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Vaccination of Rhesus Macaques with Recombinant *Mycobacterium bovis* Bacillus Calmette-Guérin Env V3 Elicits Neutralizing Antibody-Mediated Protection against Simian-Human Immunodeficiency Virus with a Homologous but Not a Heterologous V3 Motif

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Although the correlates of vaccine-induced protection against human immunodeficiency virus type 1 (HIV-1) are not fully known, it is presumed that neutralizing antibodies (NAb) play a role in controlling virus infection. In this study, we examined immune responses elicited in rhesus macaques following vaccination with recombinant *Mycobacterium bovis* bacillus Calmette-Guérin expressing an HIV-1 Env V3 antigen (rBCG Env V3). We also determined the effect of vaccination on protection against challenge with either a simian-human immunodeficiency virus (SHIV-MN) or a highly pathogenic SHIV strain (SHIV-89.6PD). Immunization with rBCG Env V3 elicited significant levels of NAb for the 24 weeks tested that were predominantly HIV-1 type specific. Sera from the immunized macaques neutralized primary HIV-1 isolates in vitro, including HIV-1_{BZ167/X4}, HIV-1_{SF2/X4}, HIV-1_{C12/X4}, and, to a lesser extent, HIV-1_{MNP/X4}, all of which contain a V3 sequence homologous to that of rBCG Env V3. In contrast, neutralization was not observed against HIV-1_{SF33/X4}, which has a heterologous V3 sequence, nor was it found against primary HIV-1 R5 isolates from either clade A or B. Furthermore, the viral load in the vaccinated macaques was significantly reduced following low-dose challenge with SHIV-MN, and early plasma viremia was markedly decreased after high-dose SHIV-MN challenge. In contrast, replication of pathogenic SHIV-89.6PD was not affected by vaccination in any of the macaques. Thus, we have shown that immunization with an rBCG Env V3 vaccine elicits a strong, type-specific V3 NAb response in rhesus macaques. While this response was not sufficient to provide protection against a pathogenic SHIV challenge, it was able to significantly reduce the viral load in macaques following challenge with a nonpathogenic SHIV. These observations suggest that rBCG vectors have the potential to deliver an appropriate virus immunogen for desirable immune elicitation.

Development of a preventive vaccine against human immunodeficiency virus type 1 (HIV-1) is urgently needed to control the spread of the virus worldwide. Although the immunological parameters that correlate with protective immunity against natural infection with HIV-1 are not fully known, it is assumed that a preventive vaccine must elicit potent, broadly reactive immunity against divergent strains of HIV-1 (25, 36, 42). Several recent studies have demonstrated that induction of virus-specific T-cell responses can confer protective immunity in nonhuman primate models, and these responses may also play a role in controlling HIV-1 replication in humans (6, 18, 19, 31, 33, 34, 38, 45, 48). Vaccine constructs containing viral *env* genes, in addition to *gag* and *pol*, have been shown to effec-

tively control replication of challenge viruses (2, 5, 10), suggesting that neutralizing antibody (NAb) responses might also contribute to protection against pathogenic infection or disease progression. Passive transfer of serum immunoglobulin from chimpanzees experimentally infected with several different HIV-1 isolates has been shown to block the establishment of a simian immunodeficiency virus (SIV)-HIV chimeric simian-human immunodeficiency virus (SHIV) infection in pig-tailed macaques (37, 46). It is not known, however, whether vaccines that actively elicit a potent NAb response can provide protection in nonhuman primates challenged with SHIV.

Previously, we demonstrated that recombinant *Mycobacterium bovis* bacillus Calmette-Guérin (rBCG), which secretes a chimeric protein consisting of the V3-neutralizing epitope of HIV-1 and α -antigen (rBCG Env V3), can induce HIV-1-specific NAb in a small-animal model (9, 15, 16). BCG was selected as a vaccine vehicle because it has several characteristics that are considered efficacious for developing a candidate

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HIV-1 vaccine (1, 49), including the ability to induce long-lasting immune responses (7). It is generally accepted that a candidate vaccine against HIV-1 must also be easily administered and affordable in developing countries, and it must be compatible with other commonly administered vaccines (35). If effective, a BCG-based recombinant HIV-1 (rBCG-HIV-1) vaccine would fulfill many of these critical requirements.

Results using other vaccine modalities, in particular, live attenuated SIV vaccines, have raised concerns about the potential for reversion to pathogenicity (3, 4), suggesting that many SIV strains may be potentially virulent. In this study, we used two distinct strains of challenge virus: SHIV-MN (29), which contains V3 sequences homologous to rBCG Env V3, and SHIV-89.6PD (12, 20, 28, 41), which is heterologous in the V3 region and highly pathogenic. We examined whether vaccination with rBCG Env V3 could effectively elicit NAb responses in rhesus macaques and whether it might effectively induce protective immunity against challenge with either SHIV-MN or SHIV-89.6PD.

