A novel synthetic peptide immunogen targeting the human immunodeficiency virus type-1 (HIV-1) coreceptor CXCR4 was evaluated for its capacity to induce CXCR4-specific antibodies with anti-HIV-1 activity in BALB/c mice andynomolgus monkeys. A cyclic closed-chain dodecapeptide mimicking the conformation-specific domain of CXCR4 (cDDX4) was prepared in which Gly-Asp, as the dipeptide forming a spacer arm, links the amino and carboxyl termini of the dodecapeptidyl linear chain (linear DX4, Asn176 to Ile185) derived from the undecapeptidyl arch (UPA; Asn176 to Cys186) of extracellular loop 2 (ECL-2) in CXCR4. Immunization of BALB/c mice with cDDX4 conjugated with a multiple-antigen peptide (cDDX4-MAP) induced conformational epitope-specific antibodies, and monoclonal antibody IA2-F9 reacted with cDDX4, but not with linear DX4, as determined by real-time biomolecular interaction analysis using surface plasmon resonance. The antibody also reacted with cells expressing CXCR4 but not with cells expressing the other HIV coreceptor, CCR5. Furthermore, the antibody inhibited the replication of HIV-1 X4 virus (using CXCR4), as shown by an infection assay using both MAGIC-5 cells and MT4 cells, but not that of HIV-1 R5 virus (using CCR5). The antibody weakly interfered with chemotaxis induced by stromal cell-derived factor-1α in THP-1 cells or moderately inhibited the chemotaxis of Molt4#8 cells under the same conditions. In addition, immunization of cynomolgus monkeys also induced cDDX4-specific antibodies with anti-HIV activity. Taken together, these results indicate that cDDX4 conjugated with a multi-antigen peptide induces the conformational epitope-specific antibodies to the undecapeptidyl arch of CXCR4 may be a novel candidate immunogen for preventing disease progression in HIV-1-infected individuals.

Human immunodeficiency virus type-1 (HIV-1) requires both CD4 and a chemokine receptor for cellular entry. Follow-

ing its binding to CD4, the viral envelope protein changes its conformation to bind to the chemokine receptor and initiates fusion with the cellular membrane (1–4). Because chemokine receptors CCR5 and CXCR4 are the main coreceptors for cellular entry of HIV-1, viral strains are classified as R5, X4, or R5X4 according to the usage of chemokine receptor (5, 6). HIV-1 R5 virus generally transmits the infection, and predominates in an early stage of infection and in long-term nonprogressors (1, 4). In contrast, HIV-1 X4 virus emerges during chronic infection, and its emergence is associated with a rapid decline in the count of CD4+ T cells and progression to AIDS (7–9). The highly active antiretroviral therapy (HAART) has a marked effect on the epidemic of AIDS, whereas the limitations of HAART, such as the emergence of drug-resistant HIV-1 variants and several inherent adverse effects, were also reported (10–12). Therefore, development of an antiviral drug that targets novel molecules is desirable (13). CXCR4 antagonists that prevent entry of X4 virus are one of the candidates. Some studies showed that blockade of CXCR4 can possibly prevent emergence of X4 virus and change the phenotype of already existing X4 virus to R5 virus (14, 15). In addition, a recent statistical study showed that long term nonprogressors have high levels of plasma stromal cell-derived factor-1α (SDF-1α), which is a ligand for CXCR4, and low CXCR4 expression levels on T lymphocytes; in advancing disease, the expression level of CXCR4 increases (16). These results indicate that CXCR4 is an attractive target, which not only inhibits the entry of X4 virus but may also delay the disease progression to AIDS, and may become the target of immunotherapeutic approaches.

