IN VIVO IMMUNOGENICITY OF SIV VACCINE CANDIDATES WITH AND WITHOUT A NEW IMMUNOMODULATOR IN RHESUS MACAQUES

INTRODUCTION

An effective HIV vaccine needs to induce broadly neutralizing antibodies and elicit polyfunctional T cells against multiple HIV antigens. New HIV vaccine strategies are needed to accomplish this goal.

The use of novel immunomodulators in combination with immunogens represents one modality of improving vaccine efficacy. Adjuvants/immunomodulators activate dendritic cells and thereby improve T cell priming and, indirectly, antibody responses. We have developed a novel native lipid-GNA complex (CLDC) that has been shown to enhance cellular immune responses against multiple antigens in several animal models of human diseases.

The rhesus macaque model of SIV infection represents the best available animal model to test HIV vaccine and intervention strategies.

Therefore, we propose to test the immunogenicity of a subunit SIV vaccine (SIVag) on a whole-animal SIV vaccine (SIVmac239 A2) administered with and without CLDC in the rhesus macaque model. The analysis included the measurement of binding and neutralizing antibody and the measurement of SIV-specific T cell responses. In addition, we determined if long-lived memory responses could be induced. A comparative analysis of the quality and quantity of vaccine-induced parameters in the presence and absence of CLDC was performed.

OBJECTIVE

To determine whether CLDC can improve the quality or quantity of anti-SIV immune responses associated with the vaccine candidates with the long-term goal of developing an effective HIV vaccine.

The current study is designed as a pilot study to test immunogenicity only.

EXPERIMENTAL DESIGN

Animal: Juvenile male rhesus macaques, n=12

Experiment 1: Primary Immunization

1. SIVag (25 µg); immunoreactivity (imm): 1:
2. SIVag/CLDC (25 µg); i.e., SIVag + SIVmac239-A2/CLDC (10 µg); i.e., 1:3

Experiment 2: Long-term Memory Induction

1. SIVmac239-A2 (10 µg) subcutaneously (s.c.): n=2
2. SIVmac239-A2/CLDC (10 µg); s.c.: n=11

T CELL RESPONSES

- SIVmac239 binding activity assay - SIVmac neutralizing antibody assay in T20ML cells: B2715.6 and SIVmac239 ClADE-1 (T. Monis, Duke University)
- MIP-1α multiplex cytometry for SIVag and SIVmac239-A2 specific T cell responses (TNFα, gamma interferon (IFNγ), and TNFα)

ANTIBODY RESPONSES

- SIVag and sIVmac239 binding activity assay
- SIVmac neutralizing antibody assay in TZM-bl cells: SIVmac251.6 and SIVmac239 ClADE-1 (T. Monis, Duke University)
- SIVmac239 ClADE-1 (T. Monis, Duke University)
- MIP-1α multiplex cytometry for SIVag and SIVmac239-A2 specific T cell responses (TNFα, gamma interferon (IFNγ), and TNFα)

Primary Immunization:
- Consistent with earlier studies, immunization with a subunit SIV (SIVag) vaccine is inefficient in inducing immunoresponses.
- Administration of a subunit vaccine with CLDC clearly results in an enhancement of cellular immune responses.
- However, the strongest and most consistent responses were achieved when animals were immunized with a whole-array vaccine with CLDC.
- Based on the results from this study, a separate experiment was performed to determine if vaccine-induced immune responses in the presence of CLDC were also more effective in inducing long-lived memory responses.

Induction of Memory Responses by Vaccination in the Presence of CLDC:
- Chemically-saturated SIVmac239 AT2 has been shown previously by Lifson et al. to be highly immunogenic in rhesus macaques.
- Our data show that binding and neutralizing antibody responses could be further increased about 50-fold by immunizing macaques with SIVmac239 AT2 in combination with CLDC.
- In addition, SIV-specific CD4+ and CD8+ T cell responses were more consistently induced and at higher frequencies in macaques receiving both SIVmac239 AT2 and CLDC compared to animals immunized with SIVmac239 AT2 alone.
- SIVspecific T and B cell responses were maintained after the last immunization, although at somewhat lower levels.
- Higher frequency of SIV-specific CD8+ T cells after the first round of immunizations with CLDC manifested itself in higher and more consistent memory responses detected several months later after immunization with SIVmac239 AT2 only.

CONCLUSIONS

- CLDC is a novel immunomodulator that is safe and does not cause any adverse reactions, even after repeated administration, in rhesus macaques.
- Rhesus macaques immunized with SIV vaccine candidates in the presence of CLDC show greatly enhanced SIV-specific T cell and antibody responses, higher SIV-specific binding and neutralizing antibody compared to animals vaccinated with the same vaccine without CLDC.
- Stronger immune responses induced after primary immunization in the presence of CLDC translate into higher and more consistent memory responses after several months.
- Multiple SIV antigens are needed to achieve optimal vaccine-induced immune responses.
- The inclusion of the envelope antigens is obviously critical for the induction of neutralizing antibodies.
- While the quantity/ frequency of SIV-specific T and B cell responses could be increased by CLDC, the quality of the immune responses was not altered by the addition of CLDC to the vaccine candidate. SIV-specific T cells produced predominantly Th1 responses, but less consistently IFNγ, and did not show cytokine activity.

FUTURE STUDIES

- Expand the current pilot studies to test the efficacy of SIV vaccines administered with CLDC in SIV challenge studies.
- Explore different SIV vaccine candidates, for example SIV antigen expressing vectors, that will be given with CLDC to enhance immune responses.
- Include multiple SIV vaccine antigens like gag, pol, env and accessory genes to broaden the responses.
- Test the vaccine efficacy of SIV/CLDC vaccines after administration by different routes (systemic versus mucosal immunization).

ACKNOWLEDGEMENTS

We thank Dr. J. Lifson, NIAID, for providing SIVag and Dr. S. Monis, Duke University, for neutralizing antibody to SIV. Dr. S. Monis and Dr. D. Montefiori, Duke University, for immunomodulator and sIVmac239 vaccine. The study was supported by NIAID R01 AI067429A.