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Topical Mucosal Vaccination with Angiotensin (1-7) and Feline Immunodeficiency Virus Induces Secretory IgA Responses

Robert A. Larsen[1], Kathleen E. Rodgers[2], W. Martin Kast[2], Diane Da Silva[2], Rosa Altomstone[1], Christopher Meeks[2], Gere S. diZerga[2] and John M. Leedom[1]*

[1]Department of Health Services of Los Angeles, Los Angeles, California, USA
[2]University of Southern California Keck School of Pharmacy, Los Angeles, CA, USA

Abstract

Background: Pre-existing mucosal generated secretory IgA antibodies may prevent transmission of HIV. The present study aimed to characterize mucosal antibodies generated following topical vaccination with a novel mucosal adjuvant, angiotensin (1-7), in combination with a killed feline immunodeficiency virus (FIV) vaccine used as a model antigen.

Methods: Female outbred cats were vaccinated with Fel-O-Vax FIV vaccine agent combined with increasing concentrations of angiotensin (1-7) [A (1-7)] applied topically to oral, vaginal and rectal surfaces weekly for six weeks. Control animals received intramuscular vaccinations with the Fel-O-Vax FIV alone or with A (1-7). Mucosal secretions were evaluated for antibody responses against FIV-p24 antigen or HIV-gp120 antigen.

Results: Topical application of whole killed FIV virus with A (1-7) induced substantial secretory IgA (SIgA)-anti-gp120 antibodies in oral, vaginal and rectal secretions across a wide ranges of A (1-7) dose levels. Intramuscular vaccination of FIV antigen with A (1-7) induced high levels of SIgA-anti-gp120 antibodies at vaginal and rectal sites. The topical application vaccination strategy elicited only very weak systemic immune responses.

Conclusion: Angiotensin (1-7) is a potent mucosal vaccine adjuvant.

Keywords: HIV; FIV; Mucosal immunity; Topical vaccination; Angiotensin (1-7)

Introduction

The AIDS epidemic is sustained largely through sexual transmission of HIV [1-4]. Often, HIV infection appears to be established with just a very few founder viruses [5-8]. As the virus breaches the mucosal barrier there is a vigorous host immune response which almost never is adequate to control disease [9-11]. Augmenting the mucosal immunological barrier prior to exposure to HIV by mucosal immunization may prevent transmission [12].

Local generation of Secretory IgA antibodies (SIgA) constitutes the largest source of antibody in the body [13]. SIgA has been demonstrated to effectively block epithelial penetration of HIV [14] and in sexually active individuals negative for HIV who live with a HIV-positive partner often appear to be protected by specific SIgA in their genital tract [15]. SIgA in saliva has been shown to cleave gp120 effectively neutralizing the ability of HIV to bind and enter host cells [16-19]. Although HIV evolves rapidly, certain regions in the gp120 surface protein remain constant and are required to maintain HIV infectious capability. Antibodies to this gp-120 constant region have broad neutralizing abilities [20-23]. Thus, SIgA antibodies to gp120 would disrupt the CD4 cell binding site complex which is essential for virus-host cell recognition steps and would prevent HIV entry into host cells and the resultant viral replication.

A (1-7) is a member of the renin-angiotensin system that is produced naturally. Renin acts on the parent protein, angiotensinogen to form the 10 amino acid peptide, angiotensin I. Angiotensin 1 is then cleaved by angiotensin converting enzyme to yield an eight amino acid peptide, angiotensin II. A (1-7) lacks the eighth amino acid of angiotensin II and is generated by the action of neutral endopeptidase activity on angiotensin I. A (1-7) interacts with the AT_{1a}, AT_{1b} and Mas receptors found in early and late hematopoietic progenitors and differentiated cell receptors found on lymphocytes (B and T cells), and monocytes [24-29]. *In vivo* investigations demonstrate A (1-7) increases humoral immunity, dendritic and macrophage cell function, all essential components in host immune responses [30-36].

The Feline Immunodeficiency Virus (FIV) is a retrovirus which occurs worldwide in domestic cats and can lead to immunodeficiency similar to HIV [37,38]. FIV shares multiple pathogenic properties with HIV. Both HIV and FIV cause cytopathic effects in lymphocytes leading to CD4+ T-lymphocyte cell depletion and opportunistic infections and both viruses infect T-lymphocytes, employ the CXCR4 co-receptor for cell entry (although this is not the primary binding receptor for HIV), and can be transmitted by blood, sexual secretions and through vertical transmission. Thus, the pathobiology of FIV in cats is not dissimilar to that of HIV in humans. FIV infection has been established in a cat model system through vaginal and rectal challenge, making FIV a suitable candidate for evaluating local immune effects on the potential to interrupt "sexual" transmission of this retroviral agent [39,40]. At present a killed FIV virus vaccine (Fel-O-Vax˚ FIV) has proven efficacy in preventing disease, but not infection [41,42].

