Original Article

Induction of immune responses to a human immunodeficiency virus type 1 epitope by novel chimeric influenza viruses

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ABSTRACT: Mucosal and systemic immune responses play an important role in the prevention of infections, including infection with human immunodeficiency virus type 1 (HIV-1). Influenza virus can efficiently induce mucosal and systemic immune responses, and thus, chimeric influenza viruses expressing the peptides derived from HIV-1 proteins have been generated to elicit immune responses against the inserted peptide. Novel chimeric influenza viruses were generated with full length of the V3-loop of gp120 or cytotoxic T-lymphocyte epitope of gag from HIV-1 inserted into the stalk of NA (NA-V3 and NA-gag, respectively) and the V3-loop was inserted into the intracellular domain of M2 (M2-V3). The immune responses of mice infected with these chimeric influenza viruses were investigated. The intranasal infection of NA-gag induced gag epitope-specific CTLs and the intranasal infection of NA-V3 and M2-V3 induced V3-specific antibodies. The serum from mice infected with NA-V3 neutralized a clinical isolate of HIV-1 and the infection of NA-V3 induced V3-specific secretory antibodies. These results suggest that intranasal infection of these chimeric influenza viruses could induce both humoral and cellular immune responses against an inserted foreign peptide and therefore could be a potential candidate for use as an HIV-1 vaccine.

Keywords: Influenza virus, HIV-1, vaccine, gag, V3-loop

1. Introduction

Recent vaccine development against human immunodeficiency virus type 1 (HIV-1) is intended to stimulate a strong cellular immune response. In the case of HIV-1 infection, HIV-specific cytotoxic T lymphocytes (CTLs) have been detected before neutralizing antibodies are generated (*1,2*). Specific CTL activity correlates with the clinical stage of disease in infected individuals (*3,4*). Therefore, it is thought that the induction of strong CTL responses against HIV-1 could be important to prevent the onset of acquired immune deficiency syndrome (AIDS). HIV-1 gag is one of the most conserved antigens of HIV-1. Many studies towards development of an HIV vaccine are based on induction of a CTL against gag because several reports have shown that long-term nonprogressors have higher levels of gag-specific CTLs (*4*). Neutralizing antibodies may play only a limited role on primary HIV replication because it has proven difficult to induce broadly neutralizing antibodies due to its unusual neutralization-resistant mechanisms, such as masking of neutralizing epitopes and the high mutation rate of HIV-1 (*5,6*). However, neutralizing antibodies controlled primary virus replication in a macaque AIDS model (*7*). Therefore, it is thought that induction of effective neutralizing antibodies would be important for preventing HIV-1 infection as well as controlling primary virus replication. The epitopes that are known to induce neutralizing antibodies include the membrane proximal external region of gp41, the CD4 binding site on gp120, complex glycans on gp120, the CD4-induced epitope in and around the gp120 bridging sheet, and the V3-loop of gp120 (*8*). The V3-loop which is critical for co-receptor recognition is a highly immunogenic region of the virus envelope. In addition, the neutralizing epitope of the V3-loop is formed by a continuous stretch of amino acids in comparison to other neutralizing epitopes. Therefore, the V3-loop has been targeted for the development of vaccines to induce neutralizing antibodies against HIV-1.

The induction of strong CTL responses and neutralizing antibodies against HIV-1 would be required for an HIV vaccine. However, inoculation of recombinant epitopes with adjuvant and inactivated viruses do not efficiently induce mucosal and cellular immune responses. A virus vector has been developed

