Al-Azhar Bulletin of Science

Volume 29 | Issue 2 Article 6

12-1-2018
Section: Botany, Microbiology and Zoology

ENHANCEMENT OF IMMUNE RESPONSE TO VACCINES AGAINST HIV BY SELF-BOOSTING

Mostafa Hussein
Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt

How to Cite This Article

Hussein, Mostafa (2018) "ENHANCEMENT OF IMMUNE RESPONSE TO VACCINES AGAINST HIV BY SELF-BOOSTING," *Al-Azhar Bulletin of Science*: Vol. 29: Iss. 2, Article 6.
DOI: https://doi.org/10.21608/absb.2018.33807

Al Azhar Bulletin of Science Vol. 29, No. 2 (December), 2018, pp. 9-17.

ENHANCEMENT OF IMMUNE RESPONSE TO VACCINES AGAINST HIV BY SELF-BOOSTING

Mostafa Ahmed Hussein

Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt

ABSTRACT

A major challenge for immunologists has been the development of vaccines able to augment both of humoral and cellular immune responses. One particularly promising approach is the prime–boost strategy, which has been shown to generate high levels of T-cell memory in animal models. In the present study, HIV-1 gp41 (662-683) loaded liposomes were used to determine how does prime–boost strategy would be beneficial for generation of humoral and cellular responses. BALB/c mice were immunized with HIV-1 gp41 (662-683) liposomes and 30 days later they received the booster doseof the same immunogen. HIV-1 gp41 (662-683) specific humoralresponse was evaluated pre-prime, 30days post-prime and 30 days post-boost by ELISA. HIV-1 gp41 (662-683) specific antibodies sharply jumped after boosting by ~40 foldcompared to that generated by priming. Similarly, self-boosting generated considerably HIV-1 gp41 (662-683) specific antigen secreting plasma cells in bone marrow. Overall, the results suggest that self-boosting is very important for generation of specific humoral response for HIV-1 gp41 (662-683). This prime-boost strategy shows a promise to induce both of specific-antibodies and specific antibody secreting cells in bone marrowfor weak immunogens like HIV-1 gp41 (662-683).

Keywords: Self-boosting, prime-boosting, HIV-1 gp41(662-683), serological response, cellular response.

1. INTRODUCTION

The prime–boost strategies are considered as a promising approach for elicitation of humoral and cellular immunity to a variety of pathogens and have demonstrated efficacy in humans **(Woodland, 2004)**. Acquired immunodeficiency syndrome (AIDS) was first reported in 1981 **(CDC, 1981)**. Two years later, the responsible retrovirus was isolated, at the same time, at National Cancer Institute in Bethesda, Maryland, USA (**Gallo*et al*., 1983)** and the Pasteur Institute in France (**Barré-Sinoussi*et al*., 1983).**This virus was later named as Human Immunodeficiency Virus (HIV) by the International Committee on Taxonomy of Viruses in 1986 (**Coffin *et al*., 1986)**.The mature HIV-1 particle is roughly round with diameter of about 100 nm. HIV-1 is enveloped with a thick lipid bilayer membrane derived from the membrane of host cells during budding process. On each HIV virion, there are only about 8–14 envelope glycoprotein spikes**(Zanetti *et al*., 2006 and Zhu *et al*., 2006)**.The HIV-1 envelope glycoprotein composed of the receptor binding domain gp120 and the fusion protein subunit gp41 which catalyzes virus entry and is a major target for therapeutic intervention and for neutralizing antibodies **(Buzon *et al*., 2010)**.Until recently, mapping and structural definition of the HIV-1 has allowed the identification of five sites of vulnerability to neutralizing antibodies on the HIV-1 trimeric complex. These five sites are (1) CD4 binding site (CD4bs), (2) N-glycan V1/V2 loop, (3) N-glycan V3 loop, (4) gp120/gp41 interface, (5) gp41(662-683) **(van Gils and Sanders, 2013; Zhang *et al*., 2016)**.

Most of effective viral vaccines are working by induction of antibodies with potent virus neutralization **(Plotkin, 2010)**. HIV-1 differs from other viruses for which successful vaccines have not been made, because as a highly mutable, integrating retrovirus, it is resistant to both immune responses and antiretroviral therapy upon establishment of a latently infected CD4+ T- cell pool **(Wei *et al*., 2003)**. The HIV-1 gp41 (662-683) known as Membrane Proximal External Region (MPER) is a highly conserved among different HIV-1

clades and tryptophan-rich hydrophobic segment **(Salzwedel *et al.*, 1999)**. HIV-1 gp41 (662-683) segment is critical for fusion and cell entry; hence its conservation is required to maintain its functions. The HIV-1 gp41 (662-683), unlike many discontinuous epitopes, houses linear epitopes for some broadly neutralizing antibodies like 2F5, 4E10, Z13e and 10E8; all isolated from HIV-1 infected persons **(Muster *et al.*, 1994; Cardoso *et al.*, 2005; Nelson *et al.*, 2007; Huang *et al.*, 2012; McCoy and Weiss, 2013)**.

