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Mucosal Administration of Completely Non-Replicative Vaccinia Virus Recombinant Dairen I strain Elicits Effective Mucosal and Systemic Immunity

N. Yoshino*,†, M. Kanekiyo†, Y. Hagiwara‡, T. Okamura†, K. Someya†, K. Matsuo†, Y. Ami§, S. Sato*, N. Yamamoto† & M. Honda†

*Department of Microbiology, School of Medicine, Iwate Medical University, Iwate; †AIDS Research Center, National Institute of Infectious Diseases, Tokyo; ‡Research Center for Biologicals, The Kitasato Institute, Saitama; and §Division of Experimental Animals Research, National Institute of Infectious Diseases, Tokyo, Japan

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Correspondence to: N. Yoshino, Department of Microbiology, School of Medicine, Iwate Medical University, 19-1 Uchimaru, Morioka, Iwate 020-8505, Japan. E-mail: nyoshino@iwate-med.ac.jp

Abstract

We studied the immunogenicity of completely replication-deficient vaccinia virus Dairen I strain recombinant encoding simian immunodeficiency virus (SIV) *gag/pol* (rDIs) in both mucosal and systemic compartments. When administered either intranasally or intragastrically, rDIs elicited enhanced levels of both SIV Gag p27-specific IgA antibodies and specific plasma antibodies, and the enhanced immunity persisted for the 1-year of observation by intranasal immunization. Increases were observed in antigen-specific IgA antibody-forming cells (AFC) in intestinal mucosal tissues and in IgG AFC in spleens. Furthermore, induction of type 1 and 2 helper cytokines in $CD4^+$ spleen T cells and of $CD8^+$ IFN-γ spot-forming cells in mucosal tissues was observed in the intranasally immunized mice. Moreover, not even high-dose rDIs generated an *SIV* gene signal in the brain tissues of immunized mice. These findings suggest that mucosal immunization with the DIs recombinant hold promise as a safe mucosal vector.

Introduction

Human immunodeficiency virus type-1 (HIV-1), like most infectious agents, gains entry into the host via the mucosal surfaces. Immunoprophylaxis by the mucosal route is an important approach to control mucosally transmitted infections. Moreover, because most generic immunization procedures for vaccinia virus require needles, mucosal vaccines pose the risk of cross-contamination due to needle reuse. But mucosal immunization would eliminate such a risk.

A number of groups have demonstrated the efficacy of poxvirus as a mucosal vaccine vector. Recombinant modified vaccinia virus Ankara (rMVA) expressing HIV-1 89.6 gp160 induced both mucosal and systemic cytotoxic T-lymphocyte responses in intrarectally immunized mice [1]. The MVA vector was tested in multiple prime-boost vaccine protection studies in macaques [2, 3]. In another study, NYVAC/SIV_{gpe}, the highly attenuated poxvirus vector NYVAC, encoding the SIV_{mak6w} *gag*, *pol*, and *env*, was administered via various routes and its immunogenicity against simian immunodeficiency virus (SIV) Gag peptide was assessed. This NYVAC study found that mucosal immunization routes were effective in inducing mucosal immune responses [4]. Obtaining the appropriate balance between safety and immunogenicity is a critical issue for the development of any vaccine. Some of vector systems, such as those based on replication-deficient poxviruses, are readily established and have undergone clinical testing in humans, mainly for safety and, to some extent, for immunogenicity [5–7].

In this study, we selected the completely replication-deficient vaccinia virus, Dairen I strain (DIs) for the vaccinia vector. A highly attenuated mutant of vaccinia virus obtained by successive 1-day egg passages of the DIE virus [8–10], DIs replicates only in chick embryo fibroblast cells and is not pathogenic to mice, guinea pigs or rabbits [8]. Although completely non-virulent, the recombinant virus efficiently produces foreign gene products [11, 12]. When systemically administered,

recombinants of DIs have induced specific immunities in mice and non-human primates [11–14]. Furthermore, the efficacy of prime-boost regimen that primed with recombinant *Mycobacterium bovis* bacillus Calmette-Guérin expressing SIV *gag* or DNA encoding SIV *gag* and *pol* genes and boosted by DIs recombinants have been shown in macaques [13, 14].

