Ⅲ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表レイアウト

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
Ito M, K. Kobayashi, and T. Nakahata.	NOD/Shi-scid IL2rg<null> (NOG) Mice More Appropriate for Humanized Mouse Models	T. Nomura et al.	"Humanized mice" in Current Topics in Microbiology and Immunology 324	Springer-Verlag	Berlin Heidelberg	2008	53-76
Koyanagi Y, Y. Tanaka, M. Ito, and N. Yamamoto.	Humanized Mice for Human Retrovirus Infection	T. Nomura et al.	"Humanized mice" in Current Topics in Microbiology and Immunology 324	Springer-Verlag	Berlin Heidelberg	2008	133-48

雑誌

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Zhang LF, Okuma K, Tanaka R, Kodama A, Kondo K, Ansari AA, and Tanaka Y.	Generation of mature dendritic cells with unique phenotype and function by in vitro short-term culture of human monocytes in the presence of interleukin-4 and interferon-beta.	Experimental Biology and Medicine			in press.

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Okuma K, Tanaka R,Ogura T, Ito M, Kumakura S, Yanaka M, Nishizawa M, Sugiura W, Yamamoto N, and Tanaka Y.	Interleukin-4-Transgenic hu-PBL-SCID Mice: A Model for the Screening of Antiviral Drugs and Immunotherapeutic Agents against X4 HIV-1 Viruses.	J Infec Dis	197	134-41	2008
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Watanabe S, Ohta S, Yajima M, Terashima K, Ito M, Mugishima H, Fujisawa S, Shimizu N, and Yamamoto N.	Humanized NOD/SCID/IL2Rgnull Mice Transplanted with Hematopoietic Stem Cells under Nonmyeloablative Conditions Show Prolonged Life Spans and Allow Detailed Analysis of Human Immunodeficiency Virus Type 1 Pathogenesis.	J Virol	81	13259-64	2007
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Ⅳ. 研究成果の刊行物・別刷

MAJOR ARTICLE

Interleukin-4–Transgenic hu-PBL-SCID Mice: A Model for the Screening of Antiviral Drugs and Immunotherapeutic Agents against X4 HIV-1 Viruses

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CXCR4-tropic (X4) human immunodeficiency virus type 1 (HIV-1) does not efficiently infect and replicate in severe combined immunodeficiency (SCID) mice reconstituted with human peripheral blood mononuclear cells, termed "hu-PBL-SCID mice," due to, at least in part, relatively low levels of expression of the CXCR4 coreceptor. To overcome this limitation, interleukin (IL)–4–transgenic hu-PBL-SCID mice were derived that spontaneously synthesized human IL-4, which has been shown to enhance CXCR4 expression and promote X4 virus infection in vitro. Experiments reported here show that (1) synthesis of human IL-4 in vivo augmented CXCR4 expression on human CD4$^+$ lymphocytes and importantly led to productive infection of not only X4 HIV-1_{NL4-3} but also multidrug-resistant primary clinical isolates and that (2) the in vivo infection could be significantly blocked by the administration of a CXCR4 antagonist. Altogether, IL-4–transgenic hu-PBL-SCID mice provide a useful model for X4 HIV-1 study and testing/screening of anti-X4 viral drugs.

HIV-1 isolates enter target cells primarily after binding to the CD4 receptor and via the CXCR4 and CCR5 coreceptors [1–5] and are classified into X4 and R5 strains, respectively [6]. The X4 isolates are frequently implicated in the decline of peripheral CD4$^+$ T cell counts characteristic of the late stage of HIV-1 infection proceeding to the development of AIDS [7].

hu-PBL-SCID mice have been extensively used as a small animal model to study HIV-1 pathogenesis [8–14]. Results from a previous study showed that, although infection of human peripheral blood mononuclear cell (PBMC)–reconstituted hu-PBL-SCID mice with a predominantly R5 HIV-1 caused intensive CD4$^+$ T cell depletion, infection of similarly reconstituted mice with the same infectious dose of an X4 HIV-1 resulted in little or no CD4$^+$ T cell depletion [11]. Thereafter, it was noted that this limitation of X4 HIV-1 infection was due, at least in part, to a decrease in the intensity of CXCR4 expression on CD4$^+$ T cells [13]. Thus, it was reasoned that the pathogenic effects of the X4 HIV-1 strains in the hu-PBL-SCID mice might be related to the relative levels of the expression of HIV-1 coreceptor (the state of activation/differentiation) on human CD4$^+$ T cells at the time of infection in these mice. This limitation has to date restricted our ability to use this mouse model for understanding the mechanisms of X4 HIV-1 pathogenesis and for the evaluation of candidate therapeutics against X4 viruses. These findings prompted us to seek alternative strategies for the development of an improved hu-PBL-SCID mouse system that is permissive for infection/replication of X4 isolates.

Human interleukin (IL)–4 has been shown to specifically enhance the cell-surface expression of CXCR4 on

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resting peripheral blood T cells [15]. Furthermore, it has been reported that human IL-4 plays an important role in rendering $CD4^+$ T cells susceptible to X4 HIV-1 infection via enhanced cell-surface expression of the CXCR4 coreceptor in vitro [15–17].

In efforts to overcome the limitation inherent with the use of hu-PBL-SCID mice for the study of X4 HIV-1 as described above, we developed an IL-4–transgenic immunodeficient mouse model that consistently secreted readily detectable serum levels of human IL-4. We show here that X4 isolates readily infect/replicate in this mouse model but not in wild-type (wt) non–IL-4–transgenic mice and that this model can now be exploited for the rapid evaluation of the therapeutic efficacy of new anti-X4 HIV-1 agents in vivo.

METHODS

IL-4–producing mice. Two strains of human IL-4–transgenic immunodeficient mice were bred on the C.B-17-*scid* [18] and BALB/cA-Rag2$^{-/-}\gamma_c^{-/-}$ (dKO) genetic background mice [19, 20] at the Central Institute for Experimental Animals (CIEA) as follows. PBMCs were isolated from a healthy human volunteer and activated in vitro with pokeweed mitogen. RNA was prepared from these PBMCs, and then cDNA was synthesized by reverse-transcriptase polymerase chain reaction (PCR). Human IL-4 cDNA was amplified from the cDNA using one set of primers: 5'-CCCGGGATCGTTAGCTTCTCCTGATAAAA-3' and 5'-GCGGCCGCTATTCAGCTCGAACACTTTGAAT-3'. The product was inserted into the PCR2.1 vector by use of the TA cloning kit (Invitrogen) and the insert sequenced. After confirmation of the sequence, IL-4 cDNA was inserted into pCMVb with a CMV promoter (Invitrogen). To produce transgenic mice, a DNA fragment containing the CMV promoter, IL-4 cDNA, and Poly(A) regions was excised with *Xho*I and *Hind*III sites of pCMVb and microinjected into the pronuclei of fertilized eggs from the 2 strains (C.B-17-*scid* and BALB/cA-dKO) of mice. These eggs were subsequently transplanted into oviducts of pseudopregnant foster recipient mice. The offspring mice were screened to confirm the insertion of the transgene into the genome by PCR, and serum from these mice was screened for levels of human IL-4 by ELISA with a commercial kit (BD). The IL-4 transgene–hemizygous mice were maintained by mating them with wt mice with the same genetic background in the specific-pathogen–free (SPF) facility of the CIEA. The mice were transferred to the SPF and biosafety level 3 facilities of the Institute for Animal Experiments, University of the Ryukyus, and were used for further experiments. The experimental protocols were approved by the Institutional Animal Care and Use Committee on the basis of the Regulation for Animal Experimentation of the CIEA and University of the Ryukyus before the initiation of the study.