The ability of the human immune system to mount a neutralizing response against HIV-1 gp41 (662-683) and their protective activity in animal models made this regiona promising target for vaccine design aiming to develop a protective neutralizing response against HIV-1 **(Montero *et al.*, 2008; Denner, 2011; Molinos-Albert, 2014)**. However, the elicitation of such neutralizing responses against the HIV-1 gp41 (662-683) is challenging likely because of its poor immunogenicity due to topological constraints **(Montero *et al.*, 2012; Kim *et al.*, 2013)**. The HIV-1 gp41 (662-683) segment is a poorly immunogenic either during natural infection or by vaccination alone or in context of gp160 (**Montero *et al.*, 2012; Kim *et al.*, 2013)**. The present paper was conducted to assess how the booster dose would help in elicitation of peptide-specific antibodies. To assess the primary and secondary immune response and how booster dose affect elicitation of peptide-specific antibodies, BALB/c mice (5 mice per group) were primed with HIV-1 gp41 (662-683) liposomes and 30 days later boosted by the same HIV-1 gp41 (662-683) liposomes.

MATERIAL AND METHODS

All experiments have been done in the Immunobiology laboratory, Dana-Farber Cancer Institute, USA.

HIV-1 gp41 (662-683) liposomes preparation

HIV-1 gp41 (662-683)loaded liposomes were prepared **(Doniu*set al.*, 2016)**by mixing the following components: N-terminally palmitoylated gp41 (662-683) of HIV-1 peptides of clade B HxB2, monophosphoryl lipid A (MPLA) from *Salmonella enterica*serotype Minnesota (Sigma-Aldrich, USA), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2- dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DOPG) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) (Avanti Polar Lipids, Alabaster, AL, USA) at molar percentages 0.5: 0.2: 40: 20: 40. Then, drying them under a nitrogen stream to obtain a thin lipid film and placing them under vacuum at room temperature for overnight. Afterwards, the lipid film was hydrated with PBS pH 7.4 and $CD4^+$ T-helper peptide LACK(Leishmania homolog of receptors for activated C-kinase), even hydration and initial liposome sizing was performed by vortexing 6 times for 30 seconds each at 5-minutes intervals, followed by 6 rounds of flash freezing in liquid nitrogen for 1 minute and thawing at 37°C water bath for 5 minutes, and finally extrusion 21 times through a 200-nm-pore-size polycarbonate membrane (Whatman Inc., USA).

Immunization

Immunizations were performed by intradermal injection with 50µL of HIV1 gp41 (662-683) loaded liposomes being delivered in each hind flank. BALB/c Mice received the first immunization(prime) on day zero and the second immunization(booster dose) on day 30. Sera were collected on day zero (pre-immunization negative control), 30 days post-priming, and 30 days post-boosting**(Figure 1)**. Mice were sacrificed on day 30 post-boost and bone marrow cells were harvested for ELISPOT.

Figure 1: Immunization strategy of BALB/c mice with HIV-1 gp41 (662-683) liposomes.

ELISA Quantification of antigen-specific antibody response

Blood (~50µl) were collected from tail veins of gently-warmed mouse (under a heat lamp) into a polypropylene microcentifuge tube. Blood was maintained at room temperature and was allowed to coagulate. Serum was then separated by centrifugation for 5 minutes in microcentrifuge at high speed (12000 rpm). Serum was collected and stored at -20 °C until assayed.

Immuno 2HB Plates with 96 wells (Thermo Fisher Scientific) were coated overnight with 2 µg/ml streptavidin diluted in PBS (50 µl/well) at 4°C for attachment of biotinylated liposomes. The wells were washed three times with blank buffer(0.1% BSA-PBS)for removing of free streptavidin and blocked for 3 hr with 200µl 1% BSA-PBS to fillover the left empty sides. Blocking solution was discarded and wells were incubated with 0.2% biotinylated polyethylene glycol (PEG) 2000-containing HIV-1 gp41 (662-683) or LACK liposomes (1:50 peptid: lipid ratio & 4:1 DOPC:DOPG ratio) at 32 µg/ml in 100µl 1% BSA-PBS for 2 hr with shaking at room temperature and then for another 2 hr at 4°C. Wells were washed again, incubated overnight at 4°C with serial dilutions of sera (1:100-1:500000) in blank buffer. For positive and negative controls, respectively, anti-MPER recombinant monoclonal antibody 2F5 and blank buffer were used**(Donius*et al.*, 2016)**.

