

## Development of Anti HIV Gp120 and HIV Gp41 Peptide Vaccines

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### Abstract

The aim of this preliminary study was to produce anti HIV antibodies against the fragment 579 601 of the HIV gp41 and fragments 308 331 and 421 438 of the HIV gp120 from the human immunodeficiency virus (HIV) in layer hens. Keyhole limpet hemocynin (KLH) was conjugated to HIV synthetic peptides by the glutaraldehyde method after they were dimerized by cysteine oxidation with dimethyl sulfide. Two healthy brown Leghorn layer hens (per immunogen) were injected intramuscularly on the breasts with KLH peptide conjugated vaccines. They were immunized on days 0, 21, 45 and 60. Enzyme linked immunosorbent assays (ELISA) were used to test for anti HIV antibodies and there was a statistical significance in the mean of optical density readings between pre immunized and post immunized animals, proving the formation of specific antibodies. These molecules can potentially be used as therapeutic agents or diagnostic reagents. The limitations of this investigation were the small number of cases, viral neutralization produced by anti HIV antibodies was not tested in cell cultures neither their capacity to inhibit the HIV entry into the CD4+ lymphocyte. We conclude that KLH peptide conjugated vaccines against regions of the gp120 and gp41 effectively produced a strong immune response in egg yolks from layer hens. Despite of limitations of this investigation we report an outcome that encourages us to design and perform a larger study, which can be done in future.

**Keywords:** Anti HIV Gp120; HIV Gp41; Peptide vaccines

### Introduction

The species traditionally chosen for antisera production are mammals especially rabbits but recently there has been a growing use of hens. Immunoglobulin (Ig) Y is the major antibody produced by birds and offers many advantages over antibodies conventionally derived from laboratory animals, for instance it does not activate the complement system [1].

The Joint United Nations Programme on human immunodeficiency virus/ acquired immunodeficiency syndrome (HIV/AIDS; UNAIDS) has reported that the Caribbean is second only to Sub Saharan Africa in world AIDS prevalence [2].

Drugs used in the treatment of HIV/AIDS are expensive and often unavailable in developing countries at low costs. The development of cheaper therapeutic agents could be one of the means of alleviating the problem. The human consumption of therapeutic antibodies, which specifically target HIV proteins, is therefore an important avenue of exploration [3,4]. The aim of this study was to produce anti HIV antibodies in layer hens for their potential use as therapeutic agents or diagnostic reagents.

### Material and Methods

All reagents used in this research were commercially available products from Sigma Aldrich Co. Experiments were repeated 3 times and had similar results.

### HIV immunogens

The immunogens used in these experiments were the keyhole limpet hemocynin (KLH) conjugated to HIV synthetic peptides (fragment 579 601 of the HIV gp41 and fragments 308 331 and 421 438 of the HIV gp120). Below are represented the amino acidic sequences of each peptide and its references.

HIV gp41 (579 601): Arg Ile Leu Ala Val Glu Arg Tyr Leu Lys Asp

Gln Gln Leu Leu Gly Ile Trp Gly Cys Ser Gly Lys [5].

HIV gp120 (308 331): Asn Asn Thr Arg Lys Ser Ile Arg Ile Gln Arg Gly Pro Gly Arg Ala He Val Thr Ile Gly Lys Ile Gly [6].

HIV gp120 (421 438): Lys Gln Phe Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro [7].

### Dimerization of HIV peptides (addition of a C terminal cysteine)

The C terminal cysteine was added to the amino acidic sequences of HIV peptides (fragment 579 601 of the HIV gp41 and fragments 308 331 and 421 438 of the HIV gp120). These fragments were dimerized by cysteine oxidation with dimethyl sulfide [8]. The peptide was dissolved in 5% acetic acid (5 mg/ml), the pH was adjusted to 6 with 1 M (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>, and dimethyl sulfoxide was added to 20% of the final volume. After 4 h at room temperature, the solute was removed and the peptide was dissolved in trifluoroacetic acid and precipitated with cold ether.

### Preparation of HIV immunogens

The fragments of different gp120 or gp41 was conjugated to keyhole limpet hemocyanin (KLH) by the glutaraldehyde method [9] as follows:

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**Received** August 29, 2013; **Accepted** October 10, 2013; **Published** October 14, 2013

**Citation:** Justiz Vaillant AA, Anderson MF, Smikle M, Wisdom B, Mohammed W, et al. (2013) Development of Anti HIV Gp120 and HIV Gp41 Peptide Vaccines. J Vaccines Vaccin 4: 206. doi: [10.4172/2157-7560.1000206](https://doi.org/10.4172/2157-7560.1000206)

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1 mg hemocynin was dissolved in 2 ml 0.1 M borate buffer, pH 10, in a 15 ml glass tube by gentle stirring, 1  $\mu$ mol of synthetic peptide and 0.2 ml 0.3% glutaraldehyde solution were added slowly with stirring at RT. It was allowed to stand for 2 hr at room temperature (RT) until a yellow coloration was observed. To block excess glutaraldehyde, 0.25 ml of 1 M glycine was added and the mixture left for 30 min at RT. The conjugate was then dialyzed against 1L 0.1 M borate buffer, pH 8.3 overnight at 4°C, then against 1L of the same buffer for 8 hr at 4°C. The dialysate was stored at 4°C.

