T-cell Epitopes Identified by *BALB/c* Mice Immunized with Vaccinia Expressing HIV-1 Gag lie within Immunodominant Regions Recognized by HIV-infected Indian Patients

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ABSTRACT

Background: Human immunodeficiency virus (HIV) antigens from transmitted strains of HIV would prove crucial in vaccine designing for prevention of HIV infection. Immune response generated by Vaccinia construct expressing the HIV-1 *gag* gene from transmitted Indian HIV-1 subtype C strain (*Vgag*) in *BALB/c* mice is reported in the present study along with the identification of epitopes responsible for induction of the immune response. **Aims:** The aim of this study was to determine immune response generated by the constructs in a mouse model and to understand the epitope specificities of the response. **Settings and Design:** This was an observational study carried out in *BALB/c* mice. **Materials and Methods:** The immunogenecity of *Vgag* construct was evaluated in *BALB/c* mice after multiple immunizations. T-cell response was monitored by the interferon-γ ELISPOT assay using HIV-1 C Gag overlapping peptides and anti-P24 antibodies were estimated by ELISA. **Statistical Analysis Used:** Graphpad prism software was used for statistical analysis and for plotting graphs. **Results:** IFN-γ-secreting T cells and antibodies were detected against HIV Gag in mice after immunization. Although after repeated immunizations, antibody-mediated immune response increased or remained sustained, the magnitude of IFN-γ-secreting T cell was found to be decreased over time. The Gag peptides recognized by mice were mainly confined to the P24 region and had a considerable overlap with earlier reported immunodominant regions recognized by HIV-infected Indian patients. **Conclusion:** Vaccinia construct with a *gag* gene from transmitted HIV-1 virus was found to be immunogenic. The Gag regions identified by mice could have important implications in terms of future HIV vaccine designing.

Key words: Cellular immune response, T-cell epitopes, *Vgag*

INTRODUCTION

Although general population prevalence in India is low (0.29%) in comparison with many African countries,[1] effective strategies including effective anti-HIV vaccine are required for preventing further spread of epidemic to the general population. Induction of HIV-specific cytotoxic T-lymphocyte (CTL) responses is considered important, in addition to neutralizing antibody response, for a candidate vaccine to be effective. But in spite of induction of good antibody or cellular immune response, different vaccine candidates have failed in preventing HIV infection or slowing down disease progression in clinical trials.[2,3]

HIV diversity has been one of the major obstacles in the development of an effective prophylactic vaccine. Among HIV genes, *gag* is relatively conserved and immune responses, particularly CTL responses, specific to *gag* have been shown to be associated with the clearance of primary viremia and control of virus multiplication.[4] *Gag*-specific CTL response is associated with slower progression of the disease.[5] Also, *Gag*-specific CD8 (+) T cells have been shown to recognize infected CD4 (+) T lymphocytes as early as 2 hours post infection, even before proviral DNA integration.[6] The number of *gag* epitopes recognized by CD8 (+) T cells is reported to be significantly associated with lower viremia in SIV-infected rhesus macaques.[6]

It has been reported that Indian subtype C sequences cluster away from subtype C sequences of non-Indian origin.[7] But,

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there have been very few studies carried out to evaluate immune response against an Indian HIV subtype C-based immunogen in animal models as well as in clinical trials. Also, the viruses used in the vaccine studies up till now were grown from chronically infected persons and hence may have been selected under immune pressure. On the other hand, viruses cultured from acute HIV infection are considered to be the crucial targets of vaccine-induced immunity,[8] as such strains represent recently transmitted HIV that has not undergone selection under immune pressure.

Among different HIV vaccine strategies, Poxvirus vectored vaccines have shown encouraging immunogenicity in HIV vaccine clinical trials.[9] Results from recently published Phase III Thai trial using Canarypox-based vaccine candidate also had shown a very modest protection among vaccines.[10] Hence, a recombinant Vaccinia virus construct expressing *gag* from recently infected individuals with HIV-1 subtype C virus of Indian origin was constructed and evaluated in *BALB/c* mice for generation of *Gag*-specific CTL and antibody response.

MATERIALS AND METHODS

HIV-1 C *gag* gene sequencing

Gag gene from HIV-1 subtype C strain from an Indian patient with acute HIV infection was sequenced. Full-length *gag* (P55) was amplified in PCR and cloned into pGEM T Easy vector as described previously.[11] Sequencing was performed by using cycle sequencing and big dye termination on an automated sequencer (Applied Biosystems Inc. 310, Perkin Elmer).