The next day, serum samples were removed, the plate was washed and goat anti-mouse–horseradish peroxidase (HRP) secondary antibody at a 1:2000 dilution was applied for 1 hr at 4°C. Plate was washed two times with 0.1% BSA PBS and two times with PBS. Bound antibody was detected by incubation with *o*-phenylenediamine (OPD) solution in citrate buffer, pH 4.5, for 10 minutes. The OPD reaction was stopped with 2.25M H_2SO_4, and the absorbance was read at 490 nm on a Victor X4 plate reader (Perkin-Elmer, USA).

ELISPOT quantification of peptide-specific antibody-secreting cells

Quantification of HIV1 gp41(662-683)-specific antibody-secreting cells (ASCs) from bone marrow (BM) was performed using ELISPOT assay **(Donius*et al.*, 2016)**. Mouse BM was collected from the combined femurs and tibias by removing the ends of the bones and flushing the cells out with PBS. BM red blood cells were further lysed by 1 ml of Ammonium-Chloride-Potassium (ACK) lysing buffer (Thermo Fisher Scientific) and the BM cells were resuspended in a buffer (0.5% BSA, 2mM EDTA in PBS).

Briefly, 96-well 0.45-µm, hydrophobic, high protein-binding Immobilon-P polyvinylidenedifluoride (PVDF) membrane plates (EMD Millipore, USA) was pretreated with 30 µl 35% ethanol and washed eight times with sterile water. Plates were coated with 100 µl per well of 100 µg/ml HIV-1gp41 (662-683) liposome diluted in PBS (1:50 peptide: lipid ratio; 4:1 DOPC: DOPG ratio) and incubated overnight at 4°C. Plates were washed six times with 0.1% BSA-PBS and blocked with 200 µl/well 1% BSA-PBS for at least 4 hr. Plates then washed once with RPMI 1640 medium supplemented with 10% FBS, glutamine, 2-mercaptoethanol, and penicillin-streptomycin, and then blocked for 1 hr at 37°C with the same medium. BM cells were strained by 70 µm-size strainer, quantified using a hemacytometer, resuspended to 1×10^7 cells/ml, restrained to 70 µm again and added to wells, in triplicate. Broadly neutralizing antibody 2F5-expressing cells were plated as positive controls. Plates incubated overnight at 37°C and 5% CO_2 in a humidified chamber. The next day, wells were washed six times with 0.1% BSA-PBS and blocked for 1.5 hr with 1% BSA-PBS and then 0.6 µg/ml of alkaline phosphatase-conjugated goat anti-mouse secondary antibodies anti-IgG diluted in 1% BSA-PBS added for 1.5 hr at room temperature. Anti-MPER broadly neutralizing antibody 2F5-secreting control cells were visualized using goat anti-human IgG– alkaline phosphatase. Plates were washed

eight times with 0.1% BSA-PBS and spots were developed by the addition of 100 µl/well 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) solution for 5 minutes. Plates were washed extensively with distilled water and dried overnight at room temperature. Spots were quantified using a CTL ImmunoSpot ELISPOT plate reader and ImmunoSpot 3 software (CTL, Shaker Heights, USA).

RESULTS

Ultimately, the quality of the humoral immune response is defined by the quantity and quality of antibodies produced in response to antigen. Using ELISA to assess how boosting modulated the final outcome of the circulating antibody in the sera. HIV-1 gp41 (662-683) loaded liposomes was used for testing how does self-boosting strategy enhance generation of cellular and humoral response to HIV-1 gp41 (662-683) segment. Sera were collected on days zero (pre-immunization control), day 30 post-prime and day 30 post-boost. Then, peptide- (HIV-1 gp41 "662-683" or LACK) arrayed liposomes used for detecting HIV-1 gp41 (662-683) or LACK specific antibodies by ELISA. Blank buffer was used as a negative control.

Peptide-specific antibody response elicited after primary and secondary immunization

ELISA optical density at 490nm reading of the primary immune response at day 30 post immunization to HIV-1 gp41 (662-683) and LACK revealed only a trace antibody response against HIV-1 gp41 (662-683) and LACK, but response was elevated by boosting on day 30. The secondary immune response at day 30 post-boost to HIV-1 gp41 (662-683) and LACK showed increasing of anti-gp41 (662-683) **(Table 1& Figure 2)** and anti-LACK response **(Table 2 and Figure 3)**. HIV-1 gp41 specific antibody 2F5 and buffer blank were used as, respectively, positive and negative controls in all ELISA plates to double check the efficiency of coating peptide/liposomes.

Table 1: ELISA optical density at 490nm of anti-HIV-1 gp41 (662-683).