MATERIALS AND METHODS

Animals. The macaques (*Macaca mulatta*) used in this study originated from China and were purchased through Japan SLC Ltd., Shizuoka, Japan. The animals were maintained according to standard operating procedures established for the evaluation of human vaccines at the Tsukuba Primate Center, National Institute of Infectious Diseases, Tsukuba, Ibaragi, Japan. The study was conducted in the P3 facility for monkeys in the Murayama Branch, National Institute of Infectious Diseases, Musashimurayama, Tokyo, Japan, and in accordance with requirements specified in the laboratory biosafety manual of the World Health Organization.

Construction of the rBCG Env V3 immunogen. rBCG strain Tokyo was produced by transfection of BCG-Tokyo 172 cells with plasmid pSO246 as described previously (21, 22, 30). The XhoI site of this plasmid was used to insert a mycobacterial codon-optimized DNA fragment encoding 19 amino acids of the Japanese HIV-1 V3 consensus sequence (NTRKSIHIGPGRFYATGS), which has a neutralization sequence identical to that of HIV-1_{MN} (16, 23, 39, 52). The resulting rBCG vector was designated rBCG Env V3. By semiquantitation of a chimeric protein consisting of the V3 peptides and α -K protein (9), the concentration of the secreted protein was estimated to range from 1 to 3 μ g/ml in the culture filtrate of rBCG Env V3 (data not shown).

Viruses. Viruses used in challenge experiments were kindly provided by Y. Lu, Harvard AIDS Institute, Cambridge, Mass. The SHIV-MN virus stock was prepared in concanavalin A-activated macaque peripheral blood mononuclear cells (PBMC) from normal animals, and the amount of virus was quantified by SIV p27 antigen enzyme-linked immunosorbent assay (ELISA) (Coulter Co., Hialeah, Fla.). The tissue culture infective dose (TCID) of the stock was measured on CEMx174 cells (AIDS Research and Reference Reagent Program, National Institutes of Health, Rockville, Md.). Stocks of HIV-1_{MN} and HIV-1_{IIIB} (AIDS Research and Reference Reagent Program) were prepared by propagating 100 50% TCID (TCID₅₀) of each virus in phytohemagglutinin-activated normal human PBMC, as described previously (17). The primary isolate, HIV-1_{MNP}, was kindly provided by J. Sullivan, University of Massachusetts Medical School, Worcester, Mass. All other viruses were obtained from the AIDS Research and Reference Reagent Program. Cell-free virus stocks were stored at -130°C until they were used.

V3-specific ELISA. HIV-1 V3 peptide-based ELISAs were used for titration and quantification of serum antibodies for detection as described previously (14). In brief, 96-well ELISA plates (MaxiSorp; Nunc A/S, Roskilde, Denmark) were coated with 100 μ l of peptide MN (DKRIHIGPGRFYTT) /well in 50 mM carbonate buffer (pH 9.3) at 5 μ g/ml overnight at 4 $^{\circ}\text{C}$. The wells were washed and treated with 5% nonfat milk in phosphate-buffered saline for 1 h at 37 $^{\circ}\text{C}$. Duplicate samples containing either control or test macaque serum at appropriate dilutions were then added at 100 μ l/well, and the plates were incubated for 1 h at 37 $^{\circ}\text{C}$. The wells were washed and incubated with a detection antibody solution consisting of peroxidase-conjugated goat anti-monkey immunoglobulin G (IgG) antibody (EY laboratories Inc., San Mateo, Calif.) at 100 μ l/well for 1 h at 37 $^{\circ}\text{C}$. After final washes with 0.05% Tween-20-phosphate-buffered saline

(PBST), peroxidase substrate was added, and the reaction was stopped by the addition of 0.5 M H₂SO₄.

IFN- γ ELISPOT assay. Enzyme-linked immunospot (ELISPOT) assays were performed using the method developed by Mothe and Watkins of the Wisconsin University Primate Center and described elsewhere (18, 33). In brief, 96-well flat-bottom plates (U-CyTech-BV, Utrecht, The Netherlands) were coated with anti-gamma interferon (IFN- γ) monoclonal antibody before being washed with PBST and blocked with bovine serum albumin. Freshly isolated PBMC were mixed with either concanavalin A or 2 μ M V3 peptide and were then incubated for 16 h at 37 $^{\circ}\text{C}$ in 5% CO₂ in anti-IFN- γ -coated plates. Once the plates had been washed, rabbit anti-IFN- γ polyclonal biotinylated detector antibodies were added, and the plates were incubated. Gold-labeled anti-biotin IgG solution (U-CyTech-BV) was added to the plates after they were washed with PBST. The plates were then incubated for 1 h at 37 $^{\circ}\text{C}$. Developed wells were imaged, and spot-forming cells (SFC) were counted using the KS ELISPOT compact system (Carl Zeiss, Oberkochen, Germany). An SFC was defined as a large black spot with a fuzzy border (33).