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to induce both mucosal and cellular immune responses. Influenza A virus is a segmented negative-strand RNA virus and may be a candidate for the development of effective vaccine vectors against various diseases. Reverse-genetics methods have been developed to manipulate the influenza virus genome (*9-11*). Influenza virus can induce the maturation of strong virus-specific CTLs and the secretion of neutralizing antibodies. Significantly, influenza virus elicits mucosal immunity which is thought to be important for preventing the infection of infectious agents, such as HIV-1, through mucosal tissues. Therefore, chimeric influenza viruses have been engineered to express foreign antigens. Influenza virus has two membrane-spanning glycoproteins, hemagglutinin (HA) and neuraminidase (NA), on the envelope. In many cases, foreign antigens have been inserted into HA or NA because HA and NA are viral membrane proteins and anti-HA and anti-NA antibodies that neutralize influenza virus are induced in infected animals. To examine the potential of chimeric influenza virus as a vaccine for HIV-1, a 12-amino-acid peptide derived from the V3-loop of gp120 was inserted into the loop of antigenic site B of the HA and a 15-amino-acid peptide derived from the V3-loop of gp120 was inserted into the stalk of NA. These chimeric viruses can induce both a humoral and a cell-mediated immune response against HIV-1 (*12,13*). Furthermore, a 8-amino-acid peptide derived from gp41 was inserted into the loop of antigenic site B of the HA and the chimeric virus could induce inserted peptide-specific IgA in respiratory, intestinal, and vaginal secretions (*14,15*).

Some chimeric influenza viruses that are candidates for HIV-1 vaccine have been generated, but no chimeric influenza viruses have been generated with the full length of the V3-loop and CTL epitope of gag inserted into viral protein. The foreign peptides were inserted into only NA or HA and whether foreign peptides can be inserted into other viral proteins is not known. In this study, three chimeric influenza A viruses were generated that expressed the V3-loop of HIV-1 env in the NA stalk (NA-V3) and in the intracellular domain of matrix protein 2 (M2), a viral membrane protein, intracellular domain (M2-V3), respectively, and the CTL epitope derived from gag (H-2K^d) in the NA stalk (NA-gag). The infection of NA-gag could induce gag specific CTL and the infection of M2-V3 could induce specific antibodies to the V3-loop by intranasal infection, and the infection of NA-V3 induced antibodies that could neutralize a clinical HIV-1 strain. These experiments demonstrated that chimeric influenza virus expressing the CTL epitope of gag could induce a cell-mediated immune response for gag and chimeric influenza viruses and that full length of the V3-loop inserted into NA and M2 could be generated and induce V3-specific antibodies by intranasal infection.

2. Materials and Methods

2.1. *Cells and viruses*

Cultures of 293T (*16*) and MAGIC-5 (*17*) cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) containing 10% fetal bovine serum (FBS). MDCK and MDBK cells were maintained in Eagle's minimal essential medium (MEM; Invitrogen) containing 10% FBS. LB27.4 cells (H-2b/d) were maintained in RPMI1640 (Invitrogen) containing 10% FCS, 5 mM β-mercaptoethanol, and 1 mM pyruvic acid. Influenza A/WSN/33 (H1N1) virus was grown at 34°C for 48 h in allantoic sacs of 11-day-old embryonated eggs, and the virus titer was determined by a plaque assay.

2.2. *Mouse infection and serum collection*

Six- to 10-week-old female BALB/c mice were housed under conventional conditions and were provided with standard diet and water ad libitum. The mice were anesthetized with diethyl ether and infected intranasally with influenza virus (1.0×10^4 PFU/20 μL) to induce antibodies. Blood was collected from the tail and was allowed to clot for 4 h at room temperature. Thereafter, the sample was centrifuged and the serum was collected. The lungs from infected mice were suspended in saponin extraction buffer (2% saponin in PBS) to permeabilize the cell membrane (*18*), and then incubated at 4°C for 18 h. The mixture was centrifuged and the supernatant was collected as a lung extract.

2.3. *Chimeric influenza viruses*

Chimeric influenza virus expressing the V3-loop of HIV-1 isolate, 2088E34t kindly provided by Dr. T. Shioda (Oosaka University) (*19*), envelope protein (Genebank: #AB002930) in the NA stalk (NA-V3) was generated based on the RNP transfection method (*20*). The pT3NAv, containing the NA gene of A/WSN/33, and A/WSN-HK virus were kindly provided by Dr. P. Palese (*20,21*). A C to G mutation was introduced into NA gene position 184 by PCR to create an *Nsi*I site in pT3NAv (pT3NAv-NsiI). To make pT3NAvENV51, the env fragment was amplified by RT-PCR from the HIV-1 genome. The amplified product was digested with *Nsi*I and inserted into the *Nsi*I site of pT3NAv-NsiI.