CXCR4 has been shown to be critical for development (17–19) but is probably dispensable in adults. However, when developing a self-antigen as a target of an immunotherapeutic approach, unexpected effects must be taken into consideration together with sufficient immune responses. Hence, it is desirable to design an immunogen that targets a restricted protein region rather than the whole protein. Therefore, a linear peptide immunogen has the advantage in that it can elicit a desired immune response against a restricted protein region, whereas it can hardly induce antibodies against the conformational epitope (20, 21). Indeed, because CXCR4 is a member of the seven transmembrane-spanning G protein-coupled receptor family, and it has conformational and flexible extracellular cell-derived factor-1α; PBMCs, peripheral blood mononuclear cells; mAb, monoclonal antibody; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; FITC, fluorescein isothiocyanate; Ig, immunoglobulin; MOE, molecular operating environment; X-gal, 5-bromo-4-chloro-3-indolyl-ß-D-galactopyranoside; MAP, multiple-antigen peptide; FCS, fetal calf serum; PBS, phosphate-buffered saline; m.o.i., multiplicity of infection.
regions, most conformational-specific anti-CXCR4 antibodies were induced by direct immunization of cells that expressed human CXCR4 (22, 23). Therefore, the antibody induced by a linear synthetic peptide against the extracellular loop region of CXCR4 could recognize a denatured protein but could not recognize the native protein. A linear peptide is inadequate for mimicking the conformational loop region of CXCR4, and mimicking the native conformational epitope by a peptide is critical for inducing the antibody against a conformational epitope. Therefore, to use the peptide immunogens for an immunotherapeutic approach targeting CXCR4, it is essential for the designed peptide immunogen to target not only the restricted region of CXCR4 but also to mimic the conformational epitope of the targeted region.

In this study, we investigated the application of a cyclic dodecapeptide peptide (cDDX4) that was designed to mimic the native conformational epitope of the undecapeptidyl arch (UPA) (24, 25) in CXCR4 for use as a novel immunotherapy for AIDS. Immunization with cDDX4 conjugated with a multiantigen peptide (cDDX4-MAP) induced conformational epitope-specific antibodies that preferentially recognized the cyclic structure of the antigen and cross-reacted with cell surface CXCR4. In addition, the induced antibody inhibited the replication of HIV-1 X4 virus but not that of HIV-1 R5 virus as determined by infection assay using MAGIC-5 cells and by a productive infection assay using MT4 cells. These results indicate that cDDX4 sufficiently mimics the conformational epitope of UPA in cell surface CXCR4 and possibly induces antibodies that inhibit cellular entry of HIV-1. In addition, immunization of cynomolgus monkeys with cDDX4-MAP also elicited cDDX4-specific antibodies with anti-HIV activity. Taken together, we propose that an immunotherapeutic approach using cDDX4-MAP as the self-antigen immunogen may be a novel strategy for AIDS therapy.

EXPERIMENTAL PROCEDURES

Cell Culture—The human T-lymphotropic virus type I-infected cell line (MT4), human T cell lines (CEM and MolT4#8), human monocytic cell lines (THP-1 and U937), and peripheral blood mononuclear cells (PBMCs) were cultured at 37 °C in 5% (v/v) CO2 in the RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). MAGIC-5 cells and CD4-transduced human glioma cell lines (NP2/CD4, NP2/CD4/CXCR4, and NP2/CD4/CXCR5) were cultured at 37 °C in 5% (v/v) CO2 in Dulbecco’s modified Eagle’s medium supplemented with 2.5% or 10% FCS, respectively.

Anti-CXCR4 Antibody Generation—The antigen and antibody were prepared using the protocol of Misiuri et al. (24). To mimic the native conformational epitope of human CXCR4, the CXCR4-derived linear dodecapeptide (linear DDX4: N2H-DNVSEADDRYIG-COOH) was synthesized using an automatic peptide synthesizer and then cyclized. The cyclic dodecapeptide (cDDX4: NVSEADDRYIG) was conjugated with a multiple-antigen peptide (MAP) through the formation of the peptide bond between the β-carboxyl group of Asp within the cDDX4 and the amino group within MAP. MAP, which is composed of 2-fold bifurcating polylysine core developed as a carrier of a peptide antigen, is capable of eliciting a strong antibody response in mice, monkeys, and humans (25). All peptides were purified by high-performance liquid chromatography (Waters), and their molecular masses were confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Burker Franzen Analytik GmbH, Bremen, Germany).