Although parenterally administered vaccines induce protective immune responses, they are less able to induce the mucosal immune responses needed to prevent infection at the site of initial contact between the host and the infectious agent [15]. However, no study has as yet been performed to determine the potential of recombinant DIs (rDIs) as a mucosal vaccine vector in experimental animals. In this study, we assessed the capability of rDIs to induce mucosal and systemic immunity in animals.

Materials and methods

Recombinant vaccinia virus vectors. We propagated rDIsSIV*gag/pol* as a candidate vaccine expressing full-length SIV *gag* and rDIs expressing LacZ (rDIsLacZ) as a control vector in chick embryo fibroblast cells and prepared them as previously described [11, 12]. Dr Bernard Moss (Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA) generously supplied the recombinant modified vaccinia virus Ankara (rMVA) expressing SIV *gag* (rMVA-SIV*gag/pol*) and non-recombinant MVA 74LVD6 as the parent strain of rMVA-SIV*gag/pol*. These vaccinia virus vectors were stored at −80 °C until used.

Mice. Five-week-old C57BL/6 mice (H-2^b) were purchased from Charles River Japan, Inc. (Yokohama, Japan). Mice were acclimated to the experimental animal facility for 1 week before being used in experiments and were maintained in the facility under pathogen-free conditions. All experimental procedures were conducted following the guidelines established by the National Institute of Infectious Diseases, Japan. The study was conducted in a biosafety-level 2 facility with the approval of an institutional committee for biosafety and in accordance with the requirements of the World Health Organization.

Immunization of mice. Several groups of mice were immunized with rDIsSIV*gag/pol* by intranasal, intragastrical or intradermal routes. Similar results were obtained when experiments were conducted for a second or third time. For intranasal immunization, the mice were lightly anesthetized with ketamine before being immunized with a 5-μl aliquot (2.5 μl/nostril) of PBS containing several concentrations of rDIsSIV*gag/pol*, rDIsLacZ, rMVA-SIV*gag/pol* or MVA 74LVD6. For intragastrical immunization, mice were deprived of food for 12 h before immunization and neutralized stomach acidity [16]. The mice were immunized with a 250-μl aliquot of PBS containing several concentrations of rDIsSIV*gag/pol* by intragastrical gavage. For intradermal immunization, the mice were lightly anesthetized with ketamine and immunized with a 50-μl aliquot of PBS containing several concentrations of rDIsSIV*gag/pol* in the inguinal region.

Sampling of plasma and fecal pellets. Blood and fecal pellets were collected 1 week after the last immunization, and then at 2 to 8-week intervals for 49 weeks. The collection and preparation methods have been previously described [17]. Samples of plasma and fecal extract were stored at −80 °C until needed.

Detection of p27-specific antibody production by enzyme-linked immunosorbent assay. Simian immunodeficiency virus Gag p27-specific antibody (Ab) titres in plasma and fecal extracts were determined by an endpoint enzyme-linked immunosorbent assay (ELISA). The assay was conducted in 96-well plates (Nalge Nunc International, Rochester, NY, USA), that had been coated with 1 μg/ml of SIV Gag p27 (recombinant Gag p27 SIV_{mac251}; ImmunoDiagnostics, Inc., Woburn, MA, USA) in PBS. Twofold serial dilutions of samples were added after blocking with 1% BSA–PBS. Horseradish peroxidase-conjugated goat anti-mouse IgG (H + L), IgM, or IgA Ab (Southern Biotechnology Associates, Inc., Birmingham, AL, USA) were used to detect the p27-specific Ab, which were then developed at room temperature with TMB+ substrate-chromogen (DAKO, Carpinteria, CA, USA). Endpoint titres were expressed as the last dilution that gave an optical density at 450 nm (OD_{450}) of ≥ 0.1 OD units above the OD_{450} of negative controls after 15-minute incubation. Incubations were terminated by addition of 0.5 M H_2SO_4.

Preparation of single-cell suspensions. Single-cell suspensions were obtained from spleen, submandibular lymph nodes (SMLN), mesenteric lymph nodes (MLN), Peyer's patches (PP), small intestinal lamina propria (i-LP), small intestinal intraepithelial lymphocytes (IEL), nasal lamina propria (n-LP), and nasopharynx-associated lymphoid tissue (NALT) ,and then prepared according to the protocols described elsewhere [17]. The cells were washed and resuspended in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, antibiotics and 55 μM 2-mercaptoethanol (Sigma-Aldrich Co., St Louis, MO, USA) (complete medium).