### Chicken immunization

Two healthy brown Leghorn layer hens (per HIV vaccine), aged approximately 6 months, were injected intramuscularly at multiple sites on the breast with the 3 peptide KLH conjugated vaccines. The chickens were immunized on day 0, with 0.2 ml of the immunogens in 0.5 ml complete Freund's adjuvant, and on days 21, 45 and 60 with 0.1 ml of the immunogen in incomplete Freund's adjuvant. The eggs were collected pre and post immunization. The water soluble fraction (WSF), which contains a high IgY content, was separated from the lipid fraction by the partial application of the Polson method (use of chloroform only) [10]. The soluble protein fraction from each egg sample was tested by indirect ELISAs for antibodies to each HIV fragment.

### Indirect ELISA for detection of anti HIV antibodies in chickens

The 96 well polystyrene microplates (U shaped bottom) were coated with 100 ng of 579 601 of the HIV gp41, fragments 308 331 or 421 438 from HIV gp120 in coating buffer for 4 h at 37°C. The microplates were washed 4 times with PBS Tween 20 and blocked with 3% non fat milk in PBS, 50  $\mu$ l/well, 1h at room temperature (RT). The microplates were washed 4 times again. Samples were added (50  $\mu$ l of WSF). After incubation for 1h at RT the microplates were washed 4 times and 50  $\mu$ l of peroxidase labelled anti IgY conjugate diluted 1:30,000 was added. The microplates were then incubated for 1h at RT, washed 4 times. Tetramethylbenzidine (TMB) solution (50  $\mu$ l) was added to each well. After a further incubation of 15 min in the dark, the reaction was stopped and read in a microplate reader at 450 nm. The cutoff value was calculated from the mean optical density (OD) of the negative control plus 0.25. The cut off points of ELISAs for the detection of anti peptide (579 601), anti peptide (308 331) and anti peptide (421 438) were 0.423, 0.4 and 0.446 respectively [1].

### Statistical analysis

Data was analyzed statistically using chi square test and difference was considered significant at  $p < 0.05$  by SPSS software (version 18).

### Results and Discussion

The results of the indirect ELISA for the detection of anti HIV gp120 and gp41 peptide antibodies are shown in Table 1. It demonstrated that all of the samples of egg yolks from immunized chickens were tested positive for the presence of both anti HIV gp120 or anti HIV gp41 antibodies, which were produced from week 3 post immunization and continued through week 8. Table 1 shows statistical analysis of immunogenicity study. There was a statistical significance in the mean of optical density readings of anti HIV antibody detection between pre immunized and post immunized animals, proving that specific antibody production took place.

The chicken and the egg system could be considered as an "large

ELISA	XOD values Pre-immunization (day 0)	XOD values Post-immunization (day 67)	P value
gp41 (579-601)	0.173	0.790	P<0.001
gp120 (308-331)	0.150	0.895	P<0.001
gp120 (421-438)	0.196	0.766	P<0.001

Table 1: Statistical analysis of immunogenicity study.

scale antibody factory". This study showed that the titre of specific antibodies in the egg yolk of immunized chickens was 3-4 times higher than that of the pre immune egg yolk samples. The concentration of anti HIV antibodies was not determined quantitatively but it is estimated that, after immunization, 22.8 g IgY/month is produced. Of this, 2,10% will be specific antibodies against an antigen [11].

It was reported the development of a humoral and mucosal immune response in rabbits fed daily doses of a murine IgA antibody, supported the hypothesis that human exposure to xenogeneic antibodies, most commonly bovine milk immunoglobulins and hyper immune egg yolks, may provoke the production of anti idiotypic antibodies that fight off the original antigen [1,4,12].

Peptides alone will not stimulate antibody production when injected into an animal. Therefore, the peptides are usually conjugated to a large protein molecule known as a "carrier". In this study we used KLH, which is a large protein, in this case peptide carrier complexes are sufficiently large to be recognized, producing strong immune responses in which participate antigen presenting cells, T and B cells.

Kramski et al. reported that colostrum derived anti HIV antibodies offer a cost effective option for preparing the substantial quantities of broadly neutralizing antibodies that would be needed in a low cost topical combination HIV 1 microbicide. Bovine colostrum contains very high concentrations of IgG, and on average 1 kg (500 g/liter) of IgG can be harvested from each immunized cow immediately after calving [13]. Similarly it is possible to produce, in chickens, hyper immune eggs to a variety of antigens due to phylogenetic distance, to which mammalian species, for instance a rabbit, may not produce [11].

This study constitute the first report on the production of anti HIV gp120 and anti HIV gp41 antibodies in layer hens. HIV hyper immune eggs could be used as another alternative therapy for HIV/AIDS individuals and it is supported by the results of the preliminary studies on the effect of the consumption of HIV hyper immune eggs by cats [1]. Alternately, these antibodies could be of a great value as immunological tools in HIV immunodetection and development of topical microbicides to prevent sexually transmitted diseases including HIV infections.

According to UNAIDS/WHO, 2 million people are newly infected with HIV 1 each year [14]. The development of an effective immunotherapy including a vaccine is important to stop the global expansion of the AIDS pandemic [15]. This study addresses the development of 3 HIV peptide vaccines, which targeted crucial epitopes of the envelop proteins: gp120 and gp41.

### Limitations of this Research

There were a small number of cases (layer hens). In addition, the viral neutralization produced by those anti HIV antibodies was not tested in viral cell cultures neither their capacity to inhibit the HIV entry into the CD4+ lymphocyte. There was also no investigation of the immunogenicity of the vaccine in a HIV animal model such as humanized mice or primates.

## Conclusion

The KLH peptide conjugated vaccines against regions of the HIV gp120 and HIV gp41 effectively produced a strong immune response in egg yolks from layer hens. Despite of limitations of this investigation we have reported an outcome that encourages us to design and perform a larger study, which can be done in future.

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