Female BALB/c mice were immunized with the cDDX4-MAP conjugate using the protocol of Galliffe and Milstein (26). The resulting hybridoma that produced the most potent supernatant was screened in 96-well plates for the reactivity to Multi-Pin Block (Chiron Technologies), which was conjugated with cDDX4 and was cloned by limiting dilution. The anti-CXCR4 antibody, IA2-F9, was recovered by ammonium sulfate precipitation and purified using a Sephadex G-150 column (Amersham Biosciences).

Real-time Biomolecular Interaction Analysis using Surface Plasmon Resonance—Recognition of the cyclic structure of cDDX4 by IA2-F9 was analyzed by the surface plasmon response technology using BIAcore2000 as described previously (24, 27, 28). The free β-carboxyl group of Asp that was used as a spacer-armed dipeptide in cDDX4 was conjugated to 5-[5-(N-succinimidylcarboxamidopentylamido)hexyl]d biotinamide through ethylenediamine. Biotinylated cDDX4 was immobilized to streptavidin-coated sensor chips. For competition assay, purified IA2-F9 was pretreated with cDDX4 (1 and 10 nmol) or linear DDX4 (1 and 10 nmol) at room temperature prior to BIAcore analysis. All the antigen-antibody interactions were analyzed in a binding buffer (0.02% KH2PO4, 0.29% Na2HPO4, 12H2O, 0.8% NaCl, and 0.02% ECI) at a constant flow rate of 50 μl/min and a constant temperature of 25 °C. The bound antibody was eluted from the biotinylated cDDX4 by a short pulse (20 μl) of 10 mM Gly-HCl (pH 2.0). This regeneration procedure did not alter the ability of cDDX4 to bind to the antibody in subsequent cycles. Kinetic analysis was performed on BIAcore2000 using BIAcore evaluation software. For detecting anti-cDDX4 antibodies in sera derived from immunized cynomolgus monkeys, the sera were dialyzed using Spectra/Per (cutoff molecular masses, 100,000; Spectrum Laboratories Inc.) according to the manufacturer’s instructions and analyzed.

Antibody and Flow Cytometry—The following antibodies were used: an anti-CXCR4 mAb (clone 12G5, BD Biosciences), an isotype-matched control antibody (Sigma), purified IA2-F9, and fluorescein isothiocyanate (FITC)-conjugated anti-mouse immunoglobulin G or anti-mouse IgM. Cells (1 × 106) were washed with washing buffer (PBS containing 2% FCS and 0.02% NaN3) and incubated with 10 μg/ml primary antibody (purified IA2-F9 or 12G5) for 30 min at 4 °C. The cells were then
R5, X4, and R5X4 viruses. The cells were seeded (1 × 10^5) expressing CD4, CCR5, and CXCR4, they are susceptible to infection by an infection assay using MAGIC-5 cells (32). Because MAGIC-5 cells trypan blue dye exclusion.

Chemotaxis was conducted in the presence of 10 nM SDF-1 or without IA2-F9 for 30 min and were placed in the upper chamber. Chemotaxis was conducted in the presence of 10 nM SDF-1α (placed in the lower chamber). After incubation for 3 h at 37°C, cells that migrated from the upper chamber to the lower chamber were quantified by trypan blue dye exclusion.

**Chemotaxis Assay**—The migration of Molt4#8 and THP-1 was assayed in 24-well cell-culture chambers using an insert with 5.0-μm pore membranes (Corning, Corning, NY) according to the protocol of Gosling et al. (29) with slight modifications. Cells (5 × 10^5) were pretreated with or without IA2-F9 for 30 min and were placed in the upper chamber. Chemotaxis was conducted in the presence of 10 nM SDF-1α (placed in the lower chamber). After incubation for 3 h at 37°C, cells that migrated from the upper chamber to the lower chamber were quantified by trypan blue dye exclusion.