Sera dilution	Pre-immunization (control)					Average	Day 30 post 1st immunization (30 days post-prime)					Average	Day 30 post 2nd immunization (30 days post-boost)					Average
	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
1:100	0.05	0.05	0.05	0.06	0.07	0.05	0.25	0.98	0.21	0.32	0.67	0.49	3.52	3.51	3.47	3.40	3.44	3.47
1:500	0.04	0.04	0.04	0.04	0.05	0.04	0.10	0.23	0.09	0.10	0.44	0.19	3.52	3.53	3.48	3.41	3.43	3.47
1:1000	0.04	0.04	0.04	0.04	0.04	0.04	0.09	0.15	0.08	0.08	0.30	0.14	3.51	3.56	3.49	3.44	3.45	3.49
1:5000	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.06	0.04	0.05	0.07	0.05	3.32	3.37	3.16	3.20	3.43	3.30
1:10000	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.05	0.04	0.05	0.05	0.05	1.94	2.30	2.15	1.63	3.16	2.23
1:50000	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.64	0.80	0.60	0.56	1.66	0.85
1:100000	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.38	0.40	0.34	0.27	1.06	0.49
1:500000	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.12	0.18	0.15	0.11	0.27	0.16

The numbers 1-5 are referring to immunized mouse code.

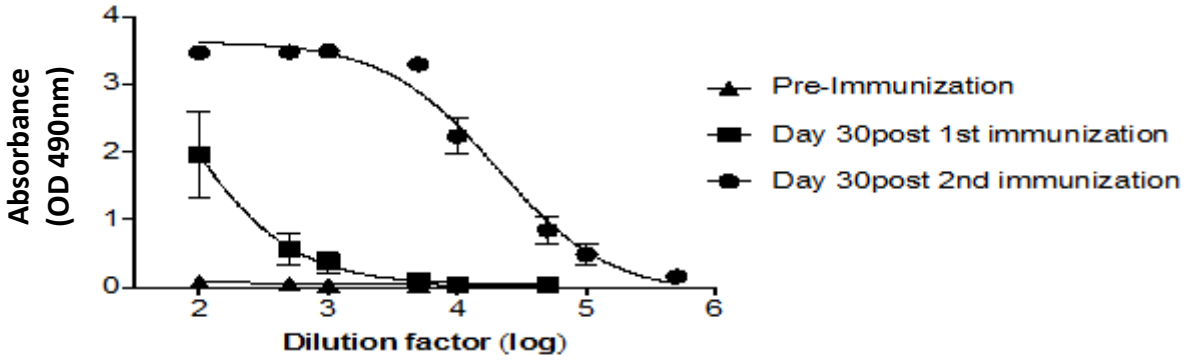

Figure 2: Anti-HIV-1 gp41 (662-683) specific antibody titer.

Table 2: ELISA optical density at 490nm of LACK.

Sera dilution	Pre-immunization (control)					Average	Day 30 post 1st immunization (30 days post-prime)					Average	Day 30 post 2nd immunization (30 days post-boost)					Average
	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
1:100	0.06	0.06	0.05	0.06	0.07	0.06	0.27	2.42	0.79	0.955	0.83	1.08	2.12	3.49	3.22	3.38	3.39	3.12
1:500	0.05	0.04	0.04	0.04	0.05	0.04	0.13	0.97	0.20	0.312	0.21	0.38	1.19	2.13	2.56	3.43	3.05	2.47
1:1000	0.04	0.04	0.04	0.04	0.04	0.04	0.10	0.67	0.14	0.155	0.17	0.27	0.66	1.16	1.17	3.44	3.08	1.90
1:5000	0.04	0.04	0.04	0.04	0.04	0.04	0.06	0.14	0.07	0.104	0.07	0.08	0.20	0.47	0.48	2.95	1.32	1.08
1:10000	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.07	0.04	0.082	0.05	0.05	0.11	0.20	0.33	2.33	0.79	0.75
1:50000	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.051	0.04	0.04	0.06	0.09	0.07	0.70	0.27	0.24
1:100000	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.05	0.06	0.25	0.12	0.11
1:500000	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.08	0.05	0.05

The numbers 1-5 are referring to immunized mouse code.

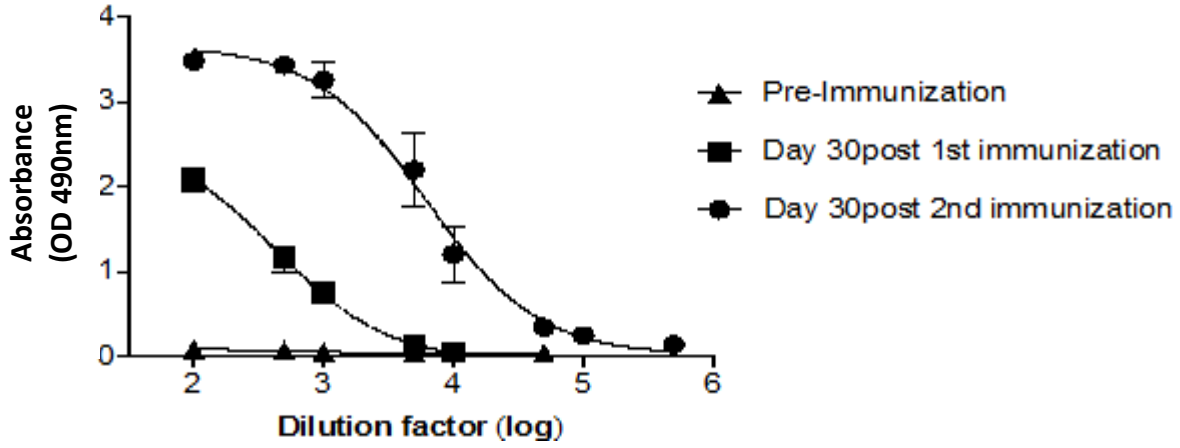

Figure 3: Anti-LACK specific antibody titer.