Chimeric influenza viruses expressing the V3-loop in the M2 intracellular domain (M2-V3) and expressing the CTL epitope derived from the HIV-1 IIIB gag protein (H-2K^d) in the NA stalk (NA-gag) were generated based on a plasmid-based reverse genetics system (*11*). Vectors for plasmid-based reverse genetics were kindly provided by Dr. Y. Kawaoka (Tokyo University). To make pHH-M2-V3, the V3 region fragment was amplified by PCR from pT3NAvENV51.

The amplified product was digested with *Nsi*I and inserted into the *Nsi*I site of pHH21-M. To make the *Nsi*I site in pHH-NA (pHH-NA-NsiI), a C to G mutation was introduced into the NA gene position 184 by PCR. To make pHH-NA-gag, a primer pair, NsiI-gag198-206f (5′-TCGCCATGCAAATGTTAAAAGAG ACCATCTGCA-3′) and NsiI-gag198-206r (5′-GATGG TCTCTTTTAACATTTGCATGGCGATGCA-3′), were hybridized and inserted into the *Nsi*I site of pHH21-NA-NsiI.

2.4. *CTL assay*

Groups of female BALB/c mice (H-2d), from 8 to 10 weeks old, were anesthetized with diethyl ether and infected intranasally with influenza virus (WSN; 1.0 × 10^4 PFU/50 µL). Spleen cells were prepared from the mice 16 days after infection, and were cultured in the presence of a peptide specific to NP (H-$2K^d$, TYQRTRALV) or gag (H-$2K^d$, AMQMLKETI; 10 µM) for 5 days, and used as effector cells. LB27.4 cells pulsed with the same peptide were incubated with increasing numbers of effector cells for 4 h (*22*), and the LDH levels in the cell culture supernatants were measured according to the manufacturer's protocol (Takara, Kyoto, Japan).

2.5. *ELISA*

Antigens diluted with coating buffer (10 mM carbonate buffer; pH 9.5) were plated onto a 96-well ELISA plate, and then incubated at 37°C for 2 h. Blocking buffer (0.05% Tween-20 and 1% BSA in PBS) was added to the plate, and then incubated at room temperature for 2 h. Serial dilutions of sera in blocking buffer were added to the plate, and incubated at 4°C for 18 h. Bound antibodies were detected with goat anti-mouse IgG antibody conjugated with alkaline phosphatase or goat anti-mouse IgA antibody conjugated with alkaline phosphatase. The plate was stained with *p*-nitrophenyl phosphate as a substrate.

2.6. *MAGI assay*

The neutralization activity was determined by an infection assay (MAGI assay) using MAGIC-5 cells (*17,23*). MAGIC-5 cells, which are derived from HeLa-CD4-LTR-β-gal (MAGI) cells and express CCR5, were seeded and cultured in a 96-well plate (1 × 10^4 cells/well). After removal of the medium, the cells were infected with the virus in the presence of sera diluted with DMEM to 1/50 for 1 h and washed with the culture medium. The cells were cultured in the medium for 48 h, and then fixed with 1% formaldeyde-0.2% glutaraldehyde in PBS for 5 min. The fixed cells were stained with X-gal. Blue-stained cells were counted under a light microscope.