Constructions of recombinant vaccinia containing HIV-1 C *gag*

The expression vector, pSC59, used in this study contained strong early/late promoter flanked by DNA sequences from vaccinia virus.[12] The coding sequence of the *gag* gene was cloned downstream to the promoter and the resulting recombinant was used to transfect vaccinia virus. The recombinant plaque was purified and expression of HIV-1 C *gag* was demonstrated by Western blot assay. For Western blot assay, CV-1 cells were infected with the recombinant vaccinia construct (*Vgag*) as well as with wild-type vaccinia virus western reserve (*WR*) at the multiplicity of infection (MOI) of 10 plaque forming units (PFU)/ cell. After 24 hours, the cells were harvested and lysed with buffer containing 0.5% TritonX-100. The lysate and supernatant of the infected cells were electrophoresed on 5% PAGE and transferred to the nitrocellulose membrane. HIV positive patient's serum was used as a source of primary antibody and anti-Human IgG conjugated with horse radish peroxidase (HRP, Biorad Laboratories) as the secondary antibody. The expression of HIV *gag* antigen was detected as a color band developed after addition of the TMB substrate [Figure 1].

Expansion and purification of recombinant vaccinia constructs

The *Vgag* and *WR* constructs were expanded using HeLa cell line and purified by sucrose density gradient centrifugation using 36% sucrose (Sigma) and the titer was estimated by plaque formation assay using the BSC-1 cell line as described elsewhere.[13]

Immunization and immunogenicity assessment schedule

5 to 8-week-old Female *BALB/c* mice were divided into groups of 10 mice each. Mice in each group were immunized subcutaneously at five different time points at a 2 weeks interval between first four vaccination and 4 weeks interval between fourth and fifth vaccinations. The mice were injected with 1×10^6 or 1×10^7 PFU/ mouse of *Vgag* (*Vgag*10^6/ *Vgag*10^7) or *WR* (*WR*10^6/ *WR*10^7) or saline at these time points.

The mice were bled retro-orbitally at 1 week following first four immunizations and 2 weeks following the fifth immunization. The serum samples were separated and stored at –20°C until they were tested for antibodies against P24. Three to four mice from each group were sacrificed by cervical dislocation 1 week following third and fourth immunizations and two were sacrificed 2 weeks after the fifth immunization. Spleen was harvested aseptically and processed for splenocyte separation for estimation of HIV 1 specific T-cell response.

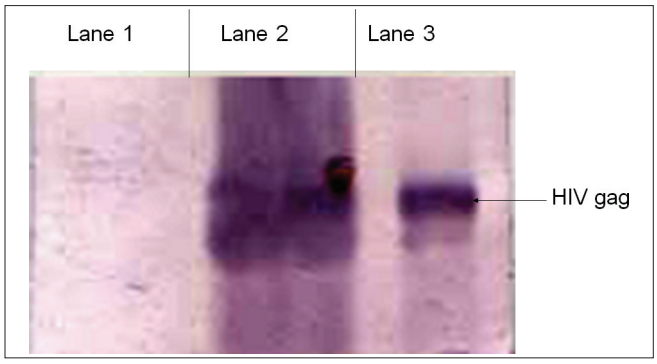


Figure 1: Western blot assay to detect expression of HIV gag by *Vgag* construct. The arrow shows *gag* expressed in lysate (lane 2) and supernatant (lane 3) of cells infected with *Vgag*. No HIV gag was detected in lysate of cells infected with *WR* (lane 1)

Experimental protocol for use of mice was approved by Institutional Biosafety Committee (IBC) of National AIDS Research Institute and Institutional Animal Ethics Committee (IAEC) of National Institute of Virology where experiments were carried out.

HIV-1 C *Gag*-specific binding antibody detection

HIV-1 C *Gag*-specific binding antibody in mouse sera was measured by the enzyme-linked immunoassay (ELISA). Ninety-six well ELISA plates (Costar) were coated with P24 antigen (Span diagnostics) at the concentration of 1 μg/ml in 0.1 M carbonate buffer (pH-9.5) overnight at 4°C. The plates were washed five times with phosphate buffer saline (PBS- pH 7.2) containing 0.05% Tween 20. The plates were blocked with 1% bovine serum albumin (Sigma) in PBS for 1 hour at 37°C. 100 μl of ten-fold diluted mouse serum samples were added in duplicate wells and incubated for 1 hour at 37°C. After washing the plate five times, 100 μl of 1:10000 diluted anti-mouse IgG peroxidase conjugate (Sigma) in PBS with 1% BSA was added and incubated for 1 hour at 37°C. After washing, the color was developed by addition of 100 μl per well of the TMB substrate (Sigma). The plates were read at 450 nm after stopping the reaction with 1N H_2SO_4. The response was considered positive if optical density (O.D.) was more than cut-off of 0.04 (Mean + 3 S.D. of O.D. of sera from saline injected mice).

Preparation of splenocytes

Mouse spleens were harvested aseptically and single cell suspension was prepared by gently crushing the spleen on sieve using a sterile plunger of a syringe. The splenocytes were separated by Ficoll hypaque (Sigma: Histopaque-1083) density gradient centrifugation and suspended in RPMI 1640 with 10% fetal calf serum, 200 mM L-glutamine and antibiotics (100 U penicillin and 100 μg/ml of streptomycin) (All the reagents were procured from Sigma). The cells were counted and percentage viability was determined by trypan blue dye exclusion. The cell viability of all the samples was above 90%.