Quantification of specific ASCs in BM after secondary immunization

The humoral response provided a global view of the total HIV-1 gp41 (662-683)-specific responses; however, a cell-specific quantification of the HIV-1 gp41 (662-683) responses was done. BALB/c mice were immunized at day 0 and day 30 with LACK loaded HIV-1 gp41 (662-683) liposomes and then sacrificed at day 30 post second immunization. The bone marrow cells were isolated and screened against HIV-1 gp41 (662-683) loaded liposomes at a peptide to lipid ratio of 1:50. All immunized mouse showed HIV-1 gp41 (662-683)-specific antigen secreting cells in bone marrow. No significant difference between quantification of HIV-1 gp41-specific antigen secreting cells generated, between immunized mice(average ~37.3 ASCs/10^6 cells) **(Table 3)**.

Table 3: ELISPOT quantification of HIV-1 gp41 (662-683)-specific plasma cells per 1 million of bone marrow on day 30 post second immunization

Immunized Mice code	Day 30 post 2nd immunization				Average
1	25	19	27	21	23
2	40	32	44	36	38
3	41	32	39	36	37
4	55	65	45	39	51
5	34	40	35	41	37.5
Total average					37.3

DISCUSSION

Several papers have highlighted the power of prime–boost strategies in eliciting protective humoral and cellular immunity to a variety of pathogens and have demonstrated efficacy in humans**(Newman, 2002; Woodland, 2004)**. An obvious approach for establishing strong humoral and cellular immunity to specific pathogens is through repeated vaccination. The idea of 'boosting' immune responses has been

around as long as vaccines and repeated administrations with the same vaccine (homologous boosting) have proven very effective for boosting humoral responses. The strategy of prime–boosting is effective for generating high levels of T-cell memory **(Ramshaw and Ramsay, 2000)**.

Coupled with recent advances in understanding of the mechanisms underlying the generation, maintenance and recall of T-cell memory, the field is poised to make tremendous progress **(Woodland, 2004)**. Over the past few years, significant effort has been directed toward developing vaccines designed to promote potent humoral and cellular immunity to these and related pathogens. The induction of long-term humoral and cellular immunity, however, is complex and require elicitation of T cell help and adjuvants. Given the complexity of antigen acquisition and delivery, as well as, possible interplay between competing B cells with specificity for LACK and those for gp41, it was crucial to determine the effects prime–boost strategies on humoral and cellular immunity against the targeted antigen HIV-1 gp41 (662-683) and CD4 helper peptide LACK. In general, immunization of specific antigen with an immunostimulators is an effective way to generate a strong immune response, mediating efficient activation of innate immune cells and antigen presentation to antigen presenting cells with a dose dependent effect **(Seder, and Hill, 2000; Bershteyn*et al*., 2012)**. The tremendous power of prime–boosting was recently further highlighted as strong enough strategy for enhancing T-memory cells aimed at prolonging protection (**Arinaminpathy *et al*., 2012)**.

In the present study, HIV-1 gp41 (662-683) loaded liposome was used to examine how second immunization is very important for modulate antibody responses of the weakly immunogenic HIV-1 gp41 (662-683) antigen. LACK was present both encapsulated in the liposomes and freely in solution in the present study. The increased HIV-1 gp41 (662-683)-specific antibody titersby second immunization compared to single immunization may be explained by increased T cell help by more LACK presentation on the HIV-1 gp41 (662-683)-specific B cells, compensating for the differences in B cell affinity. Therefore, the magnitude of the HIV-1 gp41 (662-683)-specific cellular and serological immune responses was modulated by the surface exposure of LACK as well as the quantity of LACK peptides.

The results here are similar to previous observations seen with carrier protein induced suppression of polysaccharide antigen-specific antibody responses where a population of clonal B cells specific to the carrier exhibit suppression with an effective strength inversely correlated with dose **(Schutze *et al*., 1989)**. Recently, several studies have demonstrated the efficacy of prime–boost vaccination strategies in generating cellular immunity to a variety of pathogens. These include, *M. tuberculosis* **(McShane *et al*., 2001)**, malaria **(Gilbert *et al*., 2002)**, Listeria monocytogenes **(Fensterle*et al*., 1999)**, leishmania**(Gonzalo *et al*., 2002)**, Ebola virus (**Sullivan et al., 2003)**, hepatitis C virus **(Pancholi*et al*., 2003)**, herpes simplex virus **(Meseda *et al*., 2002)**, human papillomavirus (**van der Burg *et al*., 2001)** and hepatitis B virus **(Pancholi*et al*., 2001)**. In conclusion, results show that the methods for prime–boosting with the same antigen enhance the immunogenicity of poor immunogenic B cell target antigen HIV-1 gp41(662-683) by generation of specific cellular and humoral responses.