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We describe our experience employing A (1-7) in combination with a killed FIV vaccine (Fel-O-Vax° FIV) used as the source of antigen in raising mucosal antibodies to FIV and HIV following topical application. Although the envelope glycoproteins of FIV and HIV share only minor sequence identity there are consensus sequences that exist, particularly in the V3 loop of HIV gp120 and it is predicted that both FIV and HIV secondary structure of the V3 loops of both viruses have a high degree of similarity [43-46].

Methods

Animals

Eleven (11) female outbred cats >24 weeks old that were free of FIV, feline leukemia virus (FeLV) and feline infectious peritonitis virus were employed. The animals were restrained for the application of test articles to mucous membranes and collection of blood and mucosal secretions. Sedation, if necessary, was induced by subcutaneous administration of ketamine hydrochloride (22 mg/kg) and acepromazine maleate (0.1 mg/kg). Euthanasia was accomplished with a lethal overdose of barbiturates. All activities were approved by the Institutional Animal Care and Use Committee and supervised by veterinary staff.

Vaccine preparation

Fel-O-Vax FIV (Fort Dodge Animal Health, Fort Dodge, Iowa) and A (1-7) were combined in a constant volume of 2% hydroxyethyl cellulose (2% HEC). The range of concentrations of A (1-7) ranged from 0 to 10 mg/mL with a constant 0.33 mL of Fel-O-Vax FIV suspended in each mL of the gel applied for vaccination purposes. Two control animals received intramuscular Fel-O-Vax FIV at baseline, week 3 and week 5. One control animal had mucosal vaccination with Fel-O-Vax alone suspended in the 2% HEC without A (1-7) and one control animal received intramuscular Fel-O-Vax FIV with 0.3 mg/kg of A (1-7) at baseline, week 3 and week 5.

Delivery of angiotensin (1-7) and FIV vaccine to mucosal surfaces

2% HEC, a viscous gel, was used as the vehicle for the topical delivery of angiotensin (1-7) and FIV vaccine. The topical vaccine was applied to the mucosal surface weekly for 6 consecutive weeks. 1.0 mL was applied to the muzzle for oral mucosal vaccination and 1.0 mL and 0.2 mL was instilled in the rectal and vaginal vaults, respectively for mucosal vaccination at these sites.

Measurement of local immune response

The Weck-Cel Surgical Spear (Windsor Biomedical, Newton, NH) was used to obtain secretions [47,48]. Once inserted, the sponge was allowed to remain in place for 5 minutes. The secretions were extracted by centrifugation with 300 µL of a buffer solution. The buffer solution was prepared by mixing 50 µL of 100× protease inhibitor cocktail and 250 µL of 10% Igepal (Sigma, St Louis, MO, U.S.A.) with 4.7 ml of phosphate-buffered saline (PBS) containing 0.25% bovine serum albumin (BSA, Fraction V; Sigma).

The local immune response in mucosal secretions was evaluated against the FIV-p24 or HIV-gp120 (Cell Biolabs, Inc., San Diego, CA and ImmunoDiagnostics, Inc., Woburn, MA). Plates were incubated over night at 4°C with antigen, washed and the target sample added and incubated overnight again at 4°C. The antigens consisted of 50 ng/mL FIV-p24 antigen or with 500 ng/mL of the HIV-gp120 antigen fragment (315-329) derived from the constant region of HIV

(Bachem Americans, Inc., Torrance, CA). The plates were washed and the indicator goat anti-feline IgG (Accurate Chemical & Scientific Corp., Westbury, NY, working titer 1:10,000) or goat anti-feline IgA (Accurate Chemical & Scientific Corp., Westbury, NY, working titer 1:1,000) added and incubated for 24 hours at 4°C. Following a final wash, substrate solution was added. The stop solution was added after 15 minutes for the IgG reaction and after 30 minutes for the IgA reaction. Plates were read at 450 nm. A positive titer had a relative absorbance >0.100 above the control sample.

Results

Topical application of A (1-7) and whole killed FIV virus (Fel-O-Vax FIV) did not produce substantial increases in systemic IgG antibody responses (Table 1, Serum Antibody Titers). Intramuscular (IM) vaccination with Fel-O-Vax did produce high levels of serum IgG antibodies that did not appear to translocate into mucosal secretions.