3. Results

3.1. *Pathogenesis of chimeric viruses*

Three chimeric influenza A viruses were generated, one expressing the V3-loop of HIV-1 env (residues 288 to 331) in the NA stalk (NA-V3), another expressing the V3-loop (residues 288 to 339) in the M2 intracellular domain (M2-V3), and a third expressing the CTL epitope derived from gag (residues 198 to 206; H-$2K^d$) in the NA stalk (NA-gag; Figure 1A). We planned

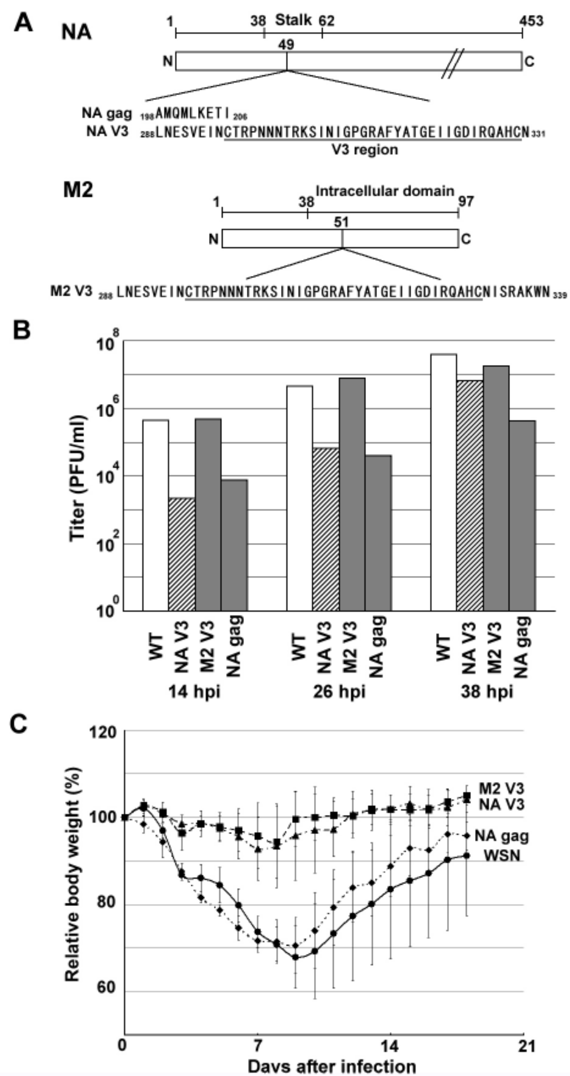

Figure 1. Chimeric influenza viruses containing the gag epitope and V3-loop. (A) Chimeric influenza viruses generated in this study. A 9-amino-acid peptide derived from gag was inserted into the stalk region of NA (NA-gag). A 44-amino-acid peptide derived from env was inserted into the stalk region of NA (NA-env). A 51-amino-acid peptide derived from env was inserted into the intracellular domain of M2. Underline indicates the V3 region in the HIV env. **(B)** Titer of the chimeric influenza viruses. MDBK cells were infected with the viruses at an MOI of 0.01 and the supernatant was collected at 14, 26, and 38 hpi. The titers in the supernatant were determined by a plaque assay. The titers represent the average of two independent experiments. **(C)** Virulence of the chimeric influenza viruses. 1.0 × 10^4 PFU of the viruses were infected into BALB/c mice. The relative body weight of the mice was calculated before infection as 100%.

to generate the chimeric virus which contained a 51-amino-acid peptide derived from env in the NA stalk but three chimeric viruses that contained a 51-amino-acid peptide, a 44-amino-acid peptide, and a 33-amino-acid peptide in the NA stalk were collected. The chimeric virus which contained the 44-amino-acid peptide was well propagated compared to the virus containing the 51-amino-acid peptide. These results suggest that the virus which contained the 44-amino-acid peptide in the stalk of NA was more stable than that containing the 51-amino-acid peptide. Thus, we used the chimeric virus containing the 44-amino-acid peptide in this study. In NA-gag, the virus that lacked the inserted nucleotide was generated after several passages from the first transfection supernatant. But, the virus which lacked the inserted sequence was not generated in several passages after plaque isolation of NA-gag from the mixture. The virus that lacked the inserted epitope might be generated in transfected cells and the inserted peptide in NA-gag was stable at least for several passages. M2-V3 and NA-V3 were stable for several passages. To examine the pathogenesis of the chimeric viruses, the virus titer of the supernatant from cells infected with the viruses was determined and the body weight of mice infected with these viruses was measured. The virus titer in the supernatant at 14 hours post infection (hpi) was 4.5×10^5 PFU/mL (wild type:WT), 2.2×10^3 PFU/mL (NA-V3), 4.9×10^5 PFU/mL (M2-V3), and 7.7×10^3 PFU/mL (NA-gag), respectively (Figure 1B). The virus titer at 38 hpi was 3.9×10^7 PFU/mL (WT), 6.6×10^6 PFU/mL (NA-V3), 1.8×10^7 PFU/mL (M2-V3), and 4.3×10^5 PFU/mL (NA-gag), respectively (Figure 1B). These results suggest that the insertion of a foreign peptide into NA and M2 tends to reduce the growth of the viruses at the early stage of replication but a significant amount of viruses could be recovered 38 h after infection. Next, BALB/c mice were infected with 1.0×10^4 PFU of WT, NA-V3, M2-V3, and NA-gag, respectively, and weight loss was monitored. As shown in Figure 1C, mice infected with either NA-gag or WT virus showed significant weight loss but mice infected with either NA-V3 or M2-V3 did not show any apparent weight loss. These results suggest that NA-V3 and M2-V3 are attenuated in BALB/c mice.