Viruses. X4 HIV-1_{NL4-3} was obtained as described elsewhere [14]. Fourteen multidrug-resistant (MDR) HIV-1 clinical isolates were obtained from HIV-1–infected patients who had been treated with highly active antiretroviral therapy (HAART). The viruses were propagated in PBMCs stimulated with phytohemagglutinin (PHA; Sigma), IL-2 (National Institutes of Health AIDS Research and Reference Reagent Program), and IL-4 (Peprotec). Three isolates from these MDR isolates that efficiently grew in the activated cells were selected for further experiments. The titers of virus stocks were determined by end-point titration using a 2-fold limiting dilution of the stock and in vitro PHA-activated human PBMCs, and the infectious units (IU) were calculated.

CXCR4 antagonist. The synthesis and purification of the CXCR4 antagonist KRH-1636 were performed at Kureha Corporation as described elsewhere [21]. As a control, the carrier tartrate was used in parallel.

Transplantation and infection. The control (wt) and the IL-4–transgenic C.B-17-*scid* mice were depleted of NK cells by the intraperitoneal (ip) injection of 0.5–1.0 mg of anti–mouse IL-2R β (TMβ-1) [22] per animal. The IL-4–transgenic and the control BALB/cA-dKO mice do not require TMβ-1 treatment because they lack NK cells [19, 20]. PBMCs were isolated from healthy human donors. Groups of 2–4-month-old IL-4–transgenic mice from each of the 2 background strains and their corresponding non–IL-4–transgenic wt mice were injected ip with PBMCs 3 days later. Groups of mice were challenged 24 h later ip with mock, HIV-1_{NL4-3}, or MDR isolates (2000 IU/500 μL/animal). For the experiments using the CXCR4 antagonist, groups of mice were administered 0.1 mL of 10 mmol/L KRH-1636, the tartrate carrier or saline ip at 1 h before and 1 day after virus infection. At 6–8 days after infection, the mice were killed, their blood was obtained by cardiocentesis, and human lymphocytes were collected from the peritoneal lavage fluids. The serum samples were assayed for levels of human IL-4 by use of an ELISA kit (R&D Systems). The human lymphocytes were analyzed using flow cytometry as described below. The remaining cells were cultured in RPMI 1640 medium (Sigma) supplemented with fetal calf serum and IL-2. The peritoneal lavage fluids, serum samples, and lymphocyte culture supernatants were examined for levels of p24 by use of an ELISA kit (Zepto Metrix).

Flow cytometry analysis. Cell samples to be analyzed by flow cytometry were initially incubated with normal human IgG for blocking of the Fc receptors. For cell-surface staining, aliquots of cells were then stained with Cy5-labeled anti-CD4 (OKT4) and phycoerythrin-labeled anti-CXCR4 (12G5; Dako) or with Cy5-labeled anti-CD3 (OKT3). For intracellular staining, after CD3 staining the aliquots of cells were fixed, permeabilized, and incubated with fluorescein isothiocyanate–labeled anti–HIV-1 Gag p24 (2C2; Y.T. et al., unpublished data). Stained samples were analyzed on a FACSCalibur flow cytometer, using Cell Quest software (BD Pharmingen). Aliquots of cells stained

Table 1. Expression of human CD4, CXCR4, and intracellular HIV-1 p24 in cells from X4 HIV-1–infected hu-PBL-SCID mice.

Category	X4 HIV-1 infection	Mice, no.	$CD4^+$ T cells, %	*P*	$CXCR4^+CD4^+$ T cells, %	*P*	$p24^+$ T cells, %	*P*
C.B-17-*scid* mice								
Control	NL4-3	6	22.1 ± 8.3	<.001	45.2 ± 4.7	<.001	0.1 ± 0.1	NS
IL-4 transgenic	NL4-3	6	66.3 ± 9.0		65.5 ± 6.1		0.1 ± 0.1	
BALB/cA-dKO mice								
Control	NL4-3	5	35.9 ± 5.1	<.01	32.6 ± 1.4	<.001	0.2 ± 0.1	<.05
IL-4 transgenic	NL4-3	3	57.4 ± 8.3		68.2 ± 3.9		3.2 ± 1.2	

NOTE. Cells in peritoneal lavage fluid from control and interleukin (IL)–4–transgenic hu-PBL-SCID mice on either the C.B-17-*scid* or BALB/cA-dKO background were labeled with appropriate monoclonal antibodies and analyzed by flow cytometry, as described in Methods. Analyzed data are shown as mean ± SD values. NS, not significant. The indicated *P* values for the comparison of control vs. transgenic mice for each category are based on Student's *t* test.

with or without each of the antibodies described above were used as controls for the purposes of establishing gates and for the determination of the frequency of positive cells.

Statistical analysis. Data obtained by flow cytometry were analyzed by Student's *t* test with GraphPad Prism (version 4.0c for Mac OS X; GraphPad Software).

RESULTS

Production of human IL-4 in IL-4–transgenic mice. Efforts to construct the IL-4–transgenic mice constitutively synthesizing human IL-4 finally led to the establishment of mice on each of the 2 immunodeficient backgrounds expressing either high or low serum levels of human IL-4 (data not shown). On the basis of preliminary data obtained on the efficiency of virus replication, all subsequent experiments were done using only the 2 strains with high serum IL-4 expression levels. We assayed for levels of human IL-4 in the serum from the $IL\text{-}4^+$ hu-PBL-SCID mice and the wt hu-PBL-SCID mice on either the C.B-17-*scid* or BALB/cA-dKO background after infection with X4 HIV-$1_{NL4\text{-}3}$. Serum from each of the IL-4–transgenic mice on either background contained significant levels of human IL-4 (~800–1800 pg/mL), whereas serum from the control mice on the same background showed nondetectable levels of human IL-4. These data demonstrate that the human IL-4 synthesized by the IL-4–transgenic mice is generated from the transgene but not from the human PBMCs transplanted in these mice.

Effect of human IL-4 on the levels of human CXCR4 and CD4 expression by cells transplanted into mice. Since human IL-4 has been previously documented to enhance the expression of CXCR4 in vitro, experiments were done to examine the expression of human CXCR4 on transplanted $CD4^+$ cells in the peritoneal lavage fluids from HIV-$1_{NL4\text{-}3}$–infected $IL\text{-}4^+$ hu-PBL-SCID mice and control hu-PBL-SCID mice on either the C.B-17-*scid* or BALB/cA-dKO background. There did not appear to be any detectable difference in the absolute amounts of cells recovered from the peritoneal lavage fluids from the IL-4–positive or IL-4–negative hu-PBL-SCID mice on either background (data not shown). Flow cytometry analysis demonstrated that the frequency of human $CD4^+$ cells from the IL-4–transgenic C.B-17-*scid* or BALB/cA-dKO mice was significantly higher than that from the control mice (table 1). As expected, there was a marked increase in the frequency of CXCR4-expressing $CD4^+$ cells from the IL-4–transgenic mice on either genetic background relative to that from the control mice (figure 1*A* and table 1). Thus, these data indicate that human IL-4 produced endogenously is functional in vivo in terms of its ability to enhance human CXCR4 expression on $CD4^+$ cells transplanted into the mice.

Increased frequency of X4 HIV-1–infected cells from IL-4–transgenic hu-PBL-SCID mice. Since the constitutive synthesis of human IL-4 in IL-4–transgenic hu-PBL-SCID mice resulted in the enhanced expression of X4 HIV-1 receptors (human CXCR4/CD4) on the transplanted cells, we reasoned that such cells were likely to be more permissive to the infection and replication of X4 HIV-1. We thus challenged the IL-4–transgenic hu-PBL-SCID mice and control hu-PBL-SCID mice bred on the C.B-17-*scid* or BALB/cA-dKO mice with HIV-$1_{NL4\text{-}3}$. Cells obtained from the peritoneal lavage fluids were analyzed for cell-surface expression of human CD3 (since HIV-1 downmodulates CD4 expression) and the presence of intracellular p24. As seen in figure 1*B* and table 1, although very few if any $CD3^+$ cells from the control or IL-4–transgenic C.B-17-*scid* mice showed p24 expression, there was a >10-fold increase in the frequency of $CD3^+$ T cells that expressed p24 from the IL-4–transgenic BALB/cA-dKO mice relative to the control mice. These data suggest that, while transgene-induced human IL-4 increases the frequency of $CD4^+CXCR4^+$ T cells transplanted into both the C.B-17-*scid* and the BALB/cA-dKO mice, only the latter demonstrates increased sensitivity to X4 HIV-1 infection and replication, at least when this assay is used (see below).