IFN-g ELISPOT assay

The T-cell response was measured using the IFN -g secretory ELISPOT assay as described previously.[14] Briefly, ELISPOT plates (Millipore, USA: MAIPS 4510) were coated with 100 μl of 10 μg/ml of an anti-mouse IFN-g monoclonal antibody (Mabtech, Sweden, Cat No: 3321-3). The plates were incubated overnight at 4°C. After blocking the plates with RPMI 1640 with 10% fetal calf serum for at least 2 hours, splenocytes were added at cell densities ranging from 2 to 5×10^5 cells/well depending on availability of cells and stimulated with 10 μg/ml of concanvalin A (positive control), or *gag* peptides. Wells containing unstimulated cells in RPMI medium were kept as negative control (mock) to measure the background response. The cultures were incubated overnight at 37°C in 5% CO_2 atmosphere. After incubation, the cells were discarded and 100 μl of biotinylated anti-IFN-g monoclonal antibody (Mabtech, Sweden: 3321-6) was added per well at 4 μg/ml concentration. The plates were incubated for 3 hours at room temperature, followed by 1 hour incubation with 100 μl of HRP–conjugated with streptavidin (Vectastain, Vector Laboratories). The spots were developed after adding 100 μl of AEC substrate (Sigma) for 4 mins at room temperature in dark. The plates were subjected to image analysis using a ELISPOT reader (AID: ELHR01) after drying overnight. The assay was considered valid if the number of spot forming units (SFU)/million cells in positive control wells exceeded 50. The response in the peptide stimulated wells was considered positive if SFU / million cells in these wells was more than cut off value (18 SFU/million cells) after subtraction of response in mock wells. The cut off value for positive response was calculated by adding 2 S.D.s to the mean of the response seen in mock wells of all the assays on vaccinated and control mice.

Peptides used for the ELISPOT assay

Overlapping 20-mer peptides with a 10-amino acid overlap for HIV-1 subtype C *gag* protein were obtained from AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH (Cat. no. 3993). The lyophilized peptides were dissolved in 10 μl of dimethyl sulfoxide (DMSO, Sigma). They were further diluted in RPMI medium so that the final concentration of each peptide in any of the pools in each ELISPOT well was 5 μg/ml. Forty-nine overlapping peptides of HIV-1C *gag* were divided into three pools (G1, G2, and G3) containing peptide # 1-16, 17-32 and 33-49 respectively. Pool G1 contained 16 peptides spanning amino acids from 1 to 170, G2 contained 16 peptides over amino acids: 160 to 325, whereas G3 had 17 peptides over amino acids: 320 to 494 of the HIV-1 *gag* region.

For the purpose of identification of individual peptides recognized by immunized mice, a matrix consisting of 14 pools, each containing seven peptides, was prepared. The matrix pools were designed in such a way that each peptide was present in two different pools as described previously.[14] Peptide common in two pools eliciting response was considered for further analysis.

Statistical analysis

Graphpad prism software was used for statistical analysis and for plotting graphs. The means and standard deviations of O.D. values for ELISA response and that of SFU/million cells for ELISPOT response of individual mouse in each group after each immunization were calculated. The means of ELISA and ELISPOT responses were compared and plotted on the graphs for monitoring humoral and cellular response elicited by immunized mice after each immunization. The standard deviations were calculated to estimate the degree of variation in each group. The responses in the two dosage groups and immunizations at different time intervals were compared by the unpaired *t* test and expressed as *P* value.

RESULTS

P24 specific antibody detection

Anti-*gag* P24 antibodies were measured in all groups of mice at 1 week following each of the first four immunizations and 2 weeks following the fifth immunization. Mice immunized with 10^6 and 10^7 PFU of *Vgag* developed the HIV-1 P24 antibodies right after the first immunization [Figure 2]. The antibody levels increased or were sustained with subsequent immunizations in the mice in both dosage groups. The antibody response was significantly higher in *Vgag*10^7 group after second immunization ($P<0.05$) in comparison with *Vgag*10^6 group.

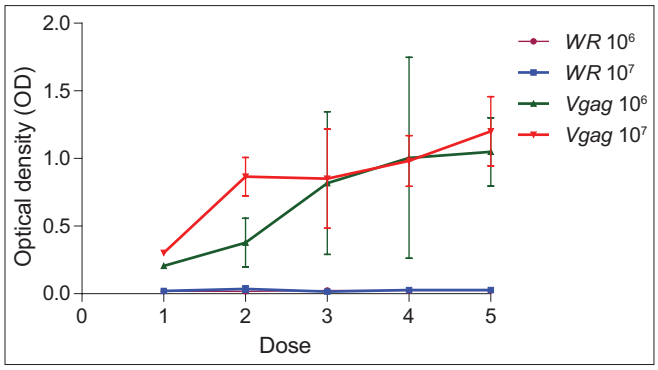


Figure 2: Humoral response against HIV-1C gag by P24 antigen ELISA. The antibody response to P24 antigen was measured in mice immunized with *WR*10^6, *WR*10^7, *Vgag*10^6, *Vgag*10^7 by ELISA. The X-axis represents doses of the immunogen and the Y-axis represents antibody response expressed as O.D. The data points and error bars represent mean and 1 S.D. of the response shown by mice in each group, respectively