3.2. *Gag-specific CTL response in mice infected with NA-gag*

A CTL assay was performed to examine the gag-specific CTL response in mice infected with NA-gag. The infection of the viruses in mice was confirmed by monitoring weight loss (data not shown). Spleen cells from mice infected with NA-gag were activated with epitope peptide and mixed with LB27.4 cells treated with epitope peptide. Cytotoxity was detected in 15.8% of target cells treated with NP epitope peptide using effector cells derived from mice infected with WT and NA-gag whereas cytotoxity was not detected using effector cells derived from mock-infected mice (Figure 2A). This result suggests that an NP-specific CTL response was detected in cells from mice infected with WT and NA-gag. Cytotoxity was detected in 34.5% of target cells treated with gag epitope peptide using effector cells derived from mouse infected with NA-gag whereas cytotoxity was detected in 10.4% or 13.2% of the target cells using effector cells derived from mock-infected mice and mice infected with WT, respectively. This result suggests that a gag-specific CTL response was detected only in cells from mice infected with NA-gag and the infection of NA-gag can induce a gag-specific CTL response.

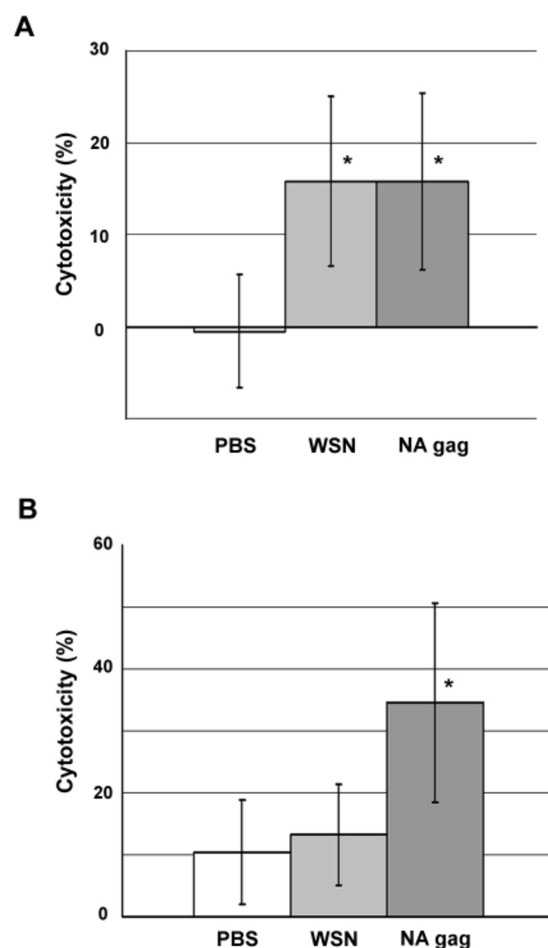

Figure 2. The induction of a specific CTL response by infection with NA-gag. (A) The induction of a specific CTL response against NP. The mice (n = 3) were mock-infected (PBS) or infected intranasally with 1.0×10^4 PFU of WT or NA-gag, and the specific CTL activity was then determined in the spleens. Spleen cells were stimulated for 6 days *in vitro* with 10 μM NP epitope peptide and examined for CTL activity against peptide-coated target cells. E:T ratios were 60:1. *, $p < 0.05$ *vs*. PBS infected mouse. **(B)** The induction of a specific CTL response against gag. The mice (n = 6: PBS and NA-gag and n = 4: WSN) were mock-infected (PBS) or infected intranasally with 1.0×10^4 PFU of WT or NA-gag, and the specific CTL activity was then determined in the spleens. Spleen cells were stimulated for 6 days *in vitro* with 10 μM gag epitope peptide and examined for CTL activity against peptide-coated target cells. E:T ratios were 60:1. *, $p < 0.05$ *vs*. PBS infected mouse.

3.3. *V3-specific antibodies in serum from mice infected with NA-V3 and M2-V3*