Detection of p27-specific Ab-forming cells by enzyme-linked immuno spot assay. Simian immunodeficiency virus p27-specific IgG, IgM and IgA Ab-forming cells (AFC) in the mucosal and systemic tissues of mice were determined by enzyme-linked immuno spot (ELISPOT) assay which was conducted in 96-well filter plates (Millititer HA; Millipore Co., Bedford, MA, USA). Plates were coated with 1 μg/ml of SIV p27 in PBS. After blocking with complete medium, single-cell suspensions of mononuclear

cells from the spleen, MLN, PP, i-LP, n-LP and NALT were added at various concentrations into each well and incubated for 4 hours at 37 °C in 5% CO_2. SIV p27-specific AFC were detected by alkaline phosphatase-conjugated goat anti-mouse IgG (H + L), IgM or IgA Ab and developed with Nitro blue tetrazolium chloride/5-Bromo-4-chloro-3-indolyl phosphate, toluidine salt (Roche Diagnostics GmbH, Penzberg, Germany). After being washed with water, the palates were dried and p27-specific AFC were quantitated with the aid of stereomicroscope.

Analysis of the cytokine production of SIV Gag peptide-specific $CD4^+$ T cells. $CD4^+$ T cells were isolated from spleen by auto MACS (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). The purified T-cell fractions were > 97% $CD4^+$ and were > 99% viable. Cells were resuspended in complete medium, and purified $CD4^+$ T cells (4×10^6 cells/ml) were cultured with or without 50 μg/ml of overlapping Gag peptides [12] in the presence of T cell-depleted, mitomycin C-treated splenic antigen-presenting cells (APC) at 37 °C in 5% CO_2. Culture supernatants were collected after 5 days, and IFN-γ, IL-4 and IL-10 were measured by ELISA kit (eBioscience, San Diego, CA, USA). The levels of Gag-specific cytokine production were calculated by subtracting the results of control cultures (e.g. without Gag peptide stimulation) from those of Gag peptide-stimulated cultures.

Analysis of IFN-γ production of SIV Gag peptide-specific $CD8^+$ T cells. To prepare APC for this assay, $CD11c^+$ dendritic cells were isolated from the spleens of naive mice by auto MACS and then resuspended into complete medium before being added into 96-well anti-mouse IFN-γ Ab-coated (R&D Systems, Inc., Minneapolis, MN, USA), sterilized nitrocellulose plates at the rate of 1×10^4 APC/well. The APC were then incubated with or without 50 μg/ml of overlapping Gag peptides for 24 hours at 37 °C in 5% CO_2. $CD8^+$ T cells were isolated from spleen, SMLN, PP, and IEL by auto MACS. The enriched $CD8^+$ cells were added to APC-cultured wells and incubated for 24 hours at 37 °C in 5% CO_2. IFN-γ spot-forming cells (SFC) were detected by ELISPOT kit (R&D Systems, Inc.) and quantitated with the aid of stereomicroscope. The number of Gag-specific IFN-γ SFC was calculated by subtracting the results of the control culture (e.g. without Gag peptide stimulation) from those of the peptide-stimulated culture. In contrast, the calculated numbers of Gag-specific IFN-γ SFC of naive mice were < $20/10^6$ cells.

Detection of rDIsSIVgag/pol in the central nervous system by nested reverse transcription PCR. To determine the dissemination of intranasally administered rDIsSIV*gag/pol* in the brain, a nested DNA-PCR and reverse transcription PCR (RT-PCR) were used to amplify a fragment of the SIV *gag* gene [18]. Mice were intranasally inoculated with 10^6 plaque-forming units (PFU) of rDIsSIV*gag/pol*. After 48 hours, brains were removed and the olfactory bulbs, cerebellum and cerebrum were sectioned. The DNA and mRNA were extracted from the olfactory bulbs, cerebellum and cerebrum by the DNeasy tissue kit (QIAGEN, KJ Venlo, The Netherlands) and the RNeasy mini kit (QIAGEN), respectively. RT-PCR was performed using One-Step RT-PCR kit (Takara Bio Inc., Shiga, Japan). Reverse transcription was carried out at 50 °C for 30 min with avian myeloblastosis virus reverse transcriptase. The initial and nested PCR protocols and the sequences of the primers have been described elsewhere [18]. After the second amplification, 10 μl of nested PCR-amplified product was run on 1.0% agarose gel, and DNA bands were visualized by staining with ethidium bromide. The lowest concentration of plasmid SIV DNA that could be detected with this PCR method in the first amplification with outer gag primer pair was 10^3 copies. Upon further amplification with nested/internal gag primers, a single copy of plasmid DNA could be routinely detected [18].