REFERENCES

Arinaminpathy, N.; Lavine, J.S.; and Grenfell, B.T. (2012): Self-boosting vaccines and their implications for herd immunity. Proc. Natl. Acad. Sci. U.S.A., 109 (49): 20154–20159.

Barré-Sinoussi, F.; Chermann, J.C.; Rey, F.; Nugeyre, M.T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vézinet-Brun, F.; Rouzioux, C.; Rozenbaum, W. and Montagnier, L. (1983): Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency

syndrome (AIDS). Science, 220(4599): 868–871.

Bershteyn, A.; Hanson, M.C.; Crespo, M.P.; Moon, J.J.; Li, A.V.; Suh, H. andIrvine, D.J.(2012): Robust IgG responses to nanograms of antigen using a biomimetic lipid-coated particle vaccine. J. Control Release, 157(3): 354–365.

Buzon, V.; Natrajan, G.; Schibli, D.; Campelo, F.; Kozlov, M.M. and Weissenhorn, W. (2010): Crystal structure of HIV-1 gp41 including both fusion peptide and membrane proximal external regions. PLoSPathog., 6(5): e1000880.

Cardoso, R.M.; Zwick, M.B.; Stanfield, R.L.; Kunert, R; Binley, J.M.; Katinger, H.; Burton, D.R. and Wilson, I.A. (2005): Broadly neutralizing anti-HIV antibody 4E10 recognizes a helical conformation of a highly conserved fusion-associated motif in gp41. Immunity, 22(2): 163–173.

CDC "Centers for disease control and prevention" (1981): Pneumocystis pneumonia--Los Angeles. Morb.Mortal. Wkly. Rep., 30: 250-252.

Coffin, J.; Haase, A.; Levy, J.A.; Montagnier, L.; Oroszlan, S.; Teich, N.; Temin, H.; Toyoshima, K.; Varmus, H.; Vogt, P. and Weiss, R. (1986): What to call the AIDS virus?. Nature, 321(6065): 10.

Denner, J. (2011): Towards an AIDS vaccine: the transmembrane envelope protein as target for broadly neutralizing antibodies. Hum. Vaccin., 7(suppl.): 4–9.

Donius, L.R.; Cheng, Y.; Choi, J.; Sun, Z. Y.; Hanson, M.; Zhang, M.; Gierahn, T. M.; Marquez, S.; Uduman, M.; Kleinstein, S.H.; Irvine, D.; Love, J. C.; Reinherz, E. L.; and Kim, M. (2016): Generation of long-lived bone marrow plasma cells secreting antibodies specific for the HIV-1 gp41 membrane-proximal external region in the absence of polyreactivity, J. Virol., 90(19): 8875–8890.

Fensterle, J.; Grode, L.; Hess, J., and Kaufmann, S.H. (1999): Effective DNA vaccination against listeriosis by prime/boost inoculation with the gene gun. J. Immunol., 163 (8): 4510–4518.

Gallo, R.C.; Sarin, P.S.; Gelmann, E.P.; Robert-Guroff, M.; Richardson, E.; Kalyanaraman, V.S.; Mann, D.; Sidhu, G.D.; Stahl, R.E.; Zolla-Pazner, S.; Leibowitch, J. and Popovic M (1983): Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). Science, 220(4599):865-867.

Gilbert, S.C.; Schneider, J.; Hannan, C.M.; Hu, J.T.; Plebanski, M.; Sinden, R., and Hill, A.V. (2002): Enhanced CD8 T cell immunogenicity and protective efficacy in a mouse malaria model using a recombinant adenoviral vaccine in heterologous prime-boost immunisation regimes. Vaccine, 20(7-8): 1039–1045.

Gonzalo, R.M.; del Real, G.; Rodriguez, J.R.; Rodriguez, D.; Heljasvaara, R.; Lucas, P., and Esteban, M. (2002): A heterologous prime-boost regime using DNA and recombinant vaccinia virus expressing the *Leishmaniainfantum* P36/LACK antigen protects BALB/c mice from cutaneous leishmaniasis. Vaccine, 20(7-8): 1226–1231.

Huang, J.; Ofek, G.; Laub, L.; Louder, M.K.; Doria-Rose, N.A.; Longo, N.S.; Imamichi, H.; Bailer, R.T,; Chakrabarti, B.; Sharma, S.K.; Alam, S.M.; Wang, T.; Yang, Y.; Zhang, B.; Migueles, S.A.; Wyatt, R.; Haynes, B.F.; Kwong, P.D.; Mascola, J.R. and Connors, M. (2012): Broad and potent neutralization of HIV-1 by a gp41-specific human antibody. Nature, 491(7424):406-412.