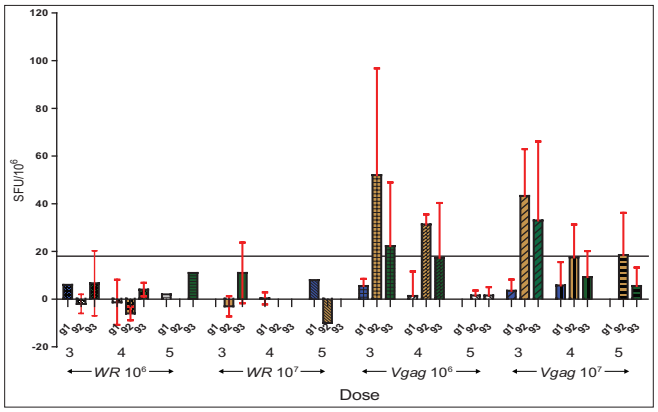


Figure 3: T-cell response determined by the IFN-g secretory ELISPOT assay. The graph shows the average mock subtracted response (SFU/million cells) on the Y-axis after third, fourth, and fifth immunizations with *Vgag*10^7, *Vgag*10^6, *WR*10^7, *WR*10^6. Each data bar indicates mean of the response in each group and the error bars indicate 1 S.D. for the response in the group. The horizontal line represents the cut-off value (mean + 2 S.D. of SFU/million cells in mock well) for considering positive response

T-cell IFN-g response

HIV-1 *Gag*-specific IFN-g secretory response was assessed after third, fourth, and fifth immunization with both *Vgag*10^6 and *Vgag*10^7. The IFN-g secreting cells were detected predominantly against peptides in the pool G2 (Average: 34.2 SFU/million cell) followed by pool G3 (Average: 18.2 SFU/million cells) in both the dose groups [Figure 3] at all time points.

Magnitude of cells producing IFN-g decreased after subsequent immunizations. In 10^6 PFU *Vgag* group, the magnitude decreased by 60% to G2 pool and by 86% to G3 pool after fifth immunization as compared to third immunization, whereas in mice immunized with 10^7 PFU, the decrease was 39% and 57% to peptide pools G2 and G3, respectively.

The magnitude of IFN-g secreting cells was sustained to pool G2 after fifth immunization in mice inoculated with 10^7 PFU, as against in mice inoculated with 10^6 PFU of *Vgag* where it declined rapidly and no response was detected after the fifth immunization to any of the pools. Thus, the overall T-cell response was found to be moderate and sustained in a dose-dependent manner.

HIV-1 *gag* peptides recognized by vaccinated mice

The IFN-g response to individual peptides was estimated using matrix ELISPOT. The sequence of the peptides used for ELISPOT assay showed 92% similarity with the *gag* sequence used in the vaccine construct. The Figure 4 shows alignment of both the sequences. A total of 15 peptides were recognized by immunized mice [Figure 5]. Among these, 12 peptides were from

p24 and 3 were from p7 region of *gag* protein. The p24 peptides identified by mouse splenocytes were clustered in three amino acid regions: Amino acids 140 to 220, 240 to 279, 310 to 349 and P7 peptides clustered in one region: Amino acids 379 to 418 of the *gag* protein. Peptides 33 and 34 (amino acids: 310-340: *QEVKNWMTDTLLVQNA NPDCKTI LK ALGPG*) were the most frequently recognized peptides followed by peptides 26 and 27(amino acids 240-270: *STLQEQIAWMTSNPPIPVGDIYKRWIILGL*).

The HIV-1C *gag* regions identified by immunized mice showed similarity with the immunodominant CTL regions identified by HIV-infected patients in India.[15] HIV-1C *gag* regions identified by immunized mice corresponding to amino acids 140-190 (*QMVHQ KLSPRTLNAWVKVIEEKAFSPEVIPMFTALSE GATPQDLNTMLNTV*), amino acids 201-220 (*LKDTINEEAAEWDRLHPVHA*), amino acids 261-279 (*IYKRWIILGLNKIVRMYSPV*) were found to overlap immunodominant CTL regions identified by HIV-infected Indian patients as shown in Figure 4.

DISCUSSION

Although vaccines have played a major role in control of many infectious diseases, vaccine for preventing HIV infection has proven to be one of the biggest challenges faced by the AIDS researchers. None of the candidate vaccine tested in clinical trials conducted has so far shown to be effective in preventing HIV infections, except for the recently concluded Thai trial based on prime- boost strategy which has shown only 31.2% efficacy.[10] This highlights the need for more efforts in basic research in HIV vaccine development and also critical evaluation of vaccine candidates in pre-clinical trials. It is also important to evaluate immune response to circulating strains of HIV subtype C, which is the most common subtype in India. Very few studies have been done to evaluate immune response generated by vaccine candidates based on Indian HIV subtype C. The present study reports the preclinical evaluation of recombinant Vaccinia construct expressing the HIV-1 Indian subtype C *gag* gene isolated from recently infected patient in the *BALB/c* mouse model.