Statistical Analysis. The results are expressed as the mean + the standard deviation (SD). Normally distributed variables were compared by using the two-tailed Student's *t* test and non-normally distributed variables by the two-tailed Mann–Whitney *U*-test. Probability value of < 0.05 was considered significant.

Results

Induction of SIV p27-specific IgA Ab responses in mucosal secretions

We ran an endpoint ELISA to determine the levels of SIV p27-specific IgA Ab in the fecal extracts by collecting fecal pellets at 1 week after the last immunization. SIV p27-specific IgA Ab were detected in the fecal extracts from all mice that had been immunized intranasally with 10^6 PFU of rDIsSIV*gag/pol* and in seven of the eight (88%) mice given 10^5 PFU of rDIsSIV*gag/pol* (Fig. 1). In contrast, p27-specific IgA Ab were detected in the fecal extracts from five of eight (63%) mice given 10^6 PFU of rDIsSIV*gag/pol* intragastrically. As a reference, SIV p27-specific IgA Ab were also detected in the fecal extracts of mice immunized intranasally with 10^3–10^6 PFU of rMVA-SIV*gag/pol* (Fig. 1). The titres of p27-specific IgA Ab in fecal extracts of mice immunized intranasally with 10^6 PFU of rDIsSIV*gag/pol* were significantly higher than in those mice immunized intranasally with the same dose of rMVA-SIV*gag/pol* ($P = 0.026$). No SIV p27-specific IgA Ab were detected in the fecal extracts of controls immunized with either rDIsLacZ or MVA 74LVD6 (Fig. 1). These results show that mucosal vaccination with rDIsSIV*gag/pol* can induce antigen (Ag)-specific humoral immunity in mucosal compartment.

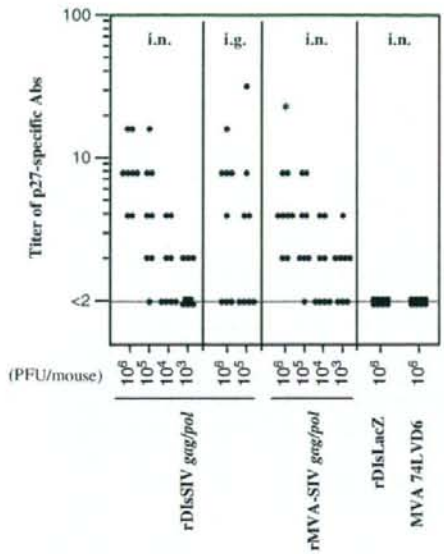

Figure 1 Titres of SIV p27-specific IgA Ab in fecal extracts of mice immunized with rDIsSIV*gag/pol* or rMVA-SIV*gag/pol*. Fecal pellets were collected at 1 week after the last immunization. The titres of p27-specific IgA Ab in the fecal extracts of mice immunized with rDIs-SIV*gag/pol* or rMVA-SIV*gag/pol* were determined using an endpoint ELISA. The data are representative of two separate experiments. Each group was compared by a two-tailed Mann–Whitney *U*-test. Significant differences between rDIs group and rMVA group are indicated by asterisks (*$P < 0.05$). in, intranasal; ig, intragastrical.

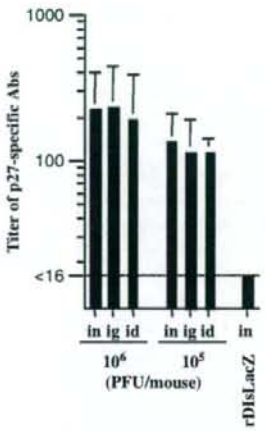

Figure 2 Comparison of SIV p27-specific IgG Ab responses in the plasma of mice immunized via different routes. Plasma samples were collected at 1 week after the last immunization. The titres of p27-specific IgG Ab in the plasma of mice immunized with 10^5 or 10^6 PFU of rDIsSIV*gag/pol* were determined using an endpoint ELISA. The data are shown as the mean titre + SD for 12 mice in each experimental group. Data are representative of three separate experiments. in, intranasal; ig, intragastrical; id, intradermal.