In vitro virus neutralization assays. GHOST cell neutralization assays were performed as previously described (8). Briefly, GHOST cells expressing either CXCR4 or CCR5 were used as targets for HIV-1 infection (50, 54). The cells were analyzed by FACSCalibur flow cytometry (Becton Dickinson, San Jose, Calif.), and 15,000 events were scored. The mean number of fluorescent GHOST cells determined from negative controls plus 2 standard deviations was considered the cutoff for a positive sample. Purified human immunoglobulin (Nihon Pharmaceutical Co., Tokyo, Japan) and saline were included as additional controls.

M8166 cell-based virus neutralization assays were also performed as described previously (16, 47). In brief, the in vitro neutralization activity of purified macaque IgG was determined using 100 TCID₅₀ of either HIV-1_{MN} or SHIV-MN in cultures of M8166 cells. The results were compared with parallel cultures to which preimmune serum IgG was added. Neutralization was expressed as percent inhibition of HIV-1 p24 or SIV p27 antigen production in the culture supernatants. Purified normal macaque IgG was used as a control.

Quantification of cell-associated viral load. Levels of cell-associated virus were quantified by limiting dilution of PBMC (from 10⁶ to 1 cells), and the virus was cocultured with M8166 cells as described previously (17). Virus released into the culture supernatant was measured by SIV p27 antigen ELISA (Coulter). The smallest number of PBMC required to produce a positive culture was considered the end point, and the titer of infectious virus was expressed as TCID₅₀ per 10⁶ PBMC.

PCR detection of proviral HIV-1 infection of rhesus macaques. PBMC with SHIV were detected by DNA PCR using a primer pair that spans the C2-V3 sequence of HIV-1_{IIIB}, followed by Southern blotting with an SE1 probe, 5'-G CAGAAGAAGAGGTAGTAATTAGAT-3' (nucleotides 7019 to 7043) (47). The positions of the oligonucleotides are numbered relative to the HIV-1_{HXB2} isolate in the ENTREZ database (National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, Md.). Viral DNA was quantified by comparison with standards derived from 8E5/LAV cells, which contain one copy of HIV-1 proviral DNA per cell (AIDS Research and Reference Reagent Program).

Competitive PCR quantitation of SHIV RNA in plasma. Quantitative, competitive reverse transcription-PCR was performed using a competitor RNA and a DNA template as previously described (18, 32, 44). The detection limit of this assay was 500 RNA copies/ml in monkey plasma (18, 32).

Sequencing of HIV-1 Env C2-V3 sequence. To determine the sequence of the HIV-1 Env C2-V3 region, mRNA was extracted from stock virus and cDNA was synthesized using a Micro-FastTrack version 2.0 kit (Invitrogen, Carlsbad, Calif.) and a cDNA cycle kit (Invitrogen) according to the manufacturer's instructions. The PCR products were cloned into a pCR II vector with a dual promoter using a TA cloning kit (Invitrogen) (47). Sequence analysis was performed using a Big Dye terminator cycle-sequencing FS kit (Perkin-Elmer, Foster City, Calif.) and automated ABI 310 sequencer (Perkin-Elmer) with Sp6 and T7 sequence primers (Invitrogen). Sequence data were compared with published HIV-1 sequences in GenBank (National Center for Biotechnology Information, National Institutes of Health).

Statistical analysis. Calculations of the geometric mean \pm standard deviation (SD) were carried out with a microcomputer. Significance was defined as a *P* value of <0.05 .