High production of X4 HIV-1 in the culture supernatants of cells from IL-4–transgenic hu-PBL-SCID mice. In an effort

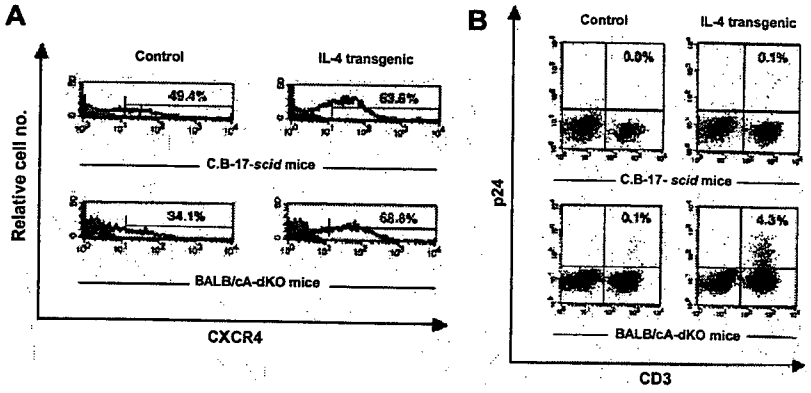

Figure 1. Enhancement of the expression of CXCR4 coreceptor and facilitation of X4 HIV-1 infection and replication in hu-PBL-SCID mice by in vivo production of human interleukin (IL)–4. Groups of hu-PBL-SCID mice, generated from IL-4–transgenic and nontransgenic (control) mice on either the C.B-17-*scid* or BALB/cA-dKO background, were injected intraperitoneally with HIV-1_{NL4-3} at ~24 h after peripheral blood mononuclear cell (PBMC) reconstitution. Six to eight days later, peritoneal lavage fluids were harvested from mice in each group, and cells were collected from the fluids by density-gradient centrifugation. *A*, Cells analyzed for the frequency and mean density of human CXCR4 expression on $CD4^+$ cells by flow cytometry. Data for analyzed cells are depicted by a thick line, and the background control profile is depicted by a thin line and gray shading. The nos. above the bars represent the percentage of positive cells. Data shown are representative of mice in each group from 3 independent experiments. *B*, HIV-1 infectivity. Cells were subjected to flow cytometry after cell-surface CD3 and intracellular p24 staining. Analyzed data are depicted as dot plots. The nos. in the graphs indicate the percentage of $CD3^+p24^+$ cells. Data displayed are representative of mice in each group from 3 independent experiments.

to determine the reason for our failure to detect levels of intracellular p24 in the IL-4–transgenic mice on the C.B-17-*scid* background and to further support the above finding, peritoneal lavage fluids were collected from mock- or HIV-1_{NL4-3}–infected $IL\text{-}4^+$ hu-PBL-SCID mice and, for purposes of control, the HIV-1_{NL4-3}–infected non–IL-4–transgenic mice on the C.B-17-*scid* background. The cells were isolated from the peritoneal lavage fluids, and an aliquot was analyzed for the frequency and the relative density of human CXCR4/CD4; the remaining aliquot was cultured in vitro. In addition, the peritoneal lavage fluids and the culture supernatants of cells at days 1–3 after culture were assayed for levels of p24 production. As displayed in table 2, although the frequency of $CXCR4^+CD4^+$ cells in the IL-4–transgenic mice was significantly higher than that in the nontransgenic mice, the mean fluorescence intensity (MFI) of CXCR4 expressed by the $CD4^+$ T cells from these mice was not increased compared with the control (because of an increase in the frequency of $CXCR4^+CD4^+$ cells with relatively low MFI; see figure 1*A*). Analysis of levels of synthesized p24 demonstrated marked differences, as shown in figure 2. Thus, although the amounts of p24 produced were modest in the peritoneal lavage fluids and the cell-culture supernatants from HIV-1–infected control mice, the levels of p24 produced by those from HIV-1–infected IL-4–transgenic mice were strikingly higher (15,429, 11,844, 1696, and 53 pg/mL in the supernatants on day 3) (mean, 48.9 vs. 7255 pg/mL; >100-fold increase). Although the levels of p24 produced by one of the IL-4–transgenic mice (mouse 12) were similar to those in the control mice, this was likely due to the much lower relative level of human IL-4 (354 pg/mL in serum) produced by mouse 12 than those from the other 3 IL-4–transgenic mice (4227, 6313, and 2356 pg/mL in serum). The present data not only document the fact that the cells from these

Table 2. Effect of the CXCR4 antagonist KRH-1636 on the expression of human CXCR4 by $CD4^+$ cells from X4 HIV-1–infected interleukin (IL)–4–transgenic hu-PBL-SCID mice.

C.B-17-*scid* mice	X4 HIV-1 Infection	CXCR4 antagonist	Mice, no.	$CXCR4^+CD4^+$ T cells, %	*P*	CXCR4 on $CD4^+$ T cells, MFI	*P*
Control	NL4-3	Mock	4	45.5 ± 9.3	<.05[a]	73.7 ± 36.0	NS[a]
IL-4 transgenic	NL4-3	Mock	4	66.7 ± 7.4	NS[b]	73.1 ± 6.2	<.05[b]
IL-4 transgenic	NL4-3	KRH-1636	4	63.0 ± 4.2		62.2 ± 4.8	

NOTE. Control or IL-4–transgenic hu-PBL-SCID mice on the C.B-17-*scid* background infected with X4 HIV-1_{NL4-3} were administered mock KRH-1636 or real KRH-1636. Cells isolated from the peritoneal lavage fluid from the mice in each group were labeled with appropriate monoclonal antibodies and subjected to flow cytometry, as described in Methods. Data analyzed are displayed as mean ± SD values. MFI, mean fluorescence intensity; NS, not significant. The indicated *P* values are based on Student's *t* test.

[a] For the comparison between control mice and IL-4–transgenic mice that received a mock CXCR4 antagonist.

[b] For the comparison between IL-4–transgenic mice that received a mock CXCR4 antagonist and IL-4–transgenic mice that received KRH-1636.

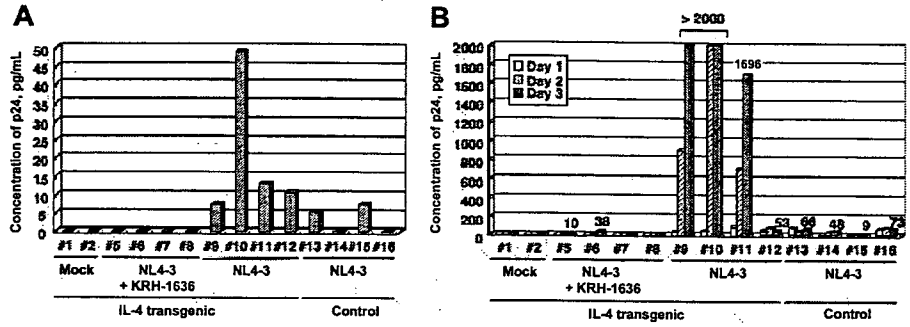

Figure 2. Efficient infection of interleukin (IL)–4–producing hu-PBL-SCID mice with X4 laboratory strain and an inhibitory effect of the CXCR4 antagonist KRH-1636 on infection. Twelve IL-4–transgenic and 4 nontransgenic (control) hu-PBL-SCID mice were generated on the C.B-17-*scid* background. Among them, 8 IL-4–transgenic and 4 control mice were infected intraperitoneally (ip) with the X4 laboratory strain (HIV-1_{NL4-3}) and 4 IL-4–transgenic mice were mock-infected at 1 day after engraftment. To evaluate the effect of KRH-1636, this drug was administered ip twice, at 1 h before and 1 day after infection of 4 IL-4–transgenic mice (NL4-3 + KRH-1636). At 8 days after infection, peritoneal lavage fluids were obtained from the mice in each group. Cells were isolated from the fluids and cultured in IL-2–containing medium. Levels of HIV-1 p24 in the peritoneal lavage fluids *(A)* and culture supernatants at days 1–3 after incubation *(B)* were quantitated for infectivity and replication efficiency by ELISA. With regard to the data on mock-infected mice, only 2 of 4 representative data are presented. The nos. listed above the bars in the graph indicate levels of HIV-1 p24 when it was detectable on day 3 (most of the values were <200 pg/mL, and select samples showed values of >2000 pg/mL). Pound signs (#) indicate mouse nos. Results shown are representative of 3 independent experiments.