Induction of SIV p27-specific Ab in plasma of mice immunized via mucosal routes

Our previous studies showed that SIV Gag-specific immune responses were induced in mice intradermally immunized with rDIsSIV*gag/pol* [12]. We assessed that IgG Ab response to SIV p27 in the plasma of immunized mice at 1 week after the last immunization. Our results clearly show that intranasal, intragastrical, and intradermal immunization with rDIsSIV*gag/pol* induced p27-specific IgG Ab in plasma (Fig. 2), with similar titres of p27-specific IgG observed for intranasal, intragastrical and intradermal groups receiving the same dose. In contrast, the titres of < 16 were observed for p27-specific IgG Ab in plasma of control mice immunized intranasally with 10^6 PFU rDIsLacZ.

Induction of p27-specific AFC in the mucosal and systemic immune systems

Once the SIV p27-specific Ab responses had been further confirmed at the cellular level, we compared the number of p27-specific AFC induced in the mucosal and systemic lymphoid tissues after intranasal and intragastrical immunization with 10^5 PFU of rDIsSIV*gag/pol*. When we quantitated p27-specific AFC in the mucosal tissues and spleens of mucosally immunized mice, we found clear evidence of p27-specific IgA AFC in the PP, i-LP, NALT and n-LP of mice immunized intranasally with 10^5 PFU of rDIsSIV*gag/pol* (Fig. 3A). The numbers of p27-specific IgA AFC in the i-LP of intragastrically immunized mice were significantly higher than in intranasally immunized mice. Conversely, intranasal immunization of rDIsSIV*gag/pol* strongly induced p27-specific IgA AFC in the NALT and n-LP. We also found the number of p27-specific IgG AFC in the spleen of mice immunized intranasally with rDIsSIV*gag/pol* to be significantly higher than in intragastrically immunized mice ($P < 0.05$) (Fig. 3B). These findings suggest that mucosally administered rDIs can act as a vector for the induction of p27-specific AFC in both mucosal and systemic tissues.

Helper cytokine profiles of SIV Gag-specific CD4$^+$ T cells

Simian immunodeficiency virus Gag-specific helper T-cell responses were assessed using cytokine-specific ELISA for culture supernatants of CD4$^+$ T cells isolated from the spleen of immunized mice. Our results demonstrated that both type 1 helper T cell (Th1) and Th2 cytokines were upregulated in overlapping Gag peptide-stimulated CD4$^+$ T cells taken from the spleen of mice immunized with 10^5 PFU of rDIsSIV*gag/pol*. Of special note, the levels of IFN-γ in mice immunized intranasally with

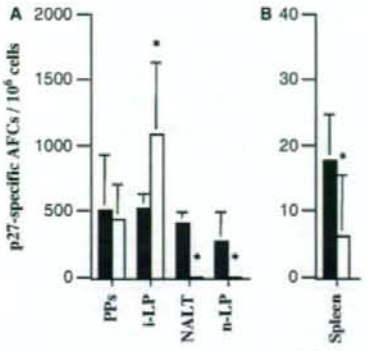

Figure 3 SIV p27-specific IgA AFC in the mucosal tissues and IgG AFC in systemic tissues of mice immunized intranasally or intragastrically with rDIsSIV*gag/pol*. The mice were killed 1 week after the last immunization. Levels of p27-specific IgA AFC in PP, i-LP, NALT and n-LP (A) and levels of p27-specific IgG AFC in spleen (B) of mice immunized intranasally (closed column) or intragastrically (open column) with 10^5 PFU rDIsSIV*gag/pol* were determined using an ELISPOT assay. The data are shown as the mean number of AFC/10^6 cells + SD for 12 mice in each experimental group. Data are representative of three separate experiments. Each group was compared by a two-tailed Student's *t* test. Significant differences between the intranasal group and intragastric group are indicated by asterisks (*$P < 0.05$).