In oral secretions, intramuscular injections of Fel-O-Vax FIV induced only low levels of SIgA-FIV-p24 antibodies (1:4 at week 8), (Table 2), Oral Secretory Antibody Titers]. SIgA-anti-HIV-gp120 antibodies were found in high concentrations with both parenteral and topical vaccination. Comparable levels of SIgA-anti-HIV-gp120 antibody titers could be achieved with exclusively topical application of the FIV antigen and A (1-7). Only low levels of IgG-anti-HIV-gp120 antibodies found in oral secretions were produced by either IM or topical methods of vaccination. The dose response observed with increasing A (1-7) concentrations in the topical vaccine was complex with an initial raise and then decline in SIgA-anti-gp120 antibody titers as A (1-7) concentrations increased.

SIgA-FIV-p24 antibodies were weakly induced in vaginal secretions by intramuscular injection of Fel-O-Vax FIV (undiluted at week 8), (Table 3), and Vaginal Secretory Antibody Titers]. However, topical application of FIV antigen with A (1-7) induced substantial SIgA-anti-HIV-gp120 antibodies (≥1:2,400). Interestingly, IgG anti-HIV-pg120 antibodies found in vaginal secretions were also elicited with either parenteral or topical vaccination.

Once again, SIgA-FIV-p24 antibodies were only weakly inducted in rectal secretions by parenteral vaccination (1:300 and negative at week 8), (Table 4), Rectal Antibody Titers]. But, high levels (≥1:1,200) of SIgA anti-HIV-gp120 were found with either parenteral vaccination or topical application of Fel-O-Vax FIV with A (1-7). IgG levels in rectal secretions were not assessed because of insufficient sample from this site.

Vaccine Strategy	IgG Anti-FIV-p24
	Week 8
Intramuscular (IM)	1:10,000
IM	1:10,000
IM+ A(1-7)	1:20,000
Topical + Fel-O-Vax vaccine and gel only	1:20
0.1 mg A(1-7)	Negative
0.2 mg A(1-7)	1:20
0.3 mg A(1-7)	1:20
0.4 mg A(1-7)	Negative
5.0 mg A(1-7)	Negative
7.5 mg A(1-7)	Negative
10.0 mg A(1-7)	Negative

Table 1: Serum antibody titers.

Vaccine strategy	SIgA anti-FIV-p24[1]				SIgA anti-HIV- gp120[2]	SIgG anti-FIV-p24[2]
	Week 2	Week 4	Week 6	Week 8	Week 8	Week 8
IM	1:256	1:32	1:16	1:4	1:512	1:4
IM	Negative	1:4	1:16	Negative	1:256	Negative
IM+ A(1-7)	Negative	1:4	1:32	1:4	1:4,096	1:4
Topical gel only	Negative	1:4	Negative	Negative	1:16[3]	Negative
0.1 mg A(1-7)	Negative	Negative	1:4	Negative	1:128	Negative
0.2 mg A(1-7)	Negative	Negative	Negative	Negative	1:128	Negative
0.3 mg A(1-7)	Negative	Negative	Negative	Negative	1:2,048	Negative
0.4 mg A(1-7)	1:256	1:4	Negative	1:16	1:128	1:16
5.0 mg A(1-7)	1:4	1:32	1:32	1:16	1:16[3]	1:16
7.5 mg A(1-7)	1:16	Negative	1:32	1:512	Negative	1:128
10.0 mg A(1-7)	Negative	Negative	Negative	Negative	Negative	Negative

[1]Undiluted was the lowest dilution tested. Elution factor 4-fold

[2]1:4 was the lowest dilution tested. Elution factor 4-fold

[3]Sample tested was from week 6.

Table 2: Oral secretory antibody titers.

Vaccine strategy	SIgA anti-FIV-p24[1]				SIgA anti-HIV gp120[2]	SIgG anti-HIV gp120[2]
	Week 2	Week 4	Week 6	Week 8	Week 8	Week 8
IM	1:2,400	1:1,200	1:300	1:300	Negative	1:9,600
IM	Negative	Negative	Negative	Negative	Negative[3]	Not tested
IM+ A(1-7)	Negative	1:300	1:300	1:1,200	1:9,600	Not tested
Topical gel only	1:1,200	Negative	1;300	Negative	Negative[3]	Negative
0.1 mg A(1-7)	1;300	1:300	1:4,800	1:1,200	Negative	Negative
0.2 mg A(1-7)	1:300	1:1,200	1:2,400	1:300	1:4,800	1:76,800
0.3 mg A(1-7)	1:19,200	1:2,400	1:300	Negative	1:2,400	1:4,800
0.4 mg A(1-7)	1:4,800	1:19,200	1:19,200	1:4,800	1:9,600	1:2,400
5.0 mg A(1-7)	Negative	Negative	Negative	Negative	Negative	Negative
7.5 mg A(1-7)	Negative	Negative	Negative	Negative	1:1,200	Negative
10.0 mg A(1-7)	Negative	1:4,800	Negative	Negative	1:38,400	Negative

[1]Undiluted was the lowest dilution tested with an elution factor of 300-fold.