RESULTS

Vaccination protocol. Twenty-four male rhesus macaques (R-01 through R-24) were enrolled in the study. Of these, 15 were subcutaneously immunized for 24 weeks with 10 mg of

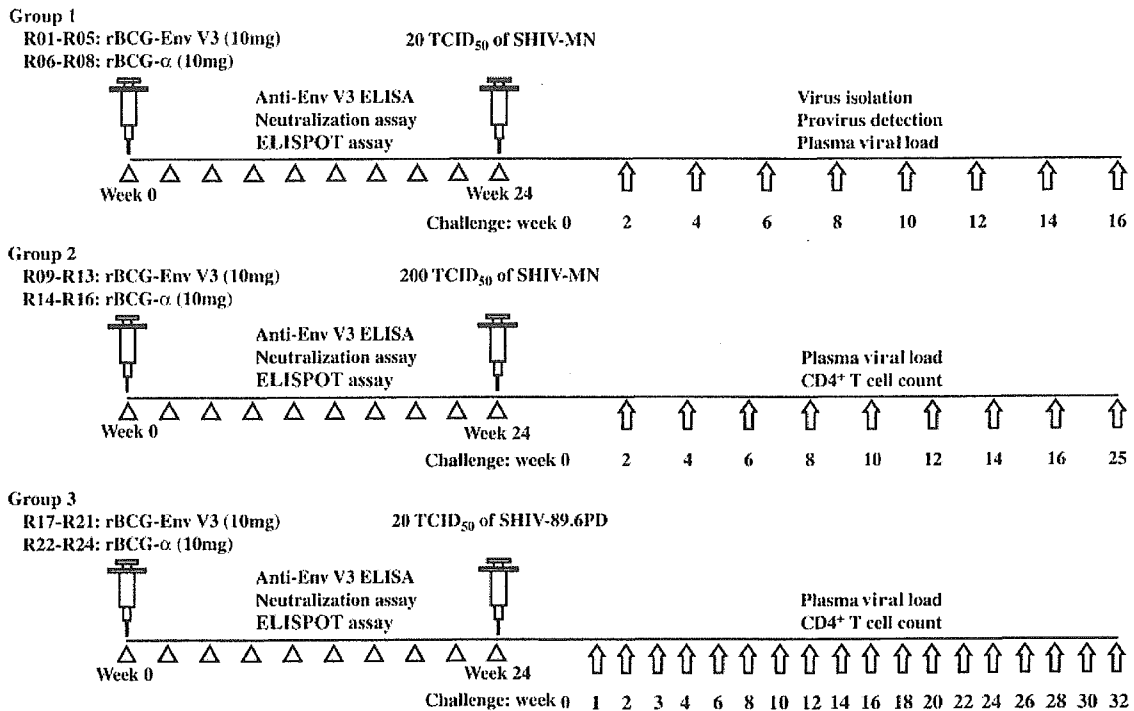


FIG. 1. Schematic representation of the experimental protocol for immunization of rhesus macaques with rBCG Env V3 and challenge with either SHIV-MN or SHIV-89.6PD. A total of 24 macaques were assigned to either the rBCG Env V3 vaccine or rBCG vector control group. The animals each received a single subcutaneous injection and were then split into three groups prior to challenge with either low-dose SHIV-MN, high-dose SHIV-MN, or SHIV-98.6PD.

rBCG Env V3 (16), which expresses and secretes a chimeric protein consisting of α -antigen and the Env V3 region of HIV-1_{MN}. The remaining nine macaques were immunized by the same route and with the same dose of rBCG α -antigen and served as vector controls. All macaques inoculated with rBCG Env V3 remained in good health following vaccination. Three of the 15 immunized macaques experienced transient redness with slight erosion localized at the injection site; however, the reaction spontaneously resolved within 3 months. Following immunization, the 24 macaques were divided into three groups, each group consisting of five immunized animals and three vector controls. The macaques within each group received an intravenous challenge with either SHIV-MN (20 or 200 TCID₅₀) or SHIV-89.6PD (20 TCID₅₀) (Fig. 1).

Vaccine-induced HIV-specific immune responses following rBCG Env V3 immunization. (i) **Neutralizing antibodies.** As described above, 15 rhesus macaques were vaccinated with a single subcutaneous inoculation of 10 mg of rBCG Env V3. Induction of HIV-1-specific immunity was measured 24 weeks later in blood samples obtained pre- and postvaccination. All 15 immunized macaques exhibited HIV-1 Env V3 peptide-binding antibody activity by ELISA at serum dilutions ranging from 1:640 to 1:10,240 (Fig. 2). Antibody responses were monophasic, peaking at 4 to 6 weeks and then gradually declining. Serum samples obtained from naive macaques were consistently negative by ELISA, while postvaccination sera did not react with a control fusion peptide of HIV gp41 (data not shown).