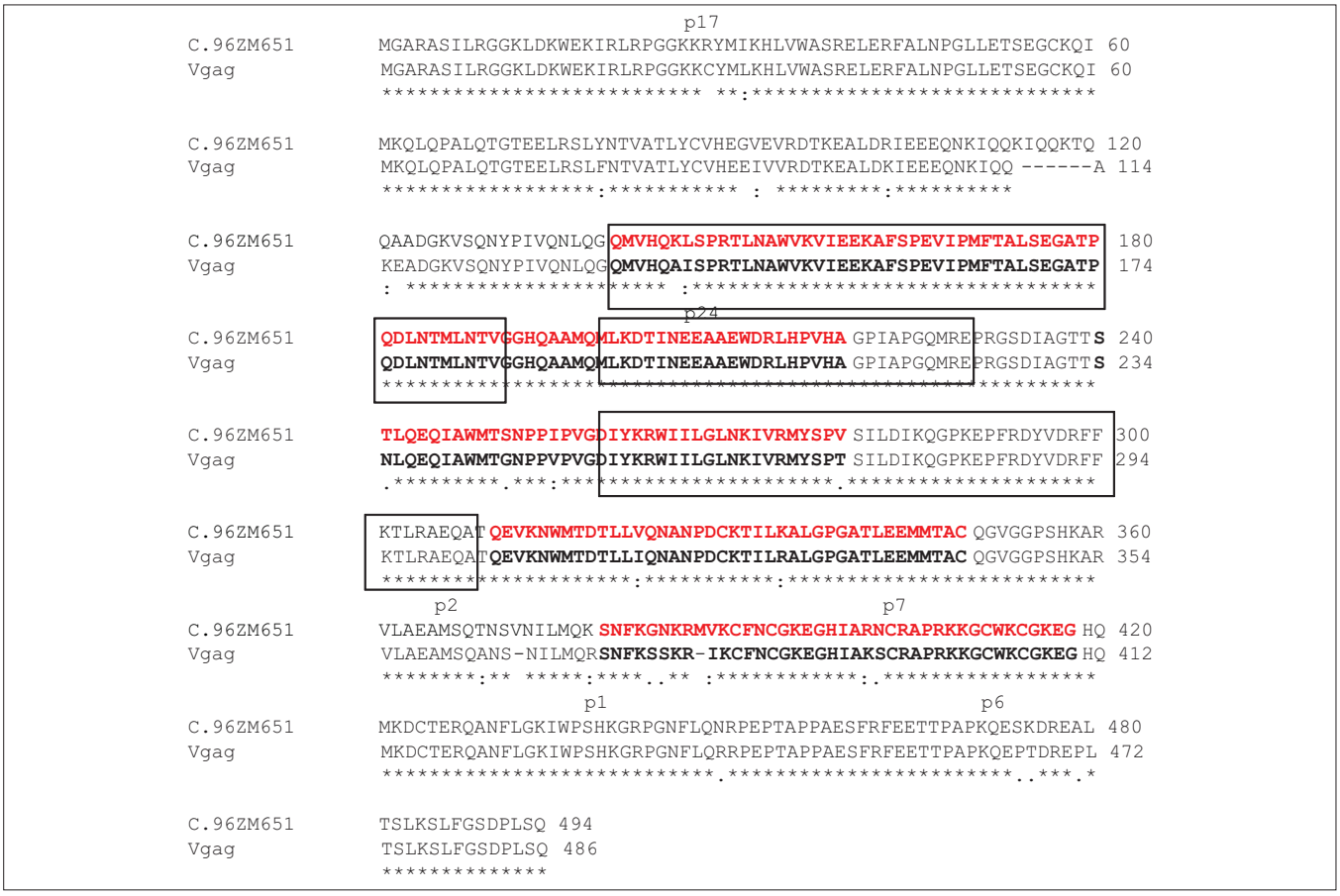
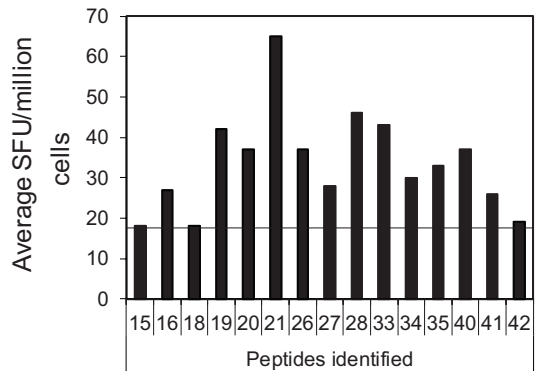


Figure 4: HIV-1 C *gag* regions identified by immunized mice. Alignment of the Vaccine *gag* sequence (*Vgag*- GenBank accession No. AY484419) with the sequence of HIV-1 C.96ZM651 which was used for overlapping peptides synthesis. (*) indicates conserved sequences; (:) indicates conserved substitutions whereas (.) stands for semi-conserved substitutions. The regions identified by immunized *BALB/c* mice are shown in red letters. The blocks correspond to the immunodominant CTL regions identified by HIV infected patients in India