IL-4–transgenic C.B-17-*scid* mice are susceptible but also demonstrate that the virus from such cells is replication competent. In addition, these findings suggest that the use of intracellular p24 levels is not a sensitive enough technique and that data using the intracellular p24 assay need to be carefully evaluated. These data also indicate that the IL-4–transgenic hu-PBL-SCID mice provide a powerful model for the study of X4 HIV-1 infection independently of the genetic background of the mice.

Inhibitory effect of the CXCR4 antagonist on infection of IL-4–transgenic hu-PBL-SCID mice with the X4 laboratory strain. In an effort to further validate that the CXCR4 coreceptor was indeed used by the X4 HIV-1 virus in the IL-4–transgenic hu-PBL-SCID mice, we used the X4 virus-entry inhibitor, CXCR4 antagonist KRH-1636. Thus, the IL-4–transgenic hu-PBL-SCID mice on the C.B-17-*scid* background were infected with X4 laboratory strain HIV-1_{NL4-3} and were either mock treated or treated with KRH-1636, and the peritoneal lavage fluids, cells in fluids, and cell-culture supernatants were examined as described above. As shown in table 2, the frequency of $CXCR4^+CD4^+$ cells in KRH-1636–treated IL-4–transgenic mice was marginally lower than that in mock-treated IL-4–transgenic mice. In addition, the MFI of CXCR4 expression by the $CD4^+$ T cells was clearly reduced by KRH-1636 administration. Importantly, treatment with KRH-1636 almost completely blocked X4 HIV-1 infection in these IL-4–transgenic mice (figure 2). These data indicate that X4 HIV-1 infection in transgenic mice is CXCR4 dependent and that our mouse model can be used to develop and test new anti-X4 HIV-1 drugs in vivo.

Therapeutic effect of KRH-1636 on the infection of IL-4–transgenic hu-PBL-SCID mice with MDR clinical isolates. The appearance of MDR HIV-1 clinical isolates has been and continues to be one of the growing problems in a significant number of patients receiving HAART and seriously limits the use of the antiviral drugs that are currently available. Thus, the development of novel adjunct or alternative therapeutics is an urgent need. Since treated patients tend to harbor significantly higher levels of either dual/mixed or X4 viruses [23] and since MDR isolates are not usually refractory to new treatment with drugs from classes that have not been used previously in patients from which the viruses were derived, we finally wanted to examine the effect of KRH-1636 on MDR HIV-1 infection in IL-4–transgenic hu-PBL-SCID mice. For this experiment, we used the IL-4–transgenic BALB/cA-dKO mice instead of the IL-4–transgenic C.B-17-*scid* mice, because the former seems more permissive to X4 HIV-1 infection than the latter, as described above. Before the in vivo study, we confirmed that the in vitro infection by 3 MDR clinical isolates could be inhibited with KRH-1636 (more than ~90% inhibition at the 5-μmol/L level). Thus, groups of IL-4–transgenic hu-PBL-SCID mice were infected with a mixture of these selected MDR isolates containing equal IU of each virus and treated with KRH-1636 or the tartrate carrier control. Thereafter, the cells obtained from the peritoneal lavage fluids were analyzed for the expression of cell-surface human CD4, CD3, and intracellular p24. The serum, peritoneal lavage fluids, and supernatants following in vitro culture of the cells for 24 h were assayed for levels of p24 production. Flow cytometry analysis after CD4 staining demonstrated a significant decline in $CD4^+$ T cells in 2 (mouse 7 and mouse 8) of 4 control-treated mice (figure 3*A;* top profile shows data from 1 of these 2 mice), which was likely due to MDR HIV-1 pathogenesis. However, importantly, no detectable depletion of $CD4^+$ T cells was observed in any of 4 KRH-1636–treated mice (figure 3*A;* bottom profile). As summarized in table 3, the difference in the frequency of $CD4^+$ T cells between the control-treated mice and the