The subsequent experiments investigated whether infection of NA-V3 and M2-V3 in mice would induce antibodies specific for the V3 region. Sera from mice infected with NA-V3 and M2-V3 was prepared and V3-specific IgG in the serum was detected by ELISA. The induction of specific antibodies in infected mice (positive mice) was evaluated by comparison of the value obtained from ELISA assays using the serum from infected mice with the maximum value using sera from mock-infected mice. HA-specific IgG was detected in serum from 100% (57 of the positive mice/57 of the infected mice) of mice infected with WSN and V3-specific IgG was not detected in the serum from the mice infected with WSN (Figure 3). HA-specific IgG was detected in the serum from 100% (76 of the positive mice/76 of the mice infected with NA-V3 and 20 of the positive mice/20 of the mice infected with M2-V3) of the mice infected with NA-V3 and M2-V3 and V3-specific IgG was detected from 33% (25 of the positive mice/76 of the mice infected with NA-V3) and 25% (5 of the positive mice/20 of the mice infected with M2-V3), respectively (Figure 3). These results suggest that the infection of NA-V3 and M2-V3 can induce specific antibodies for the V3 region.

3.4. *Secretory antibodies in mice infected with NA-V3 and M2-V3*

Secretory antibodies are important for preventing viral infection. Therefore, V3-specific IgA was measured in serum and lung extracts from mice infected with NA-V3 and M2-V3 by ELISA. V3-specific IgA in serum was detected from 3% (2 of the positive mice/76 of the mice infected with NA-V3) and 5% (1 positive mouse/20 of the mice infected with M2-V3), respectively (Figure 3). HA-specific IgA was detected in the lung extract from 94% (29 of the positive mice/31 of the mice infected with WSN), 98% (54 of the positive mice/55 of the mice infected with NA-V3), and 100% (20 of the positive mice/20 of the mice infected with M2-V3), respectively (Figure 4). No V3-specific IgA was detected in the extract from mice infected with WSN and was detected in the extract from 18% (10 of the positive mice/55 of the mice infected with NA-V3) and 5% (1 positive mouse/20 of the mice infected with M2-V3), respectively (Figure 4). These results suggest that an infection with NA-V3 and M2-V3 can induce the secretion of V3-specific IgA in lungs.

3.5. *Neutralization of clinically isolated HIV-1*

A neutralization assay was performed to determine whether these antibodies have neutralization activity