**MAGIC-5 Assay**—The antiviral activity of IA2-F9 was determined by an infection assay using MAGIC-5 cells (32). Because MAGIC-5 cells express CD4, CCR5, and CXCR4, they are susceptible to infection by R5, X4, and R5X4 viruses. The cells were seeded (1 × 10^5 cells per well) and cultured in a 48-well plate for 24 h. After removal of the medium from each well, the cells were incubated for 30 min with IA2-F9 at the indicated concentrations and were infected with the virus in the presence of DEAE-dextran (20 μg/ml) for 2 h and washed with the culture medium. The cells were cultured in the medium containing IA2-F9 for 48 h, fixed with 1% formaldehyde-0.2% glutaraldehyde in PBS for 5 min, washed, and then stained with X-gal. The number of blue-stained cells was counted under a light microscope. Control experiments were carried out under identical conditions in the absence of the antibody.

For determining anti-HIV activity of sera at 0 and 10 weeks derived from immunized cynomolgus monkeys, antibodies were purified from serum using an NAb™ Protein L Spin kit (Pierce Biotechnology, Rockford, IL) according to the manufacturer’s instructions. For determining anti-HIV activity of sera at 25 and 27 weeks, sera were dialyzed with PBS(−) using Spectra/Per (cutoff molecular mass, 100 kDa; Spectrum Laboratories Inc.) and adjusted to a concentration corresponding to a 1/10 dilution of sera with PBS(−).

**Productive Infection Assay**—MT4 cells (1 × 10^5) were preincubated with IA2-F9 at the indicated concentrations for 30 min, and then HIV-1 X4 virus (m.o.i. = 0.01) was inoculated to these cells. After washing three times with PBS, the cells were incubated with IA2-F9 (0, 1, and 10 μg/ml) for 72, 96, and 120 h. Then the culture supernatant of each cell was collected, and the p24 antigen level was measured by antigen-capture enzyme-linked assay using a RETRO-TEK HIV-1 p24 antigen enzyme-linked immunosorbent assay kit (ZeptoMetrix Corp.) according to the manufacturer’s instructions.

**Immunization Schedule**—All the cynomolgus monkeys were housed in individual cages and maintained according to the rules and guidelines of the National Institute for Infectious Diseases for experimental animal welfare. Three cynomolgus monkeys, 4–6 years old, were immunized intraperitoneally at 0 and 1 week with 300 μg of cDDX4-MAP in complete Freund’s adjuvant and boosted subcutaneously at 6 weeks with 300 μg of cDDX4-MAP in incomplete Freund’s adjuvant. Furthermore, these monkeys were reboosted subcutaneously at 25 weeks with 300 μg of cDDX4-MAP in Freund’s incomplete adjuvant. Another three cynomolgus monkeys were immunized with MAP following the same immunization schedule. Blood samples were obtained at 0, 10, 25, and 27 weeks, which were then subjected to BIACore analysis and MAGIC-5 assay.

**RESULTS**

**Design and Synthesis of cDDX4**—The hypothetical structure model of CXCR4 was based on its homology with rhodopsin (30), and energy-minimized with the Molecular Operating Environment, MOE (Chemical Computing Group Inc., Montreal, Quebec, Canada) (Fig. 1A). The extracellular loop-2 (ECL-2) region of CXCR4, and its structure deduced by MOE (Figs. 1B-1 and 2) has a unique arch structure consisting of 11 amino acid residues (UPA) on the basis of the Cys109 residue of ECL-1 by a disulfide bond (Swiss-Prot P30991, Fig. 1B-1). The cDDX4 moiety designed to mimic the

![Fig. 2. MALDI-TOF MS spectra of linear DDX4 and cDDX4.](image) The spectra exhibit two peaks at m/z 1317.7 and 1335.2: the upper peak corresponds to the ion derived from linear DDX4, and the lower peak to the ion derived from cDDX4. The matrix was a saturated solution of α-cyano-4-hydroxycinnamic acid in solution of acetonitrile-water (1:2, v/v) containing 0.1% trifluoroacetic acid. The fraction with a molecular mass of 17.5 corresponding to H₂O was deleted after cyclizing the linear DDX4 with the peptide bond.
Antitargeting SDF-1