Kim, M.; Song, L.; Moon, J.; Sun, Z-Y.; Bershteyn, A.; Hanson, M.; Cain, D.; Goka, S.; Kelsoe, G.; Wagner, G.; Irvine, D. and Reinherz, E.L. (2013): Immunogenicity of membrane-bound HIV-1 gp41 membrane-proximal external region (MPER) segments is dominated by residue accessibility and modulated by stereochemistry. J. Biol. Chem., 288(44): 31888–31901.

McCoy, L.E. and Weiss, R.A. (2013): Neutralizing antibodies to HIV-1 induced by immunization. J. Exp. Med., 210(2): 209–223.

McShane, H.; Brookes, R.; Gilbert, S.C. and Hill, A.V. (2001): Enhanced immunogenicity of CD4+ T-cell responses and protective efficacy of a DNA-modified vaccinia virus Ankara prime-boost vaccination regimen

for murine tuberculosis. Infect. Immun., 69(2): 681-686.

Meseda, C.A.; Elkins, K.L.; Merchlinsky, M.J. Weir, J. P. (2002): Prime-boost immunization with DNA and modified vaccinia virus ankara vectors expressing herpes simplex virus-2 glycoprotein D elicits greater specific antibody and cytokine responses than DNA vaccine alone. J. Infect. Dis., 186(8): 1065–1073

Molinos-Albert, L.M.; Carrillo, J.; Curriu, M.; Concepción, M.L.; Marfil, S.; García, E.; Clotet, B. and Blanco, J. (2014): Anti-MPER antibodies with heterogeneous neutralization capacity are detectable in most untreated HIV-1 infected individuals. Retrovirology, 11: 44.

Montero, M.;Gulzar, N. and Klaric, K-A.(2012): Neutralizing epitopes in the MPER of HIV-1 gp41 are influenced by the transmembrane domain and the plasma membrane. J. Virol., 86: 2930–2941.

Montero, M.; van Houten, N. E.; Wang, X. and Scott, J. K. (2008): The membrane-proximal external region of the human immunodeficiency virus type 1 envelope: dominant site of antibody neutralization and target for vaccine design. Microbiol Mol. Biol. Rev., 72(1): 54–84.

Muster, T.; Guinea, R.; Trkola, A.; Purtscher, M.; Klima, A.; Steindl, F.; Palese, P. and Katinger, H. (1994): Cross-neutralizing activity against divergent human immunodeficiency virus type 1 isolates induced by the gp41 sequence ELDKWAS. J. Virol., 68: 4031–4034.

Nelson, J.D.; Brunel, F.M.; Jensen, R.; Crooks, E.T.; Cardoso, R.M.; Wang, M., Hessell, A.; Wilson, I.A.; Binley, J.M.; Dawson, P.E.; Burton, D.R. and Zwick, M.B. (2007): An affinity-enhanced neutralizing antibody against the membrane-proximal external region of human immunodeficiency virus type 1 gp41 recognizes an epitope between those of 2F5 and 4E10. J. Virol., 81(8): 4033-4043.

Newman, M.J. (2002): Heterologous prime-boost vaccination strategies for HIV-1: augmenting cellular immune responses. Curr.Opin.Investig. Drugs, 3: 374–378.

Pancholi, P.; Lee, D.H.; Liu, Q.; Tackney, C.; Taylor, P.; Perkus, M., Andrus, L.; Brotman, B.and Prince, A.M. (2001): DNA prime/canarypox boost—based immunotherapy of chronic hepatitis B virus infection in a chimpanzee. Hepatology, 33(2): 448-454.

Pancholi, P.; Perkus, M.; Tricoche, N.; Liu, Q., and Prince, A.M. (2003): DNA immunization with hepatitis C virus (HCV) polycistronic genes or immunization by HCV DNA priming-recombinant canarypox virus boosting induces immune responses and protection from recombinant HCV-vaccinia virus infection in HLA-A2. 1-transgenic mice. J. Virol., 77(1): 382-390.

Plotkin, S. (2010): Correlates of protection induced by vaccination. Clin. Vaccine Immunol., 17(7): 1055–1065.

Ramshaw, I.A. and Ramsay, A.J. (2000): The prime-boost strategy: exciting prospects for improved vaccination. Immunol., 21: 163 – 165.

Salzwedel, K.; West, J.T. and Hunter, E. (1999): A conserved tryptophan- rich motif in the membrane-proximal region of the human immunodeficiency virus type 1 gp41 ectodomain is important for Envmediated fusion and virus infectivity. J. Virol., 73: 2469–2480.

Schutze, M.P.;Deriaud, E.;Przewlocki, G. and LeClerc, C. (1989): Carrier-induced epitopic suppression is initiated through clonal dominance. J. Immunol., 142: 2635–2640.