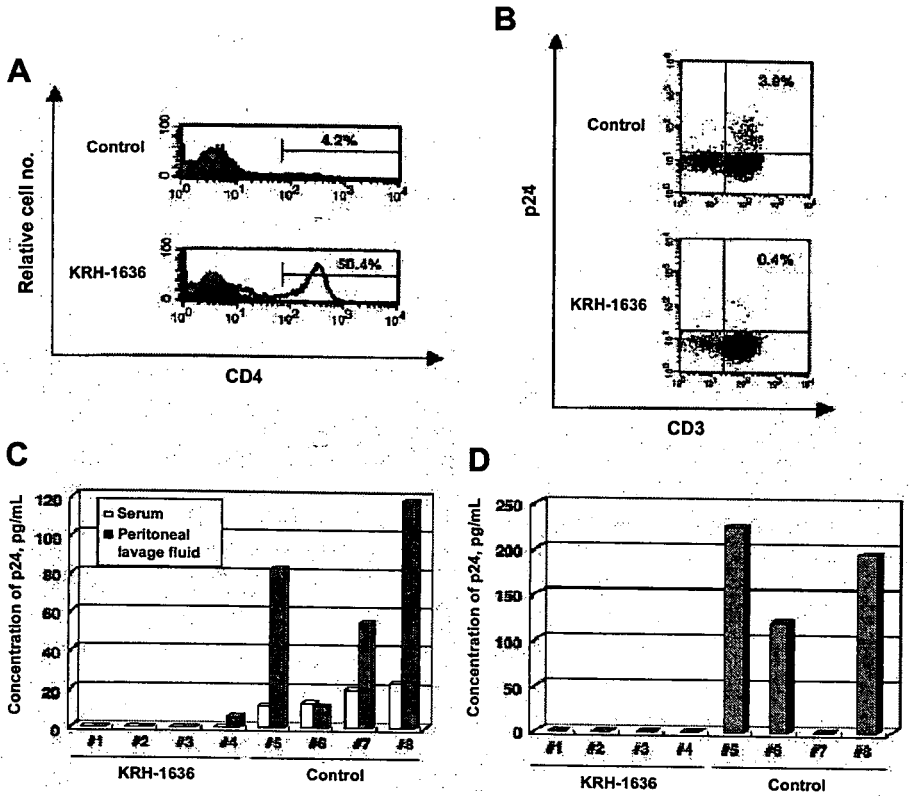

Figure 3. Prophylactic effect of KRH-1636 on infection and pathogenesis by multidrug-resistant (MDR) HIV-1 clinical isolates. Eight interleukin (IL)–4–transgenic hu-PBL-SCID mice (BALB/cA-dKO) were infected intraperitoneally (ip) with a mixture of MDR HIV-1 clinical isolates at 1 day after human peripheral blood mononuclear cell transfer. In an effort to assess the effect of KRH-1636 on HIV-1 infection, this agent or tartrate (control drug) was administered to 4 mice per group ip twice, at 1 h before infection and 1 day after infection. At 7 days after infection, serum and peritoneal lavage fluids were harvested from mice in each group, and cells were collected from the fluids. *A*, Cells examined for human CD4 expression by cell-surface staining and standard flow cytometry. Representative data from a single mouse from the control-treated or the KRH-1636–treated HIV-1–infected mice are shown. The frequency of $CD4^+$ T cells is depicted by a thick line, and the background control is depicted by a thin line with gray shading. The nos. above the bars indicate the percentage of positive cells. *B*, Aliquot of the peritoneal lavage cells analyzed by flow cytometry for the frequency of $CD3^+$ T cells that were positive for the intracellular presence of HIV-1 p24. Representative data of cells from the control-treated and the KRH-1636–treated HIV-1–infected mice are shown. The nos. in the graphs indicate the percentage of $CD3^+p24^+$ cells. *C*, Concentrations of p24 in serum and peritoneal lavage fluid. Concentrations were determined by ELISA to quantify MDR HIV-1 infection and replication efficiency. Pound signs (#) indicate mouse nos. *D*, Levels of in vitro p24 production. The remaining cells were cultured in a microtiter plate containing $IL\text{-}2^+$ medium for ~24 h, and the culture supernatants obtained were assayed for levels of in vitro p24 production by ELISA. Pound signs (#) indicate mouse nos. Results shown are representative of 3 similar independent experiments.

KRH-1636–treated mice was not significant. However, the MFI of CD4 expression was significantly decreased in the control-treated mice (229.3 vs. 296.3; $P < .05$). Results of CD3/p24 staining showed that the frequency of $CD3^+p24^+$ cells was markedly inhibited in the KRH-1636–treated mice, compared with that in the carrier-treated control mice (figure 3*B* and table 3). Furthermore, levels of HIV-1 p24 in the serum samples, peritoneal lavage fluids, and culture supernatants from the KRH-

Table 3. Effect of KRH-1636 on infection and pathogenesis by multidrug-resistant (MDR) HIV-1 clinical isolates in interleukin (IL)–4–transgenic hu-PBL-SCID mice.

BALB/cA-dKO mice	X4 HIV-1 infection	CXCR4 antagonist	Mice, no.	$CD4^+$ T cells, %	*P*	$CD4^+$ T cells, MFI	*P*	$p24^+$ T cells, %	*P*
IL-4 transgenic	MDR	Control	4	14.7 ± 11.9	NS	229.3 ± 33.0	< .05	3.2 ± 0.8	< .01
IL-4 transgenic	MDR	KRH-1636	4	31.3 ± 15.7		296.3 ± 25.2		0.8 ± 0.7	

NOTE. IL-4–transgenic hu-PBL-SCID mice on the BALB/cA-dKO background were infected with MDR HIV-1 clinical isolates and administered tartrate (control) or KRH-1636. Cells in peritoneal lavage fluid from the mice in each group were stained with appropriate monoclonal antibodies and analyzed by flow cytometry, as described in Methods. Data shown here are mean ± SD values. MFI, mean fluorescence intensity; NS, not significant. The indicated *P* values for the comparison between control mice and mice that received KRH-1636 are based on Student's *t* test.

1636–treated HIV-1–infected mice were almost completely reduced relative to those in the control mice (figure 3*C* and 3*D*). Note that the failure to detect the in vitro production of p24 in mouse 7 might result from depletion of $CD4^+$ T cells (figure 3*D*). These data demonstrate that the CXCR4 antagonist KRH-1636 has a marked degree of prophylactic effect on infection with pathogenic MDR clinical isolates in vivo.

DISCUSSION

Humanized mice that have served as valuable small animal models include the SCID-hu Thy/Liv mouse [24–28]. This mouse model, generated by implanting human hematopoietic tissues (human fetal thymus/liver) under the kidney capsule, has been used for the study of HIV-1 and is known for permissiveness to X4 HIV-1 infection [26–28]. However, the use of this model is limited by the fact that the implants are of human fetal organ origins that are not easily available. On the other hand, the hu-PBL-SCID mouse model provides another surrogate in vivo HIV-1 infection assay system. Although this model has led to a number of successful studies of HIV-1 [8–14], there was still a limitation in that it was difficult to demonstrate X4 HIV-1 infection and replication in such mice. Thus, to add extra value to the use of this mouse system for the study of HIV-1, in the present study we developed novel human IL-4–transgenic hu-PBL-SCID mice that enable CXCR4-using HIV-1 strains to efficiently infect and replicate in these mice.

Human IL-4 has low homology with murine IL-4 both at the gene and protein levels, accounting for the lack of cross-reactivity of this cytokine in the 2 species in vitro [29–32]. Results of the experiments reported here indicate that the high efficiency of X4 HIV-1 infection in the IL-4–transgenic hu-PBL-SCID mice was, at least in part, secondary to enhanced expression of viral receptors induced by human IL-4 synthesized endogenously. Interestingly, although there was no apparent increase in the number of cells recovered from the engrafted transgenic mice, there was a significant increase in the number of $CD4^+$ T cells recovered (1.5–3-fold). It is thus possible that the other cell lineages migrate from the peritoneal cavity to other tissues of the mice, resulting in enrichment of the $CD4^+$ T cell lineage. However, further studies of other tissues are needed to clarify this issue. Furthermore, our preliminary experiments indicate that the IL-4–transgenic hu-PBL-SCID mice remain permissive to R5 strain infection (data not shown).

In this report, we created 2 types of novel hu-PBL-SCID mice by transplanting human PBMCs into IL-4–transgenic C.B-17-*scid* and BALB/cA-dKO mice. The data obtained show that hu-PBL-SCID mice using the IL-4–producing BALB/cA-dKO mice appeared more permissive to X4 HIV-1 infection than did those using the IL-4–producing C.B-17-*scid* mice, at least as determined by the presence of intracellular p24. Although the reasons for this difference remain to be determined, it should be noted that, whereas the BALB/cA-dKO mice were derived by double mutation with defects in both the recombinase-activating gene 2 (Rag-2) and the gene encoding the γ_c chain of select cytokine receptors [19, 20], the C.B-17-*scid* mice have only the Rag-2 mutation [18]. Thus, although the Rag-2 mutation prevents the normal maturation of T and B lymphocytes, the γ_c chain mutation abrogates the expression of functional receptors for IL-2 and other cytokines, preventing the expansion of lymphocytes, including NK cells, which play an important role in the innate immune response such as nonspecific rejection of xenogeneic grafts. It is thus possible that the C.B-17-*scid* mice maintain a low but significant residual level of NK cell function, which may play a role in the difference noted above even though they were administered significant levels of anti–IL-2Rβ antibody. Since the BALB/cA-dKO mice are completely deficient in NK cell lineage and function, they are more immunodeficient than the C.B-17-*scid* mice, suggesting that the level and type of immunodeficiency in the BALB/cA-dKO mice may facilitate better engraftment and more efficient viral infection and propagation within these mice. These select defects of the BALB/cA-dKO mice might render the IL-4–transgenic mouse model on this background more valuable and ideal for studies of X4 HIV-1.

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A CD63 Mutant Inhibits T-cell Tropic Human Immunodeficiency Virus Type 1 Entry by Disrupting CXCR4 Trafficking to the Plasma Membrane

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We have discovered that an N-terminal deletion mutant of a membrane protein, CD63, (CD63ΔN) blocks entry of CXCR4-using, T-cell tropic human immunodeficiency virus type 1 (X4 HIV-1) by suppressing CXCR4 surface expression. This suppression was observed for CXCR4 but not for CD4, CCR5, CD25, CD71 or other tetraspanin proteins. The suppression of CXCR4 expression on the plasma membrane appeared to be caused by mislocalization of CXCR4 and exclusive transportation of CXCR4 toward intracellular organelles, mainly late endosomes/lysosomes. Our data suggest that CXCR4 trafficking can be modified in terms of its recruitment to the plasma membrane without enhancing the degradation or arresting vesicular transport of CXCR4.