Molt4#8 and THP-1 were used with or without IA2-F9. Antigen T cells was investigated. MT4 cells, a human T cell line, were used in the experiment, the inhibitory effect of IA2-F9 on HIV-1 replication was determined by an infection assay using MAGIC-5 cells. MT4 cells, a human T cell line, were inoculated with LAV-1 (X4 virus), JRFL (R5 virus), and MAGIC-5 cells were generated from CD4- and CXCR4-positive HeLa-CD4-LTR-β-gal (MAGI) cells by transfection with a CCR5 expression plasmid to confer susceptibility to infection with not only X4 virus but also R5 virus. MAGIC-5 cells were generated from CD4- and CXCR4-positive HeLa-CD4-LTR-β-gal (MAGI) cells by transfection with a CCR5 expression plasmid to confer susceptibility to infection of not only X4 virus but also R5 virus. MAGIC-5 cells were inoculated with LAV-1 (X4 virus), JRFL (R5 virus), or 89.6 (R5X4 virus), in the absence or presence of IA2-F9 at indicated concentrations. IA2-F9 inhibited infection of LAV-1 but not those of JRFL and 89.6 (Fig. 6).

To verify the antiviral activity of IA2-F9 in an alternative experiment, the inhibitory effect of IA2-F9 on HIV-1 replication in T cells was investigated. MT4 cells, a human T cell line, were infected with HIV-1 X4 virus, LAV-1 (m.o.i. = 0.01) in the presence of IA2-F9 at varying doses, and the spread of infection was monitored based on the accumulation of p24 antigen in culture supernatants. IA2-F9 at 10 μg/ml inhibited HIV-1 LAV-1 infection at the peak of virus production in the control experiment as shown in Fig. 7. These results indicate that cDDX4-MAP could induce the antibody with anti-HIV activity, and suggest the potential of immunization with cDDX4-MAP in AIDS therapy.

Immunogenicity of cDDX4-MAP in Cynomolgus Monkeys—To verify whether cDDX4-MAP could induce CXCR4-specific antibodies with anti-HIV-1 activity in nonhuman primates as well as rodents, an experiment was performed using cynomolgus monkeys immunized following the time schedule shown in Fig. 8A. Three cynomolgus monkeys were immunized with cDDX4-MAP in Freund’s complete adjuvant or Freund’s incomplete adjuvant by intraperitoneal subcutaneous injection. Another three cynomolgus monkeys were immunized with MAP as the control. The cDDX4-immobilized BlAcore sensor chip was used for detecting cDDX4-specific antibodies in immunized monkeys. cDDX4-bound antibodies were detected in the sera from three monkeys 10 weeks after the initial immu-
nization (Fig. 8B). On the other hand, no significant responses were detected in MAP-immunized monkeys, which are the control (monkeys 4–6). These results indicate that cDDX4-MAP could induce CXCR4-specific antibodies not only in BALB/c mice but also in cynomolgus monkeys.

Anti-HIV Activity of Partially Purified Antibodies Derived from cDDX4-MAP-immunized Monkeys—Anti-HIV activity of antibodies that were induced by cDDX4-MAP was investigated. MAGIC-5 cells were inoculated with HIV-1 LAV-1 in the presence or absence of an Ig-containing fraction derived from the sera of cDDX4-MAP-immunized monkeys or MAP-immunized monkeys at 0 and 10 weeks. The Ig-containing antibodies from cDDX4-MAP-immunized monkeys (monkeys 1 and 2 in Fig. 9A) significantly inhibited HIV-1 infection but not those from monkey 3, as shown by the comparison between monkeys at 0 and 10 weeks. On the other hand, all sera from cDDX4-MAP-immunized monkey at 27 weeks inhibited HIV-1 replication. The Ig-containing antibodies and sera from MAP-immunized mon-
Chemokine receptor CXCR4 is a coreceptor for cellular entry of HIV-1 X4 virus and has been shown to be critical for development (17–19) but is probably dispensable in adults. However, when developing a self-antigen as target of an immunotherapeutic approach, unexpected effects must be taken into consideration. Therefore, for an immunotherapeutic approach to CXCR4, it is required to design an immunogen that targets a restricted region in CXCR4 rather than the whole CXCR4. A peptide immunogen strategy was used as an immunotherapeutic approach to CXCR4. Benefits of a peptide immunogen strategy were (i) its immunogenicity can be controlled by polymerization or conjugation with small carrier molecules, (ii) it can induce antibodies against a very restricted region that includes the biologically active epitope, and (iii) its chemical purity can be exactly defined and is cost-effective to produce (20, 21). Therefore, the peptide immunogen strategy appears to be the best approach to inducing antibodies against CXCR4.