Seder, R.A. and Hill, A.V. (2000): Vaccines against intracellular infections requiring cellular immunity. Nature, 406: 793–798

Sullivan, N.J.; Geisbert, T.W.; Geisbert, J.B.; Xu, L.; Yang, Z.Y.; Roederer, M.; Koup, R.A.; Jahrling, P.B. and Nabel, G.J. (2003): Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates. Nature, 424(6949): 681–684.

Van der Burg, S.H.; Kwappenberg, K.M.C.; O'neill, T.; Brandt, R.M.P.; Melief, C.J.M.; Hickling, J.K.andOffringa, R. (2001):. Pre-clinical safety and efficacy of TA-CIN, a recombinant HPV16 L2E6E7 fusion protein vaccine, in homologous and heterologous prime-boost regimens. Vaccine, 19(27): 3652-3660.

Van Gils, M.J. and Sanders, R.W. (2013):

Broadly neutralizing antibodies against HIV-1: templates for a vaccine. Virology, 435: 46–56.

Wei, X.; Decker, J.M.; Wang, S.; Hui, H.; Kappes, J.C.; Wu, X.; Salazar-Gonzalez, J.F.; Salazar, M.G.; Kilby, J.M.; Saag, M.S.; Komarova, N.L.; Nowak, M.A.; Hahn, B.H.; Kwong, P.D. and Shaw, G.M. (2003): Antibody neutralization and escape by HIV-1. Nature, 422(6929): 307–312.

Woodland, D.L. (2004): Jump-starting the immune system: prime-boosting comes of age. Trends Immunol. 25(2):98-104.

Zanetti, G.; Briggs, J.A.; Grunewald, K.; Sattentau, Q.J. and Fuller, S.D. (2006): Cryoelectron tomographic structure of an immunodeficiency virus envelope complex in situ. PLOS Pathog., 2(8):e83.

Zhang, Z.; Guan, Q.; Yuan, H. (2016): HIV-1 Broadly Neutralizing Antibodies: Identification, Development and Vaccine Evaluation. J. AIDS Clin. Res., 7(12): 636.

Zhu, P.; Liu, J.; Bess, J.Jr.; Chertova, E.; Lifson, J.D.; Grisé, H.; Ofek, G.A.; Taylor, K.A. and Roux, K.H. (2006): Distribution and three-dimensional structure of AIDS virus envelope spikes. Nature, 441(7095): 847–852.

الملخص العربي

تحسين الاستجابة المناعية للقاحات ضد فيروس الإيدز عن طريق الجرعة التعزيزية بذات اللقاح

مصطفي أحمد حسين

قسم النبات والميكروبيولوجي – كلية العلوم (بنين) – جامعة الأزهر – مدينة نصر – القاهرة – مصر

تطوير لقاحات قادرة علي إثارة استجابات مناعية خلطية وخلوية تحدي كبير. يعتبر تعزيز اللقاحاتأحد الأساليب الواعدة لتحسين الاستجابات المناعية للقاحات حيث ثبت أنها تولد مستويات عالية من الخلايا التائية الذاكرة. في هذه الدراسة، تم استخدام الجسيمات الشحمية (الليبوسوم) المحملة بالجليكوبروتين-٤١ (٦٦٢-٦٨٣) لفيروس الأيدز كلقاح نموذجي لإختبار مدي كفأة استراتيجية التعزيز بذات اللقاح لتوليد إستجابات مناعية خلطية وخلوية. تم تحصين فئران البالب-س بهذا اللقاح وتم التعزيز بنفس اللقاح بعد ٣٠ يوم من الجرعة الاولي. تم تقييم الاستجابات المناعية الخلطية قبل الجرعة الاولي وبعد ٣٠ يوم من الجرعة الاولي و٣٠ يوم من جرعة التعزيز. أظهرت نتائج الأليزا أن الاجسام المضادة الخاصة بالجليكوبروتين-٤١ (٦٦٢-٦٨٣) لفيروس الأيدز قد ارتفعت بشكل دراماتيكي بعد الجرعة التعزيزية بالمقارنة بالجرعة الاولية. وعلى نحو مشابة، فقد افرز التعزيز وبشكل ملحوظ خلايا بلازما في النخاع العظمي خاصة بالجليكوبروتين-٤١ (٦٦٢-٦٨٣) لفيروس الأيدز. عموما، فان النتائج تشير الي ان استراتيجية الجرعة التعزيزية مهمة جدا لتوليد استجابة مناعية خلطية خاصة. تحث هذه الدراسة الي استخدام استراتيجية الجرعة التعزيزية لتوليد اجسام مضادة خاصة وخلايا بلازما خاصة مع اللقاحات الضعيفة مناعيا مثل الجليكوبروتين-٤١ (٦٦٢-٦٨٣) لفيروس الأيدز.