15: *QMVHQKLSPRTLNAWVKVIE (QMVHQAISPRTLNAWVKVIE),*
16: *TLNAWVKVIEEKAFSPEVIP (TLNAWVKVIEEKAFSPEVIP),*
18: *MFTALSEGATPQDLNTMLNT (MFTALSEGATPQDLNTMLNT),*
19: *PQDLNTMLNTVGGHQAAMQM (PQDLNTMLNTVGGHQAAMQM),*
20: *VGGHQAAMQMLKDTINEEAA (VGGHQAAMQMLKDTINEEAA),*
21: *LKDTINEEAAEWDRLHPVHA (LKDTINEEAAEWDRLHPVHA),*
26: *STLQEQIAWMTSNPPIPVGD (SNLQEQIAWMTGNPPVPVGD),*
27: *TSNPPIPVGDIYKRWIILGL (TGNPPVPVGDIYKRWIILGL),*
28: *IYKRWIILGLNKIVRMYSPV (IYKRWIILGLNKIVRMYSPT),*
33: *QEVKNWMTDTLLVQNA (QEVKNWMTDTLLIQNA),*
34: *LLVQNANPDCKTILKALGPG (LLIQNANPDCKTILRALGPG),*
35: *KTILKALGPGATLEEMMTAC (KTILRALGPGATLEEMMTAC),*
40: *SNFKGNKRMVKCFNCGKEGH (SNFKSSKR-IKCFNCGKEGH),*
41: *KCFNCGKEGHIARNCRAPRK (KCFNCGKEGHIAKSCRAPRK),*
42: *IARNCRAPRKKGCWKCGKEG (IAKSCRAPRKKGCWKCGKEG)*

Figure 5: Peptides identified by immunized mice in IFN-g ELISPOT matrix. The graph shows m agnitude of response as measured by SFU/million cells (Y-axis) to 15 peptides (X axis) identified in the ELISPOT assay matrix by immunized *BALB/c* mice. The figure also shows sequences of peptides used in ELISPOT assays and corresponding *Vgag* sequences as mentioned in the brackets

The vaccine construct induced an HIV Gag-specific antibody and cell-mediated response after immunization with 10^6 and 10^7 PFU dosages, but failed to boost the immune response after subsequent immunizations. The failure to boost the immune response against vector expressed foreign antigen after repeated immunizations is likely to be due to induction of stronger immune response against viral vector proteins. It has been reported that immunity to vaccinia vectors induced by earlier immunizations resulted in significant inhibition of T-cell response to foreign antigens expressed by them upon subsequent immunizations.[16] Higher dose of vaccine probably could circumvent the vector specific immune response resulting in persistence of HIV-1 *gag* specific IFN-g response as seen after fifth immunization in *Vgag*10^7 group. Persistence of T-cell immune response was observed in the case of adenovirus vector with the highest dose of 10^{11} particle units (PUs) as compared to lower doses of 10^9 and 10^{10} PUs in human volunteers.[17] However, safety issues and development of tolerance may be the concerns for using still higher doses of vaccine candidates. Also, higher doses of Vaccinia have been reported to result in development of predominant Th2 type of immune response instead of Th1 type which plays critical role in induction of cell-mediated immunity.[15] Alternatively, the use of different combinations of vectors in prime/boost strategy and/or different routes of immunizations could be considered to circumvent the problem of vector-specific immune response.[18] Non-replicating vectors like modified vaccinia ankara (MVA) may also be used as they are safe and the strength of immune response directed against these vectors is much less than that against replication competent vaccinia vectors.[15] Codon optimization for optimal presentation of antigens by genes carried in vaccine candidate also would help to generate better immune response as compared to their naïve counterparts.[19]

Magnitude of IFN-g secretory cells as determined by the ELISPOT assay was found to be decreased after fourth and fifth immunizations in both the dosage groups as compared to third immunization. The decline may be because of apoptosis of activated cells caused by re-immunization within shorter interval.[20] As against T-cell response, antibody response against *gag* either increased or was sustained after subsequent immunizations in the both dosage groups. The similar results with higher antibody response to foreign antigen expressed by the vectors after subsequent boosters were also demonstrated in the study by Ramirez *et al*.[16] Failure of boosting of T-cell response with marginal increase in antibody response to HIV proteins after third repeated immunization of MVA has also been observed in macaques.[21] The higher antibody response seen may be as a result of B-cell adjuvanting capabilities of poxviruses through induction of TNF-a and IL-6, both of which have been shown to support plasma cell survival.[22]

HIV-1C Gag-specific T-cell response in immunized mice was found to be clustered within three regions of p24 corresponding to the amino acids 140 to 220, 240 to 279, 310 to 349, and one region of P7 corresponding to amino acids 379 to 418 of the *gag* protein. One of the earlier studies carried out for identification of *gag* epitopes in *BALB/c* mice has also identified different *gag* regions spanning between amino acids 165 to 315 after immunization with the HIV *gag* protein alone and found that Tat was responsible for broadening of immune response against *gag*.[23] Another study in *BALB/c* mice carried out in India could identify epitopes distributed throughout the length of *gag*.[24] However, there was difference in the vector used as well as the epitopes identified were determined after second immunization in that study, whereas we have reported the responses after third vaccination.