The peptide immunogen, cDDX4-MAP, was designed and prepared to mimic the conformational epitope of UPA in CXCR4, and antibodies were induced in BALB/c mice and cynomolgus monkeys. The UPA (from Asn176 to Cys 186) of CXCR4 was selected as the target in the study because of its unique amino acid sequence. Amino acid sequence alignment shows that the amino terminus and the extracellular domains (ECL-1, ECL-2, and ECL-3) are homologous in the CXC receptor family, but the amino acid sequence of UPA (from Asn176 to Cys 186) of CXCR4 is specific (Table I, upper part, identical amino acid residues of UPA are shown in red), and highly conserved in the human, cynomolgus monkey, and rhesus monkey (Table I, bottom part). Furthermore, studies using site-directed mutagenesis of CXCR4 revealed that the amino acid residues Asp20 and Tyr21 in the amino-terminal domain and Glu172, Glu173, Asp181, Asp182, Arg183, and Tyr184 in UPA had no effect on SDF-1α binding as well as the intracellular Ca2+ influx (31, 33). Therefore, the immunogen designed based on UPA in CXCR4 may induce anti-CXCR antibodies with little effect on CXCR4 signaling. Consistent with our strategy, the monoclonal antibody induced by cDDX4-MAP inhibits weakly (Molt4#8 cell migration shown in Fig. 5) or moderately (THP-1 cell migration shown in Fig. 5) SDF-1α-induced chemotaxis.

Mimicry of the conformation of the native protein is most important for inducing an antibody against the conformational epitope. Hence, an antibody induced by a linear peptide could recognize the denatured protein but could not recognize the native protein. On the other hand, an antibody that recognizes the conformational epitope of an antigen could not recognize the denatured protein. Indeed, the anti-CXCR4 antibody 12G5, induced by immunization of Sup-T1 cells that were chronically infected with SIVmac variant CP-MAC (23), could recognize cell surface CXCR4 as determined by fluorescence-activate cell sorting analysis but could not detect denatured CXCR4 as determined by Western blot analysis. To mimic the native conformational epitope of UPA in CXCR4, cDDX4 was prepared by cyclization of the decapptide (176^NVSEADDRY185) derived from the UPA sequence by insertion of the spacer-armed dipeptide (Gly-Asp). The spacer-armed dipeptide was used not only to conjugate cDDX4 to MAP but also to mimic the UPA in CXCR4 without inducing structural distortion.

The structural alignment between the hypothetical structural model of UPA in CXCR4, for which the structure of
rhodopsin was used as the template, and that of cDDX4 indicates that cDDX4 may possess the conformational property similar to that of an energy-minimized UPA structure. Because the conformational stability of a peptide antigen in vivo is also a key factor for generating antibodies against the native protein, the peptide antigen was cyclized not by a disulfide bond but by a peptide bond. Because the peptide bond is more robust and stable than a disulfide bond for cyclization, cDDX4 can maintain its conformation to a certain degree. Furthermore, as shown in the study of a thrombin-specific inhibitor, a cyclic peptide antigen seems to be more resistant to proteolytic degradation (34). Thus, cyclization of cDDX4 not only mimics the native conformational epitope of UPA in CXCR4, but also may enhance immunogenicity of cDDX4 itself by becoming resistant to proteolytic degradation.

The monoclonal antibody, IA2-F9, generated by immunization of BALB/c mice with cDDX4-MAP, showed conformational specific reactivity, as determined by BIAcore analyses, and specific binding to cell-surface CXCR4. These results indicate that IA2-F9 is a conformational epitope-specific antibody that cross-reacted with cell surface CXCR4. It is known that CXCR4 exhibits conformational heterogeneity (22), but IA2-F9 significantly reacted to all CXCR4-expressing cells that were used in this study. Although further investigation is required, UPA may be a conserved conformational region in CXCR4 that exhibits conformational heterogeneity. Furthermore, these results indirectly indicate that cDDX4 may accurately mimic the conformational epitope of UPA in CXCR4.