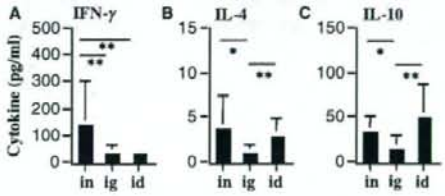

Figure 4 Th1/Th2 cytokine production of SIV Gag overlapping peptide-stimulated $CD4^+$ T cells of mice immunized with 10^5 PFU rDIsSIV*gag/pol*. $CD4^+$ T cells were isolated from spleen at 1 week after the last immunization. Culture supernatants were harvested and then analysed for the production of IFN-γ (A), IL-4 (B), and IL-10 (C) by ELISA. The levels of Gag-specific cytokine production were calculated by subtracting the results of the control culture from the peptide-stimulated culture. The data are shown as the mean concentration + SD for 12 mice in each experimental group. Data are representative of three separate experiments. Each group was compared by a two-tailed Student's *t* test. Significant differences are indicated by asterisks (*$P < 0.05$, **$P < 0.005$). in, intranasal; ig, intragastrical; id, intradermal.

rDIsSIV*gag/pol* were significantly higher than in those intragastrically or intradermally immunized (Fig. 4A). However, no preferential association was noted between Th2 and either the intranasal or intradermal group (Fig. 4B, 4C). In contrast, the intragastrical group showed significantly lower levels of Th2 cytokines than did the intranasal or intradermal group.

IFN-γ production of SIV Gag-specific $CD8^+$ T cells

Cytotoxic T-lymphocyte activity in viral infection has been shown to be of central importance for host defence. To assess the mucosal induction of cellular immunity, we assessed the $CD8^+$ IFN-γ -producing cells in the immunized mice. Because non-specific activated $CD8^+$ cells produced IFN-γ, the number of SIV Gag-specific IFN-γ SFC was calculated (see *Materials and methods*). In the IEL of mice immunized intranasally with rDIsSIV*gag/pol*, the numbers of IFN-γ SFC in unstimulated condition were 70–460 cells/10^6 $CD8^+$ cells. The numbers of IFN-γ SFC in Gag peptide-stimulated condition were 150–1385 cells/10^6 $CD8^+$ cells. There were significant differences between stimulated and unstimulated group. It was demonstrated that SIV Gag-specific IFN-γ-producing $CD8^+$ cells appeared in both systemic and mucosal compartments in the intranasally immunized mice and were particularly abundant in the IEL of the mice (Fig. 5).

Kinetics of p27-specific Ab in plasma

IgA, IgG and IgM class-specific endpoint ELISA was used to investigate an SIV p27-specific Ab in the plasma of mice immunized intranasally with 10^5 PFU of rDIsSIV*gag/pol*. Up until 11 weeks after the last immunization, the titres of p27-specific IgA Ab were low (mean values < 50). Without further boosting, increased p27-specific IgA Ab titres were observed after 11 weeks and were maintained in the plasma for approximately 1 year (49 weeks) at a mean value between 48 and 92 (Fig. 6A). Levels of p27-specific IgA Ab did not correlate with those of IgG and IgM Ab; p27-specific IgG Ab were maintained in the plasma for 1 year, and increases in IgG Ab titres were observed until 17–21 weeks after the last immunization. After peaking, the mean titre in

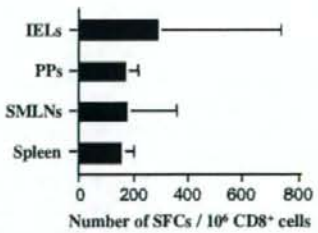

Figure 5 IFN-γ production of SIV Gag overlapping peptide-stimulated $CD8^+$ cells isolated from mucosal and systemic components of mice immunized with rDIsSIV*gag/pol*. $CD8^+$ cells were isolated from IEL, PP, SMLN and spleen at 1 week after the last immunization. IFN-γ production was assessed by ELISPOT assay. The number of Gag-specific IFN-γ SFC was calculated by subtracting the results of the control culture from those of the peptide-stimulated culture. The data are shown as the mean number of SFC + SD for 12 mice in each experimental group. Data are representative of three separate experiments.