Key words: cell surface expression, CD63, CXCR4, HIV-1, ligand-independent trafficking

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It has been formerly shown that some cellular factors have the ability to suppress retroviral replication. Restriction factors, such as APOBEC3G and Trim5α, play a significant role in controlling human immunodeficiency virus type 1 (HIV-1) infection (1), and it is predicted that other cellular factors will also influence HIV-1 replication. Innovative approaches should, therefore, bring to light, as-yet untested, antiviral factors. We have previously reported a cDNA-library-expressing lentiviral vector system used to isolate an inhibitor of HIV-1-induced cytopathic effect (CPE) (2).

HIV-1 infects T cells and macrophages that express surface CD4 and chemokine receptors. CXCR4 is a G-protein-coupled chemokine receptor that acts as a receptor for stromal-cell-derived factor 1 (SDF-1) and one of the co-receptors for HIV-1 (3). SDF-1 has been shown to inhibit X4 HIV-1 infection, probably by promoting removal of CXCR4 from the cell surface via ligand-induced endocytosis (4,5). The endocytosis of CXCR4 from the cell surface occurs through clathrin-coated pits, which results from the binding to β-arrestin and perhaps adaptor protein complex-2 (AP-2) (5–7). The ubiquitination of CXCR4 at the plasma membrane has been shown to facilitate a sorting event leading to its lysosome-dependent degradation (8,9). In general, membrane proteins are synthesized in the endoplasmic reticulum (ER) and the folded proteins are transported through the Golgi apparatus, and undergo modification such as glycosylation. At the *trans*-Golgi network (TGN), mature proteins are subsequently sorted and packaged into specific vesicles destined for the plasma membrane or the endosomes. In the case of lysosome-associated proteins (LAMP), there are two alternative routes from the TGN, one to the plasma membrane and the other to the lysosomes (10). However, it is unclear whether or not CXCR4 can be targeted directly from the TGN to the lysosome. In addition, there has not been a report of any molecule that affects trafficking of CXCR4 to the plasma membrane.

CD63 is a membrane protein belonging to the tetraspanin superfamily, consisting of four transmembrane domains (TM1-4) and two extracellular (EC) domains (1–2). It is widely expressed on the surface of many cell types, forming tetraspanin-enriched microdomains (TEMs) on the plasma membrane with other tetraspanin proteins (11). It is also present in secretory vesicles (12,13) and the membranes of the late endosomes and lysosomes (14,15). At the plasma membrane, CD63 is known to interact with molecules such as integrins (16), a tissue inhibitor of metalloproteinase-1 (17) and syntenin-1 (18), and is thought to regulate signal transduction pathways required for cell adhesion, motility and survival. It was also reported that CD63 is involved with endocytosis of its interaction partners such as the β-subunit of H, K-ATPase (HKβ) (19) or membrane-type 1 matrix metalloproteinase (MT1-MMP) (20). However, its physiological role is still not well understood (21–23).

In this study, by using our screening strategy (2), we identified an N-terminal deletion mutant of CD63 that

belongs to a novel class of HIV-1 entry blockers. This CD63 mutant appeared to suppress CXCR4 on the cell surface by changing CXCR4 intracellular trafficking probably after its dispatch from the TGN. In addition, we found that wild-type CD63 also functions to suppress CXCR4 cell surface expression, which may be a physiological function of the protein.

Results

Isolation of gene encoding an anti-HIV-1 protein

Using a bicistronic lentiviral vector, encoding cDNA and a humanized recombinant green fluorescent protein (hrGFP), we generated a human peripheral blood leukocyte (PBL) cDNA library-transduced CD4 CXCR4 T-cell line (MT-4) (2). Although most cells were killed after X4 HIV-1 (HIV-1_{NL4-3}) infection, some hrGFP (cDNA) cells were found to proliferate continuously for more than 30 days post-infection (dpi), from which it was inferred that these cells were transduced with an anti-HIV-1 cDNA. From these cells, we independently isolated two cDNA clones, each containing a 3′ fragment of *cd63* cDNA spanning nucleotide positions 193 or 228 to the 3′ poly-A sequence (Figure S1). These cDNAs (designated clone 12.03 and clone 12.22, respectively) encode a C-terminal fragment of CD63 that contains 156 amino acid residues (amino acid positions 83–238) initiating from the third methionine (Figure S1). We then confirmed that both clone 12.03 cDNA- and clone 12.22 cDNA-transduced MT-4 cells (hrGFP) were resistant against HIV-induced CPE (data not shown) and found that there is little expression of HIV-1 antigen on these cells after HIV-1 inoculation (Figure 1A). Thus, we isolated cDNAs as a new inhibitor gene of HIV-1-induced CPE.

To reproduce this inhibition via transduction of the *cd63* gene, we next prepared a wild-type *cd63* cDNA (CD63wt)-expressing lentiviral vector, as well as an N-terminal deletion mutant (CD63ΔN)-expressing lentiviral vector (Figure 1B) encoding a C-terminal fragment of CD63 identical to those of the isolated cDNA clones mentioned above (Figure S1). Western blotting analysis using an anti-CD63 monoclonal antibody (mAb), confirmed that CD63ΔN-expressing lentiviral vector and the two originally-isolated cDNA expressed peptides with identical molecular weight (MW) (data not shown). As CD63 has a tyrosine-based lysosomal sorting motif (LSM; amino acid positions 233–238) that binds to AP-2 μ and AP-3 μ subunits (15), we also prepared an LSM-deleted CD63ΔN mutant (designated CD63ΔNL) (Figure 1B). FLAG-tagged wild-type CD63 (FLAGCD63wt), CD63ΔN (FLAGCD63ΔN), or CD63ΔNL (FLAGCD63ΔNL) DNA was transfected into HeLa-derived MAGIC-5 cells (CXCR4 CD4 CCR5), and expression was confirmed by Western blotting using an anti-FLAG mAb. This revealed that these proteins were heavily glycosylated (Figure 1C). Immunofluorescent analysis using these DNA showed that CD63wt and CD63ΔN was predominantly distributed in the perinuclear region and some CD63ΔN were found in intracellular vesicles (Figure 1D). The majority of CD63ΔNL, however, appeared to accumulate at the plasma membrane with some intracellular staining (Figure 1D, third panel).