The P24 regions corresponding to amino acids 140-190, 201-220, 261-279 identified by immunized mice have been found to overlap with the P24 regions identified in the HIV-1 C-infected patients from India and South Africa.[14,25] The results of these experiments indicate that the immune response raised against *Vgag* construct targets the same regions of *gag* protein as human volunteers. The regions identified in response to HIV strains from acute HIV infection would prove important in understanding the actual crucial targets of vaccine-induced immunity. The overall sequence of *gag* in the construct showed more than >90% similarity with consensus C sequence as well as with other reported Indian subtype C *gag* sequences.[26] Although the identified regions of *gag* did not show much conservation when compared with other reported Indian subtype C *gag* sequences, the identified regions showed more than 90% conservation among the recently isolated strains of HIV indicating importance of using sequences from recently isolated strains in vaccine designing.[11]

The majority of the epitopes identified by vaccinated mice belonged to the P24 region. It has been observed that HLA alleles with a low relative hazard (RH) of disease progression preferentially present p24 epitopes.[27] Also the different regions of *gag* protein identified by mice in the present study were found to overlap with different T helper and CTL epitopes identified in different studies indicating probable use of these sequences for wider application.[28-30] Also, these regions could be more antigenic containing different murine as well as human epitopes and could be concentrated for different vaccine studies.

CONCLUSION

In summary, induction of *Gag*-specific T cell and antibody response was demonstrated in *BALB/c* mice immunized with recombinant vaccinia expressing HIV-1 subtype C *gag* of Indian origin from recent HIV infection. Although the quantum of response was rather low, the use of this vaccine in prime booster strategy along with DNA would result in better and sustainable response. The extensive overlap between the epitopes recognized by infected humans and vaccinated mice indicates that the response observed to immunization by the *Vgag* construct may have relevance in terms of vaccine development. Further studies with these immunogenic regions of HIV-1 *gag* may be critical for HIV vaccine development for prevention of HIV infection in human.

REFERENCES

1. Department of AIDS control, Ministry of Health and family welfare, Annual report 2009-2010. Available from: http://www.nacoonline.org/upload/AR%202009-10/NACO_AR_English%20 corrected.pdf [Last cited on 2010,Nov 25].
2. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, *et al.* Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): A double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet 2008;372:1881-93.
3. Kittikraisak W, van Griensven F, Martin M, McNicholl J, Gilbert PB, Chuachoowong R, *et al.* Blood and seminal plasma HIV-1 RNA levels among HIV-1-infected injecting drug users participating in the AIDSVAX B/E efficacy trial in Bangkok, Thailand. J Acquir Immune Defic Syndr 2009;51:601-8.
4. Klein MR, van Baalen CA, Holwerda AM, Kerkhof Garde SR, Bende RJ, Keet IP, *et al.* Kinetics of *Gag*-specific cytotoxic T lymphocyte responses during the clinical course of HIV-1 infection: A longitudinal analysis of rapid progressors and long-term asymptomatics. J Exp Med 1995;181:1365-72.
5. Wagner R, Leschonsky B, Harrer E, Paulus C, Weber C, Walker BD, *et al.* Molecular and functional analysis of a conserved CTL epitope in HIV-1 p24 recognized from a long-term non progressor: Constraints on immune escape associated with targeting a sequence essential for viral replication. J Immunol 1999;162:3727-34.
6. Sacha JB, Chung C, Rakasz EG, Spencer SP, Jonas AK, Bean AT, *et al. Gag*-Specific $CD8^+$ T lymphocytes recognize infected cells before AIDS-virus integration and viral protein expression. J Immunol 2007;178:2746-54.
7. Gaschen B, Taylor J, Yusim K, Foley B, Gao F, Lang D, *et al.* Diversity considerations in HIV-1 vaccine selection. Science 2002;28:2354-60.
8. Derdeyn CA, Decker JM, Bibollet-Ruche F, Mokili JL, Muldoon M, Denham SA, *et al.* Envelope-constrained neutralization-sensitive HIV-1 after heterosexual transmission. Science 2004;303:2019-22.
9. Mwau M, Cebere I, Sutton J, Chikoti P, Winstone N, Wee EG-T, *et al.* A human immunodeficiency virus 1 (HIV-1) clade A vaccine in clinical trials: Stimulation of HIV-specific T-cell responses by DNA and recombinant modified vaccinia virus Ankara (MVA) vaccines in humans. J Gen Virol 2004;85:911-9.
10. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, *et al.* Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med 2009;361:2209-20.
11. Kurle S, Tripathi S, Jadhav S, Agnihotri K, Paranjape R. Full-Length *gag* sequences of HIV Type 1 Subtype C recent seroconverters from Pune, India. AIDS Res Hum Retrovir 2004;20:1113-8.
12. Chakrabarti S, Sisler J R, Moss B. Compact, synthetic, vaccinia virus early/late promoter for protein expression. BioTechniques 1997;23:1094-7.
13. Earl PL, Cooper N, Wyatt LS, Moss B, Carroll MW. Preparation of cell cultures and Vaccinia virus stocks: Curr Protoc Mol Boil 1993;24(Supplement 15): 16.16.1-5.
14. Thakar MR, Bhonge LS, Lakhashe SK, Shankarkumar U, Sane SS, Kulkarni SS, *et al.* Cytolytic T lymphocytes (CTLs) from HIV-1 subtype C infected Indian patients recognize CTL epitopes from a conserved immunodominant region of HIV-1 *gag* and Nef. J Infect Dis 2005;192:749-59.
15. Ramírez JC, Gherardi MM, Esteban M. Biology of attenuated modified Vaccinia Virus Ankara recombinant vector in mice: Virus fate and activation of B- and T- cell immune responses in comparison with the Western Reserve strain and advantages as a vaccine. J virol 2000;74:923.
16. Ramírez JC, Gherardi MM, Rodríguez D, Esteban M. Attenuated modified vaccinia virus ankara can be used as an immunizing agent under conditions of preexisting immunity to the vector. J Virol 2000;74:7651-5.
17. Robinson HL, Weinhold KJ. Phase 1 clinical trials of the National Institutes of Health Vaccine Research Center HIV/AIDS candidate vaccines. J Infect Dis 2006;194:1625-7.
18. Belyakov IM, Moss B, Strober W, Berzofsky JA. Mucosal vaccination overcomes the barrier to recombinant vaccinia immunization caused by preexisting poxvirus immunity. Proc Natl Acad Sci U S A 1999;96:4512-7
19. Carnero E, Li W, Borderia AV, Moltedo B, Moran T, García-Sastre A. Optimization of human immunodeficiency virus *gag* expression by Newcastle disease virus vectors for the induction of potent immune responses. J Virol 2009;83:584-97.
20. Bronte V, Wang M, Overwijk WW, Surman DR, Pericle F, Rosenberg SA, *et al.* Apoptotic death of CD81 T lymphocytes after immunization:

induction of a suppressive population of Mac-11/Gr-11 Cells. J Immunol 1998;161:5313-20.
21. Amara RR, Villinger F, Staprans SI, Altman JD, Montefiori DC, Kozyr NL, *et al.* Different patterns of immune responses but similar control of simian immunodeficiency virus 89.6P mucosal challenge by modified vaccinia virus Ankara (MVA) and DNA/MVA vaccines. J Virol 2002;75:7625-31
22. Hutchings CL, Gilbert SC, Hill AV, Moore AC. Novel protein and poxvirus-based vaccine combinations for simultaneous induction of humoral and cell-mediated immunity. J Immunol 2005;175:599-606.
23. Cellini S, Fortini C, Gallerani E, Destro F, Cofano EB, Caputo A, *et al.* Identification of new HIV-1 *Gag*-specific cytotoxic T lymphocyte responses in *BALB/c* mice Virol J 2008;5:81.
24. Kumar S, Seth P. Immunogenicity of recombinant modified vaccinia ankara viruses (rMVA) expressing HIV-1 Indian subtype C *gag*-protease and env-gp120 genes in mice. Viral Immunol 2004;17:574-9.
25. Novitsky V, Rybak N, McLane MF, Gilbert P, Chigwedere P, Klein I, *et al.* Identification of human immunodeficiency virus type 1 subtype C *Gag*-, Tat-, Rev-, and Nef-specific elispot-based cytotoxic T-lymphocyte responses for AIDS vaccine design. J Virol 2001;75:9210-28.
26. Gupta RM, Prasad VV, Rai A, Seth P. Analysis of HIV Type 1 Subtype C full-length *gag* gene sequences from India: Novel observations and plausible implications. AIDS Res Hum Retrovir 2005;21:1066-72.
27. Rolland M, Heckerman D, Deng W, Rousseau CM, Coovadia H, Bishop K, Broad and *Gag*-biased HIV-1 epitope repertoires are associated with lower viral loads. PLoS ONE 2008;3:e1424.
28. Livingston B, Crimi C, Newman M, Higashimoto Y, Appella E, Sidney J, *et al.* A rational strategy to design multiepitope immunogens based on multiple Th lymphocyte epitopes. J Immunol 2002;168:5499-506.
29. Geels MJ, Dubey SA, Anderson K, Baan E, Bakker M, Pollakis G, *et al.* Broad cross-clade T-cell responses to *gag* in individuals infected with human immunodeficiency virus Type 1 non-B clades (A to G): Importance of HLA anchor residue conservation. J Virol 2005;79:11247-58.
30. Frahm N, Korber BT, Adams CM, Szinger JJ, Draenert R, Addo MM, *et al.* Consistent cytotoxic-T-lymphocyte targeting of Immunodominant regions in human immunodeficiency virus across multiple ethnicities. J Virol 2004;78:2187-200.

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