mice

Quantitative ELISPOT assays were performed as previously described [20] to measure IFN-γ-producing splenocytes in response to groups of overlapping peptides kindly provided by the AIDS Reference Reagent Program. Gag, Env, Tat + Rev + Nef pools of peptides were used at a concentration of 2 μg/ml in duplicate wells of cells. Con-A-stimulated cells (0.5 μg/well) were used as positive controls and cells lacking stimulation with the peptides were used as negative controls. Spots were counted with a stereo-microscope and reported as the number of spot forming splenocytes (SFS)/10^6

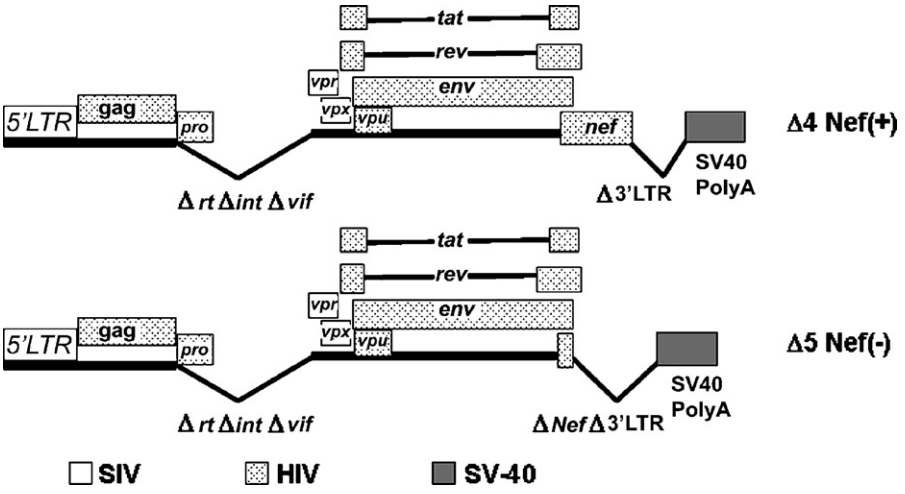

Fig. 1. Schematic representation of Δ4 Nef(+) and Δ5 Nef(−) DNA constructs. HIV and SIV structural, regulatory, and accessory genes are indicated in boxes. Expression of viral genes is driven by the 5′LTR of SIV and RNA stop transcription is insured by the SV40 polyA signal. Δ5 Nef(−) was derived from Δ4 Nef(+) DNA by additional deletion of *nef* coding sequences.