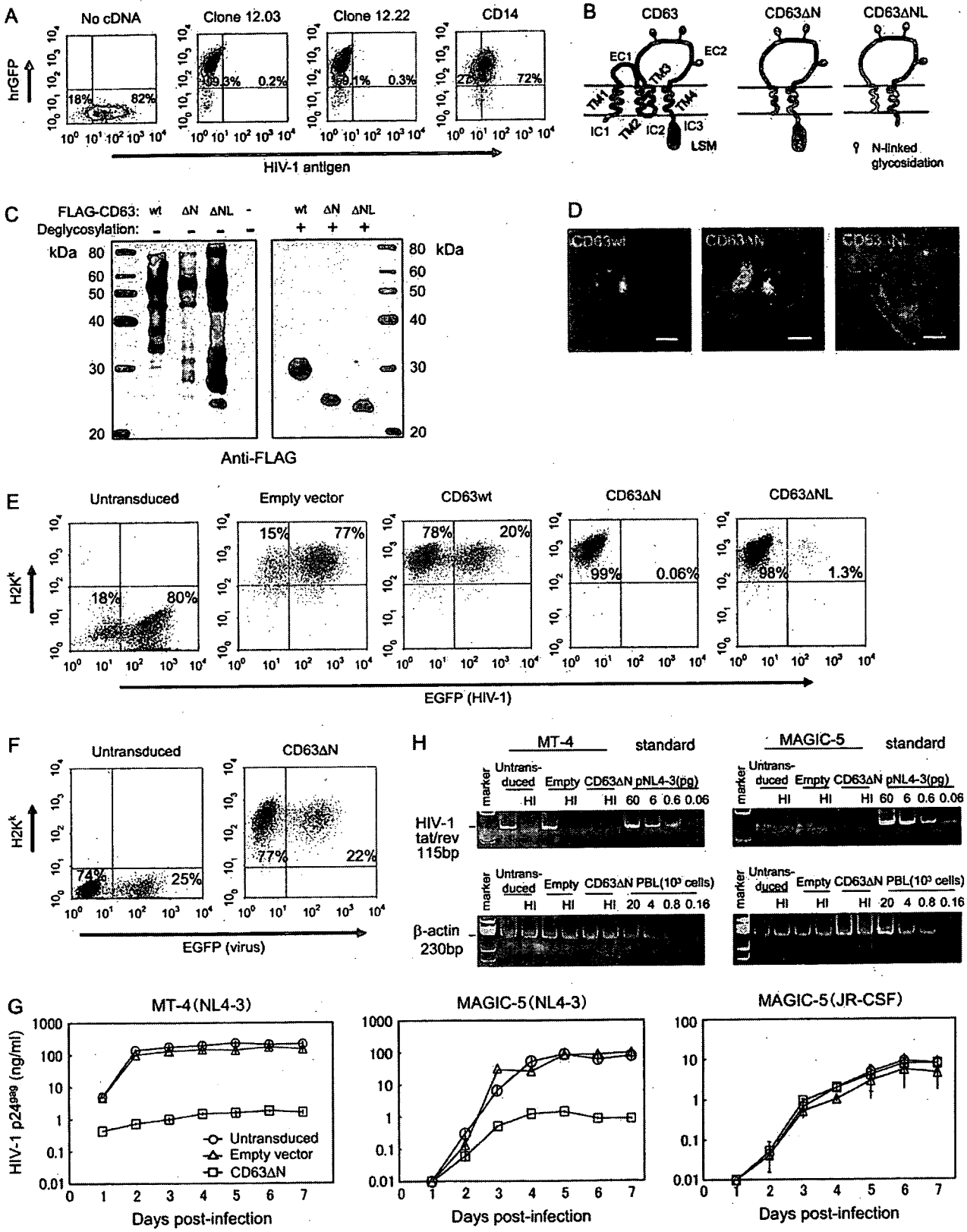
Inhibition of HIV-1 infection by transduction of cells with CD63 and CD63 mutants

To examine anti-HIV-1 activity of CD63-transduced cells further, we generated ectopic CD63wt- or CD63 mutant-expressing cells using a bicistronic $H2K^k$-expressing lentiviral vector. Transduction of the gene by this lentiviral vector did not substantially affect cell growth (data not shown). We then evaluated anti-HIV activity using an enhanced GFP (EGFP)-expressing X4 HIV-1 (NL-EGFP) (24). Flow cytometric analyses indicated that HIV-1 infection was clearly inhibited in the CD63wt- and CD63 mutant (i.e. CD63ΔN and CD63ΔNL)-transduced cells compared with untransduced or empty vector-transduced cells. In CD63ΔN-transduced cells, in particular, HIV-1 infection was severely inhibited (Figure 1E, fourth panel). However, when we used a pseudotyped HIV-1 (where the HIV-1 envelope protein was replaced with that of amphotropic Moloney murine leukemia virus, MLV), the infectivity reduction was not observed (Figure 1F), suggesting that this inhibition is virus envelope protein dependent.

We confirmed that CD63ΔN was also able to protect transduced cells against wild-type X4 HIV-1 infection. Following X4 HIV-1_{NL4-3} infection, enzyme-linked immunosorbent assays (ELISA) revealed that the level of HIV-1 $p24^{gag}$ protein in the culture supernatant of CD63ΔN-transduced MT-4 and MAGIC-5 cells was approximately 100-fold lower than that of either empty vector-transduced or untransduced cells (Figure 1G, left and center panels). However, in the case of CCR5-using HIV-1 (R5 HIV-1; HIV-1_{JR-CSF}) infection, there was no apparent effect of CD63ΔN on the concentration of $p24^{gag}$ in the culture supernatant (Figure 1G, right panel). These data suggest that replication of X4 HIV-1 was specifically inhibited by CD63ΔN. In addition, the level of newly synthesized X4 HIV-1 cDNA in the CD63ΔN-transduced cells was clearly lower (Figure 1H), suggesting that entry of X4 HIV-1 was inhibited by CD63ΔN. Taken together, these results suggest that ectopic CD63wt and CD63ΔN specifically inhibit X4 HIV-1 entry. Therefore, we hypothesized that expression of the X4 virus-specific co-receptor molecule, CXCR4, might be downregulated by ectopic expression of CD63wt and CD63ΔN.

Suppression of CXCR4 surface expression by CD63- and its mutant transduction

To examine the correlation between levels of CXCR4 surface expression and the transduction efficiency of CD63wt and its mutants, we transduced CD63 into cells in different multiplicity of infection (MOI) using a bicistronic $H2K^k$-expressing lentiviral vector. Flow cytometric analysis using an anti-$H2K^k$ mAb confirmed that higher MOI increased transduction efficiency (data not shown). The flow cytometric analyses using an anti-CXCR4 mAb (A-80)



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indicated that the level of CXCR4 surface expression on CD63wt-transduced MT-4 cells clearly decreased with increased MOI (Figure 2A). A much more obvious suppression of CXCR4 surface expression was found on CD63ΔN-transduced cells (Figure 2A). The suppression of CXCR4 surface expression was further confirmed using another anti-CXCR4 mAb (12G5) (25), which reacts with a different epitope (data not shown). The CD63ΔN-induced suppression of CXCR4 surface expression was only partially impaired by LSM deletion (CD63ΔNL in Figure 2A), and a CD63 mutant lacking LSM (CD63ΔL) still has suppressive activity (data not shown). Suppression of cell surface expression of CXCR4 was also found in 293T cells co-transfected with CXCR4 and CD63ΔN DNA (data not shown).

Suppression of surface expression in CD63wt or CD63 mutant-transduced MT-4 cells was detectable only in CXCR4, but not in CD25 [interleukin (IL)-2 receptor α chain] (Figure 2B), or CD71 (transferrin receptor) molecules (Figure 2C). The suppression of CXCR4, but not CCR5, CD4 or CD71 was also found in CD63ΔN-transduced MAGIC-5 cells (Figure 2D). In addition, the suppression of other tetraspanin proteins such as CD9, CD53, CD81, CD82 and CD151 was not found in CD63ΔN-transduced MAGIC-5 cells (data not shown). Fluorescent microscopic analysis on live cells, using a third anti-CXCR4 mAb (A-145), confirmed the loss of surface CXCR4 on CD63ΔN-transduced MAGIC-5 cells (Figure 2E). Significant suppression was also seen in human CD4 T cells derived from peripheral blood mononuclear cells (PBMC), which are natural target cells for HIV-1 (Figure 2F). To further confirm the CXCR4 suppression, we assessed the ability of the transduced cells to migrate in response to SDF-1 stimulation. We treated CD63ΔN-transduced MAGIC-5 cells with SDF-1 and found a severe suppression of chemotaxis response in CD63ΔN-transduced cells compared with that of empty vector-transduced cells (Figure 2G). From these results, we concluded that ectopic CD63, especially CD63ΔN, induces significant downregulation of CXCR4 surface expression, because it (i) protects cells against X4 HIV-1 infection, (ii) renders cell surface expression of CXCR4 undetectable when analyzed using 3 anti-CXCR4 mAbs or (iii) renders cells unable to respond to SDF-1.