[2]1:4 was the lowest dilution tested with an elution factor of 300-fold.

[3]Sample tested was from week 6.

Table 3: Vaginal secretory antibody titers.

Vaccine strategy	SIgA anti-FIV-p24[1]				SIgA anti-HIV gp120[2]
	Week 2	Week 4	Week 6	Week 8	Week 8
IM	1:300	Negative	Negative	Negative	1:19,200
IM	Not Tested	1:2,400	Negative	1:300	1:1,200[3]
IM+ A(1-7)	Not Tested	1:300	Negative	1:300	1:1,200[3]
Topical gel only	1:300	Negative	Negative	Negative	Negative[3]
0.1 mg A(1-7)	1:300	1:19,200	1:300	1:300	1:9,600
0.2 mg A(1-7)	1:300	1:4,800	Negative	Negative	1:2,400
0.3 mg A(1-7)	1:300	1:300	Negative	Negative	1:19,200
0.4 mg A(1-7)	1:1,200	1:300	1:300	1:1,200	1:9,600
5.0 mg A(1-7)	1:1,200	Negative	Negative	Negative	1:1,200[3]
7.5 mg A(1-7)	Negative	Negative	1:2,400	Negative	1:38,400[3]
10.0 mg A(1-7)	Negative	Negative	Negative	Negative	1:2,400[3]

[1]Undiluted was the lowest dilution tested with an elution factor of 300-fold.

[2]1:4 was the lowest dilution tested with an elution factor of 300-fold.

.Sample tested was from week 6.

[4]Secretory IgG antibodies were not tested at this mucosal site due to insufficient sample.

Table 4: Rectal secretory antibody titers.

Discussion

Vaccines that act at mucosal surfaces offer several potential advantages: 1) virus could be trapped and inactivated at the mucosal surface prior to spread into the host's cells, 2) locally produced immune responses (external to the host) are expected to be more effective in preventing HIV infection since it would effectively block access to the systemic immune system and avoid the consequences of HIV replication in immune cells within the host, 3) mucosal application of a vaccine is painless, easily accomplished and can be widely distributed

to locations with limited medical resources and 4) with A(1-7) used as an adjuvant to stimulate local immune responses it has been possible to stimulate mucosal SIgA while having minimal effect on IgG levels in mucosal secretions. This latter effect may be critical since it is believed that serum IgG may facilitate entry of HIV into host cells and IgA (the monomeric form) may inhibit IgG antibody-dependent cellular cytotoxicity [49-51]. The possible impact of mucosal antibodies on HIV entry into host cells and correlates with rates of HIV transmission is not known.

Our studies have not demonstrated that the mucosal antibodies raised can prevent a FIV infection following a live virus challenge and such investigations are needed, but induction of SIgA antibody production at the mucosal surface following topical vaccination which is sustained for the two month period of observation is promising. Optimizing strategies for mucosal vaccination such as more refined assessments of A (1-7) effects and antigen concentrations, priming with topical application followed by parenteral vaccination (prime/boost) and the schedule of vaccination also require additional study. It is noteworthy that each mucosal surface (vaginal, oral and rectal) responded differently to the topical application of our vaccine materials suggesting that differing dose levels of A (1-7) and antigen may be needed at each mucosal site. A (1-7) appears to increase trafficking of antibody producing cells to mucosal surfaces, where parental Fel-O-Vax FIV alone does not. Thus, this immune adjuvant seems to particularly suit to altering mucosal immunity thereby enhancing the mucosal barrier to infection at the specific site of acquisition of infection.

Progress toward an effective HIV vaccine has been stymied for a quarter century. Adding A (1-7) to vaccine antigen to enhance mucosal immunity against HIV is a novel approach to stimulate mucosal immunity. We believe that A (1-7) recruits hematopoietic stem cells to the mucosal surface at the time of antigen presentation thereby contributing to increase and robust local SIgA antibody production, although an alternative hypothesis is that A (1-7) may promote recruitment and maintenance of plasma B cells in the mucosal environment. Angiotensin(1-7) as an immune adjuvant holds great promise as it is a naturally occurring compound, has a long pharmaceutical shelf life (>10 years), is inexpensive to manufacture and has proven safety in humans with diabetes [52], cancer [53] and HIV making it an ideal candidate for rapid deployment as a vaccine adjuvant.

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