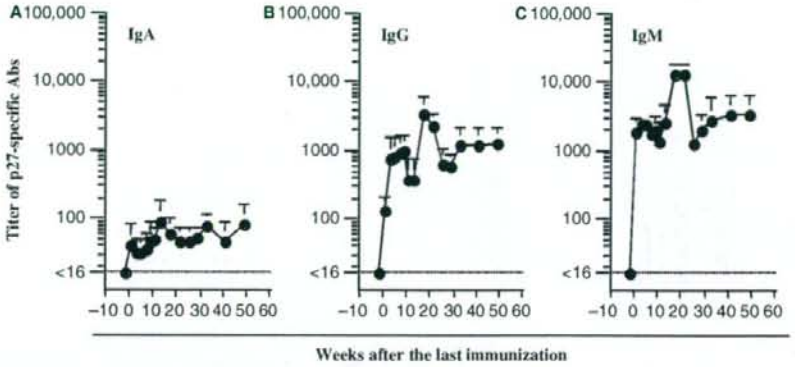

Figure 6 Sequential assessment of SIV p27-specific IgA, IgG, and IgM Ab in plasma of mice that have been intranasally immunized with 10^5 PFU rDIsSIV*gag/pol*. Plasma p27-specific IgA (A), IgG (B), and IgM (C) Ab were determined using an endpoint ELISA. The data are shown as the mean titres + SD for 12 mice in each experimental group. Data are representative of three separate experiments.

intranasally immunized mice declined to a value between 580 and 1300 (Fig. 6B). The p27-specific IgM Ab were observed in the plasma of intranasally immunized mice throughout the 1-year period (Fig. 6C). Titre kinetics was similar for p27-specific IgM Ab and IgG Ab. Thus, it is important to note that the p27-specific plasma Ab responses were maintained for 1 year after the last immunization via the nasal route.

We quantitated p27-specific IgG, IgM and IgA AFC both in systemic tissues such as spleen and in mucosal tissues such as MLN, PP, and i-LP of the mice at 49 weeks after the last immunization. Our results clearly demonstrated that high numbers of p27-specific IgG, IgM and IgA AFC in mice immunized intranasally with 10^5 PFU of rDIsSIV*gag/pol* were maintained even for 1 year after immunization (Fig. 7). Moreover, the mean titres of p27-specific IgA Ab in fecal extracts from mice at 49 weeks were 54 ± 18 (n = 12).

Analysis of viral dissemination in the central nervous system

Adverse reactions to vaccinia virus can occur regardless of pre-existing susceptibilities [19]. Of the adverse events known to occur after smallpox vaccination [20, 21], the most serious is post-vaccinal encephalitis (PVE) [20]. Although the pathogenesis of PVE is unknown, vaccinia viruses were isolated from brain tissues in PVE cases [22, 23]. As the nasopharynx resides in close proximity to the brain, we sought to determine whether rDI is disseminated from the nasal cavity to the brain. The olfactory bulbs, cerebellum and cerebrum of mice immunized intranasally with 10^6 PFU of rDIsSIV*gag/pol* were assessed using both nested DNA PCR and RT-PCR. The SIV *gag* gene was not detected in the olfactory bulbs, cerebellum or cerebrum of any of the eight mice (data not shown).

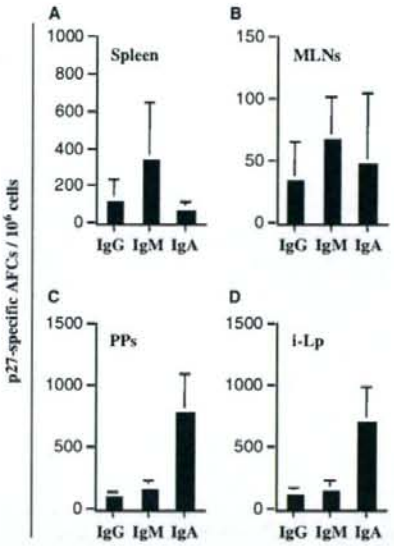

Figure 7 SIV p27-specific AFC in mucosal and systemic tissues of mice immunized intranasally with 10^5 PFU rDIsSIV*gag/pol* at 49 weeks after the last immunization. Levels of p27-specific IgG, IgM and IgA AFC in spleen (A), MLN (B), PP (C), and i-LP (D) of mice were determined using an ELISPOT assay. Data are shown as the mean number of AFC/10^6 cells + SD for 12 mice in each experimental group and the data are representative of three separate experiments.