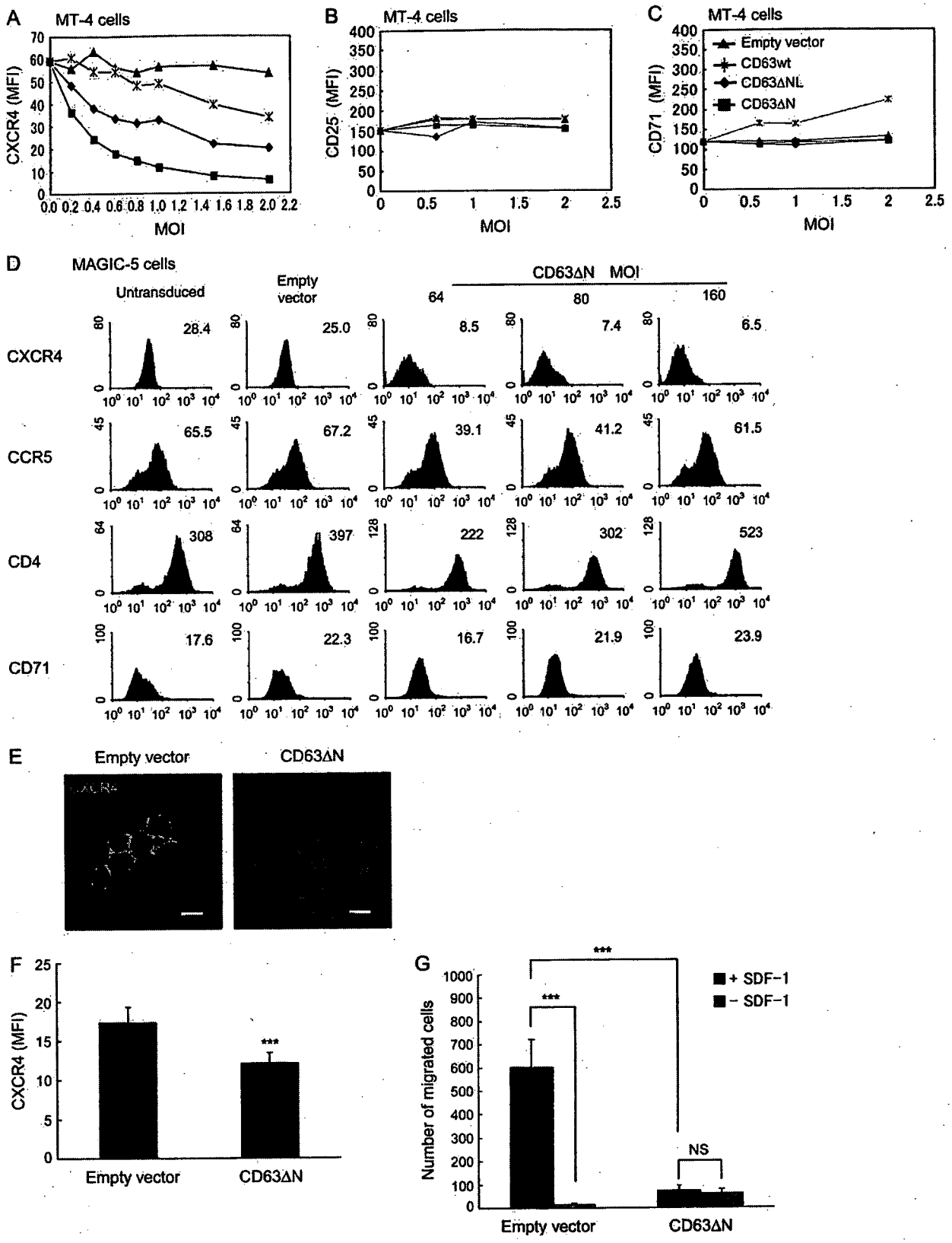
Involvement of CD63 in regulation of CXCR4 cell surface expression

To clarify whether physiological CD63 plays a role in the regulation of CXCR4 surface expression, we next depleted endogenous CD63 in empty vector-transduced cells using three small interfering RNA (siRNA) oligonucleotides against *cd63*. Immunofluorescent analysis indicated a clear depletion of intracellular CD63 by these siRNAs compared with control siRNA (Figure 3A). Flow cytometric analysis also indicated a significant reduction in CD63 surface expression by these siRNAs (Figure 3B). Levels of CXCR4 surface expression on *cd63*-depleted cells were clearly higher than that on control cells (Figure 3C), indicating that depletion of CD63 resulted in an increased level of CXCR4 surface expression. Combined with the data from CD63wt-transduced cells (Figure 2A), we deduced that CD63 may also negatively regulate CXCR4 surface expression.

Induction of CXCR4 mislocalization by CD63ΔN

To address how CD63ΔN suppresses CXCR4 surface expression, we considered the following possibilities: (i) suppression of CXCR4 mRNA or protein expression;

Figure 1: Specific inhibition of X4 HIV-1 infection by CD63 mutants. A) Little expression of HIV-1 antigen was detected in MT-4 cells transduced with the two putative CD63 C-terminal cDNAs (clone 12.03 and clone 12.22). Four days after HIV-1_{NL4-3} infection, HIV-1 antigen expression on transduced cells was examined using anti-HIV-1 human sera. The level of hrGFP indicates the efficiency of cDNA transduction (*y*-axis). The numbers in each quadrant indicate the percentage of HIV antigen ( ) and ( ) cells. CD14 is a control having some level of anti-HIV-1 activity as we have previously reported (2). The results of one of three, independently conducted, experiments are shown. B) Structure of CD63 and its mutants. CD63 is comprised of four transmembrane domains (TMs), a small extracellular loop (EC1), a four amino acid intracellular loop (IC2), and a large extracellular loop (EC2), as well as 11 amino acid N-terminal and a 10 amino acids C-terminal tail (containing the LSM). C) CD63 and its mutants are heavily glycosylated. MAGIC-5 cells were transfected with FLAGCD63wt or FLAGCD63-mutant DNA. Cell extracts were incubated in the presence (right panel) or absence (left panel) of deglycosidase and then subjected to Western blot analysis using an anti-FLAG mAb. D) Localization of CD63wt and CD63 mutants. MAGIC-5 cells were transfected with FLAGCD63wt or FLAGCD63-mutant DNA, stained with an anti-FLAG mAb, and analyzed by confocal microscopy. Images were acquired through band-pass filters (BPF) 500–520 nm (FLAG: green) and BPF 420–470 nm (Hoechst; nuclei staining: blue). Scale bars, 10 μm. E) Inhibition of HIV-1 infection by transduction of CD63wt or CD63 mutants. CD63wt-, CD63ΔN-, and CD63ΔNL-transduced MT-4 cells were challenged with NL-EGFP at a MOI of 0.1. Three days after HIV-1 infection, cells were stained with an anti-H2K^kmAb, and analyzed by flow cytometry. Surface expression of H2K^kgave an indication of transduction efficiency (*y*-axis). The numbers in each quadrant indicate the percentage of EGFP ( ) and ( ) cells. The results of one of three, independently conducted, experiments are shown. F) No inhibition of MLV Env-pseudotyped HIV-1 infection by transduction of CD63ΔN. CD63ΔN-transduced MT-4 cells were also challenged with an amphotropic MLV Env-pseudotyped EGFP-expressing HIV-1. The numbers in each quadrant indicate the percentage of EGFP ( ) and ( ) cells. The results of one of three, independently conducted, experiments are shown. G) Lower X4 HIV-1 production in CD63ΔN-transduced cells. The amount of HIV $p24^{gag}$ in cell culture supernatant was followed using ELISA after wild-type HIV-1 infection (left panel: X4 HIV-1_{NL4-3}-infected MT-4 cells; center panel: X4 HIV-1_{NL4-3}-infected MAGIC-5 cells; and right panel: R5 HIV-1_{JR-CSF}-infected MAGIC-5 cells). H) Blocking of X4 HIV-1 entry in CD63ΔN-transduced cells. The level of synthesized HIV-1 cDNA in cells was semiquantified by PCR. HI indicates heat inactivated HIV-1. HIV-1 plasmid DNA was used for marker standards (left: X4 HIV-1_{NL4-3}-infected MT-4 cells; and right: X4 HIV-1_{NL4-3}-infected MAGIC-5 cells). β-actin serves as a control.



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