HIV-1 X4 virus emerges during chronic infection, and its emergence is associated with a rapid decline in the count of CD4(+) T cells and disease progression to AIDS (7–9). Recent statistical studies showed that long term nonprogressors have a high plasma SDF-1 level and a low CXCR4 expression level on T lymphocytes, and in advancing disease, the expression level of CXCR4 increases and plasma SDF-1 level decreases (16). In addition, some studies showed that the blockade of CXCR4 may prevent the emergence of X4 virus and change the phenotype of already-existing X4 virus to R5 virus (14, 15). Therefore, blocking CXCR4 is thought to be effective in inhibiting progression to AIDS. IA2-F9 inhibited the replication of HIV-1 X4 virus as shown by the infection assay (MAGIC-5 assay, Fig. 6) and productive infection assay (HIV-1 p24 productive infection assay, Fig. 7). These results indicate that cDDX4 is the antigen that could induce anti-CXCR4 antibodies with anti-HIV-1 activity.

To investigate the immunogenicity of cDDX4-MAP in a nonhuman primate, cynomolgus monkeys were used in this study. Three cynomolgus monkeys were immunized four times with cDDX4-MAP. Immunization with cDDX4-MAP was well tolerated by all monkeys. No clinically significant changes in vital signs were observed in all monkeys immunized with cDDX4-MAP. In addition, there were no serious adverse events and toxicity for at least 1 year after immunization. Previous reports indicate the safety of the small-molecule CXCR4 inhibitors such as ALX40–4C and AMD3100 in humans (35, 36). Under limited conditions, these results support that the immunotherapeutic approach to HIV targeting CXCR4.


Immunotherapeutic Approach to HIV Targeting CXCR4

Fig. 9. Anti-HIV activity of serum antibodies derived from cDDX4-MAP-immunized monkeys. MAGIC-5 cells (HeLa-CD4"CXCR4"CCR5") were inoculated with the HIV-1 X4 virus LAV-1 in the presence of serum antibodies purified from the serum obtained at 0 week (black) or 10 weeks (gray) (A, upper figure), or the serum diluted at 1:10 serum obtained at 25 weeks (black) or 27 weeks (gray) (B, bottom figure). The number of blue-stained cells is expressed as percentage (%) relative to the number of blue-stained cells at 0 week for each monkey. No significant cytotoxicity of serum antibodies was observed.

Three monkeys produced antibodies to cDDX4-MAP as determined by BLAcore analyses, and at 10 weeks, the antibodies from monkeys 1 and 2 significantly inhibited HIV-1 infection as determined by MAGIC-5 assay, but not the antibodies from one monkey (monkey 3). However, at 27 weeks, the serum form monkey 3 also exhibited anti-HIV-1 activity. Although the reason for the difference in anti-HIV activity among the three monkeys is still unknown, the MAP moiety of the cDDX4-MAP antigen was shown to produce an unknown enhancer of HIV infectivity, because the enhancement of HIV infectivity was estimated by immunization with MAP alone. The balance in production of antibodies and an unknown enhancer, and individual differences among the monkeys may also account for differences in HIV-1 infectivity.

More recently, anti-HIV-1 agents to another chemokine receptor, CCR5, have been developed (24, 38), because individuals who lack CCR5 are resistant to HIV-1 infection and are healthy without any serious side effects (39–41). However, because individuals who progress to AIDS have X4 virus (1), development of a therapeutic strategy that targets CXCR4 is also indispensable (42–43). It is also known that individuals who have autoantibodies against CCR5 are resistant to HIV infection, although the induction mechanisms are still unclear (44). Therefore, we propose that an immunotherapeutic approach that uses cDDX4-MAP as a peptide immunogen may be a novel candidate strategy against HIV/AIDS progression.

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A Novel Cyclic Peptide Immunization Strategy for Preventing HIV-1/AIDS Infection and Progression

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