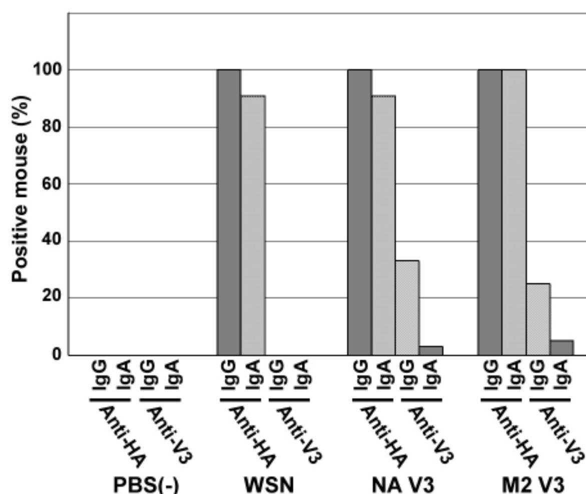

Figure 3. The induction of systemic antibodies by infection with NA-env and M2-env virus. The mice were infected intranasally with 1.0×10^4 PFU of each virus. Serum was collected 12 weeks after infection. Anti-HA IgG and IgA and anti-V3 IgG and IgA in the serum were detected by ELISA.

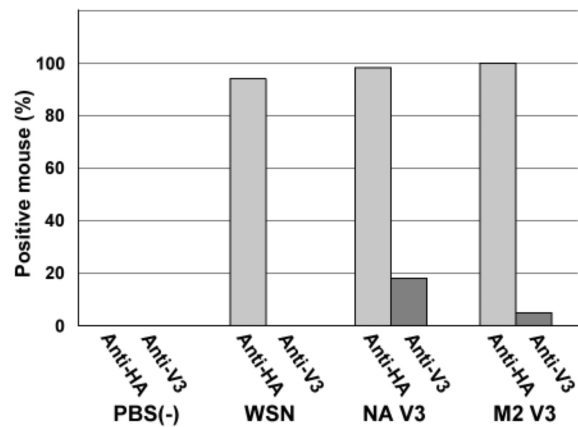

Figure 4. The induction of mucosal antibodies by infection with NA-env and M2-env virus. The mice were infected intranasally with 1.0×10^4 PFU of each virus. Lung extracts were prepared 12 weeks after infection. Anti-HA IgA and anti-V3 IgA were detected by ELISA.

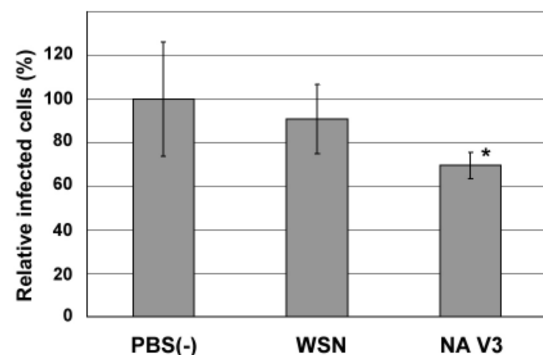

Figure 5. Neutralizing activities against a clinically isolated strain of HIV. The sera from the mice infected with each virus was diluted 50-fold and co-cultured with an isolated strain of HIV, YU-20, for 1 h. The efficiency of HIV infection was estimated by a MAGI assay. The infectious units resulting from the serum of the PBS infected mouse were calculated as 100%. *, $p < 0.05$ *vs*. PBS mock-infected and WSN infected mouse.

against clinically isolated HIV-1. The cells were infected with the clinically isolated HIV-1 in the presence of serum from the mouse infected with NA-V3. MAGIC-5 cells that were derived from MAGI cells were used to stain infected cells by MAGI assay (*17,23*). No neutralization activity to HIV-1 was detected in serum from the mouse infected with WSN (Figure 5). The number of infected cells decreased to 69.5% when the cells were infected with the HIV-1 in the presence of the serum from mice infected with NA-V3 (Figure 5). This result suggests that the V3-specific antibodies induced in mice infected with NA-V3 and the sera from mice infected with NA-V3 partially neutralized the infection of clinically isolated HIV-1.