Discussion

In this study, p27-specific IgA Ab in fecal extracts and IgG Ab in plasma were confirmed in mice immunized intranasally or intragastrically with rDIsSIV*gag/pol*. The compartmentalization within the mucosal immune system places constrains on the choice of vaccination route

for inducing effective immune responses. Oral immunization induces substantial Ab responses in the small intestine [24, 25]. We also found that the numbers of p27-specific IgA AFC were strongly induced in the small intestine by intragastrical route. Conversely, intranasal immunization results in Ab responses in the upper airway mucosa and regional secretions without evoking an immune response in the gut [26, 27]. Although the numbers of p27-specific IgA AFC in i-LP of mice intranasally immunized rDIsSIV*gag/pol* were significantly lower than intragastrical group, the titres of p27-specific IgA Ab in fecal extracts of mice immunized intranasally or intragastrically were roughly equivalent. Intranasal immunization of rDIsSIV*gag/pol* dominantly induced p27-specific IgA AFC in nasal tissues, and these cells might powerfully produce dimeric or polymeric IgA Ab. Polymeric IgA Ab removed from the circulation into bile by the hepatic polymeric immunoglobulin receptor/secretory component-driven. A large portion of murine gut IgA Ab is derived from blood rather than mucosal production [28–30]. Taken together, this study showed that intranasal immunization of rDIs, induced not only potent plasma IgA Ab response but also a good intestinal IgA Ab level despite a poorer cellular IgA Ab response in intestinal mucosa.

One of the keys to vaccine development is the longevity and stability of immune memory. We have shown that p27-specific IgG, IgM and IgA Ab and p27-specific AFC remain detectable for at least 1 year after intranasal administration of replication-deficient rDIsSIV*gag/pol*. Interestingly, IgM Ab against p27 in plasma were detected throughout the period of Ab monitoring, and significant numbers of p27-specific IgM AFC were observed in spleen even 1 year after immunization. Significantly more p27-specific IgM AFC were observed in spleens, suggesting that they may originate from those central lymphoid tissues in immunized mice. Based on the results of the current study, we theorize that SIV Gag continued to be produced in the mice in sufficient quantities to reactivate IgM Ab production in the absence of viral replication and reinfection. Others have shown that hepatitis B virus core Ag-specific or hepatitis C virus capsid Ag-specific IgM Ab are found in the plasma of individuals in the chronic phase of hepatitis B or C viral infection [31, 32]. We have not assessed long-term rDIs vector persistence in mucosal tissues, and we have not yet managed to elucidate the mechanism governing prolonged p27-specific Ab induction. However, we showed that rDIs were able to induce long-term immunity against an inserted foreign Ag in intranasally immunized mice.

Intranasal vaccination has caused unexpected complications. Although DIs vector did not detected in the brain of mouse in this study, it is not clear about the safety for human brain. Intranasal vaccination with rDIs may pose risks such as PVE. It must concern the possibility that nasal vaccines could enter the central nervous system, because of the anatomical proximity of the nasal cavity to the brain. On the other hand, although oral delivery has become an accepted route for administration of polio-vaccine, the gastrointestinal tract presents several formidable barriers to candidate vaccines. In general, oral immunization is relatively inefficient at evoking an IgA Ab response in the distal segments of the large intestine or female genital tract mucosa [24, 25]. Of special interest for possible vaccination against HIV and other sexually transmitted infections, not only intravaginal but also intranasal immunization has been found to give rise to substantial IgA and IgG Ab responses in human cervicovaginal mucosae [24, 27, 33]. This study demonstrated that both intranasal and intragastrical immunizations of rDIs induce Ag-specific mucosal immune responses. However, we could not settle which is the better strategy of vaccine administration to prevent HIV infection in this study.

Of course, much more intensive examination of the efficacy and safety of the mucosal rDIs vaccine are needed before use in humans. Nonetheless, in this study we demonstrated that it is an effective vector for the induction of Ag-specific mucosal immunity in the mucosally immunized mouse model, and represents an important step towards the development of an effective mucosal rDIs vaccine against HIV.

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