4. Discussion

Chimeric influenza viruses expressing a part of the V3-loop inserted into NA or HA were generated and examined for their immunogenicity in mice. In this paper, the novel chimeric influenza viruses possessing full length of the V3-loop or CTL epitope of gag were generated and examined for their immunogenicity. These results, for the first time, demonstrated that a foreign peptide could be inserted into the M2 intracellular domain and the chimeric influenza virus could induce antibodies against the inserted peptide. M2 functions as a proton channel and the intracellular domain of M2 is essential for the function of the M2 channel (*24,25*). In M2-V3, the V3-loop was inserted into the region that connects the transmembrane domain to the C-terminal amphipathic helix (*26*). Therefore, the inserted peptide did not apparently affect the proton channel function of M2. NA-V3 was highly attenuated in mice but NA-gag was not (Figure 1C). The length of NA stalk affects the host range of the influenza virus (*27,28*). NA has neuraminidase activity and, thus, is important for releasing progeny virus from the cells. The virus which has mutations in segment 6 to reduce the synthesis of NA mRNA was attenuated in mice, suggesting that the activity of NA is one important factor for attenuation in mice (*29*). In addition, the WSN strain can replicate without trypsin because the NA of the WSN strain binds to serum plasminogen (*30*). The insertion of a foreign peptide into the NA stalk affects the activities of NA and, consequently, the pathogenesis of these chimeric viruses for mice is changed. The virus containing FLAG tag sequence (7 amino acids) in NA stalk was attenuated in mice (*28*) but NA-gag was not. These results suggest that not only length but also amino acid sequence of NA stalk affects the activity of NA.

The infection of NA-gag could induce a gag-specific CTL response (Figure 2). The cytotoxicity against cells expressing the gag epitope was about 25% and this rate was lower than that of a previous study (*31*). The cytotoxicity against cells expressing NP epitope was induced by the infection of NA-gag but the rate was also lower than that of a previous study (*32*). In this study, CTLs were detected using non-RI method. The sensitivity for detection of cell death with a non-RI method is slightly lower than that with an RI-method, and thus, the cytotoxicity in this study is slightly lower than that in the previous study. In spite of slightly lower sensitivity, a non-RI method is used for CTL detection because of its safety. It was reported that the specific CTL against NP epitopes in influenza infected mice was detected by a non-RI method (*33*). Thus, we concluded that specific CTLs against the NP epitope and gag epitope were induced by the infection of NA-gag.

Secretory IgAs were induced in 20% of mice infected with NA-V3 and in 5% of mice infected with M2-V3 (Figure 4). The V3-loop was inserted into the intracellular domain of M2 in M2-V3 but into the extracellular domain of NA in NA-V3. Mucosal immunity is important for preventing viral infection and neutralizing antibodies are induced to the domains that are outside of the viral membrane. Thus, the infection of M2-V3 could not induce secretory IgAs efficiently in comparison to that of NA-V3. In addition, the chimeric virus expressing the gag epitope inserted into the stalk of NA could induce a gag-specific CTL response (Figure 2). Chimeric influenza virus expressing a foreign peptide inserted into the stalk of NA is a candidate for an effective vaccine against various diseases because this chimeric virus can induce both systemic and mucosal immunity. The infection of M2-V3 induced anti-V3 IgG in serum (Figure 3). The vaccination of M2-V3 would not prevent mucosal infection of HIV-1 but would prevent infection in blood. The infection of M2-V3 could not induce secretory IgA against V3 efficiently but chimeric influenza virus expressing different foreign peptides inserted into the stalk of NA and the intracellular domain of M2 could be generated and the infection of these chimeric viruses could induce immune responses against both foreign peptides. Although the percentage of IgA-induced mice was limited in the present experiment, a combination with any adjuvant will increase the immunological response of mice. Further experiments are presently on going to explore this possibility.

This study demonstrated that about 50-amino-acids of a foreign peptide could be inserted into the intracellular domain of M2 and the stalk of NA and these chimeric influenza viruses could induce immune responses against foreign peptides. The improvement of reverse-genetics methods to manipulate the influenza virus genome generates influenza viruses that express the full-length of foreign proteins (*34,35*). However, the advantage of chimeric influenza viruses with foreign peptides inserted into viral proteins is that the immune response against chimeric influenza virus is almost the same as that against wild type influenza virus.

Therefore, a chimeric influenza virus vaccine could be safely used in a manner similar to that of an attenuated influenza virus vaccine. The chimeric influenza virus may therefore be a potentially promising vaccine candidate, not only against HIV, but also against other infectious diseases.

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