Antibodies were purified from the macaque sera to remove factors that might interfere with certain bioassays (51). The purified antibodies were then tested in vitro for the ability to neutralize SHIV-MN infection in M1866 cells (Fig. 3). Antibodies induced in macaques vaccinated with rBCG Env V3 strongly neutralized both the challenge SHIV-MN (grown in rhesus PBMC) and a T-cell line-adapted (TCLA) laboratory strain, HIV-1_{MN}. A mean 50% inhibitory concentration (IC₅₀) of 0.05 to 0.5 μ g of IgG/ml was measured against SHIV-MN, and a mean IC₉₀ of \sim 3.0 μ g of IgG/ml was observed against HIV-1_{MN}. Neutralizing activity was detected in serum samples obtained 4 to 6 weeks after vaccination and was maintained for at least 24 weeks. Preimmune serum IgG from nine macaques immunized with vector alone, and IgG from three additional naive macaques (data not shown), did not neutralize either virus.

(ii) **Neutralization responses against primary HIV-1 isolates.** To further assess the specificity of antibodies in immune sera, neutralizing activity was evaluated against a panel of seven primary HIV-1 isolates using GHOST cells expressing either CCR5 or CXCR4 (Table 1). Purified IgG from macaques in each of the three immunization groups was able to effectively neutralize HIV-1_{BZ167/X4}, HIV-1_{SF2/X4}, and HIV-1_{CI2/X4} (Table 1 and Fig. 4), with mean IC₅₀ values of 5 to 7, 4 to 7, and 5 to 15 μ g/ml, respectively. By comparison, neutralization of HIV-1_{MNP/X4} required \sim 10-fold more serum IgG, with a mean IC₅₀ of 50 μ g/ml. Three additional isolates, HIV-1_{SF33/X4}, HIV-1_{SF33/R5}, and the clade A isolate HIV-1_{V1313/R5},

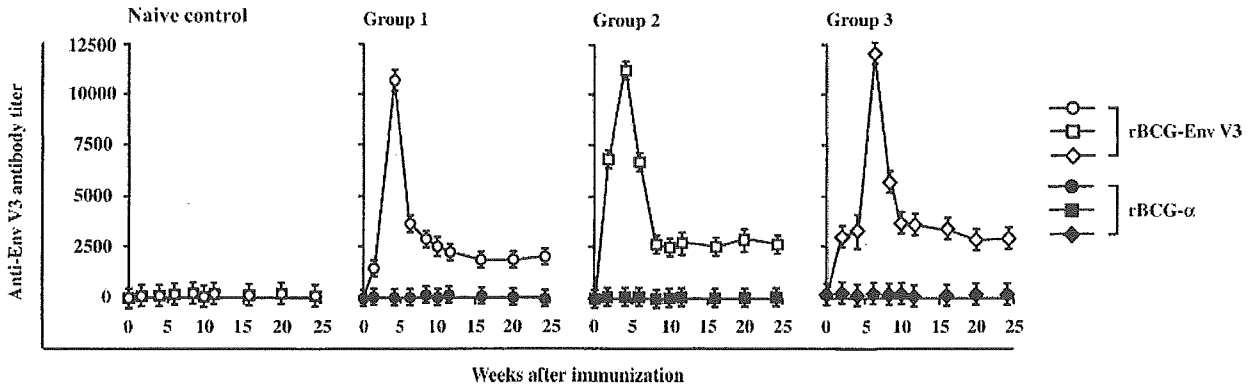


FIG. 2. Serum anti-V3 antibody titers determined by peptide-based ELISA. Preimmune and immune sera from macaques inoculated with rBCG Env V3 were collected and stored at -80°C until they were used. Sera from naïve macaques were used as controls. Data using preimmune sera were within the control levels (data not shown). The results are expressed as the means \pm SD of four independent assays.

were not neutralized with serum IgG concentrations up to 50 $\mu\text{g/ml}$ (Table 1). Preimmune sera had no neutralizing activity against any of the isolates. Thus, antibodies present in sera from the immunized macaques were able to neutralize primary HIV-1 isolates, including HIV-1_{BZ167}, HIV-1_{SF2}, and HIV-1_{C12}, in assays using GHOST cells that express CXCR4 with 10- to 50-fold-higher sensitivity than that of the dual-tropic (X4-R5) TCLA strain HIV-1_{MNP}. Among the neutralization-sensitive viruses, the V3 sequence motifs of HIV-1_{BZ167} and

HIV-1_{SF2} shown in Fig. 5 did not correlate with the observed neutralization profiles of HIV-1 Env V3.

(iii) **V3 peptide-specific T-cell responses.** Table 2 offers a comparison of the virus-specific T-cell response levels determined by IFN- γ ELISPOT analysis in immunized animals with the neutralization data provided in Fig. 2. Of the 15 animals immunized with rBCG Env V3 (180 and 160 SFC/ 10^6 PBMC at 6 weeks postimmunization [p.i.], respectively), only R-09 and R-10 showed very low levels of SFC activities at the time of

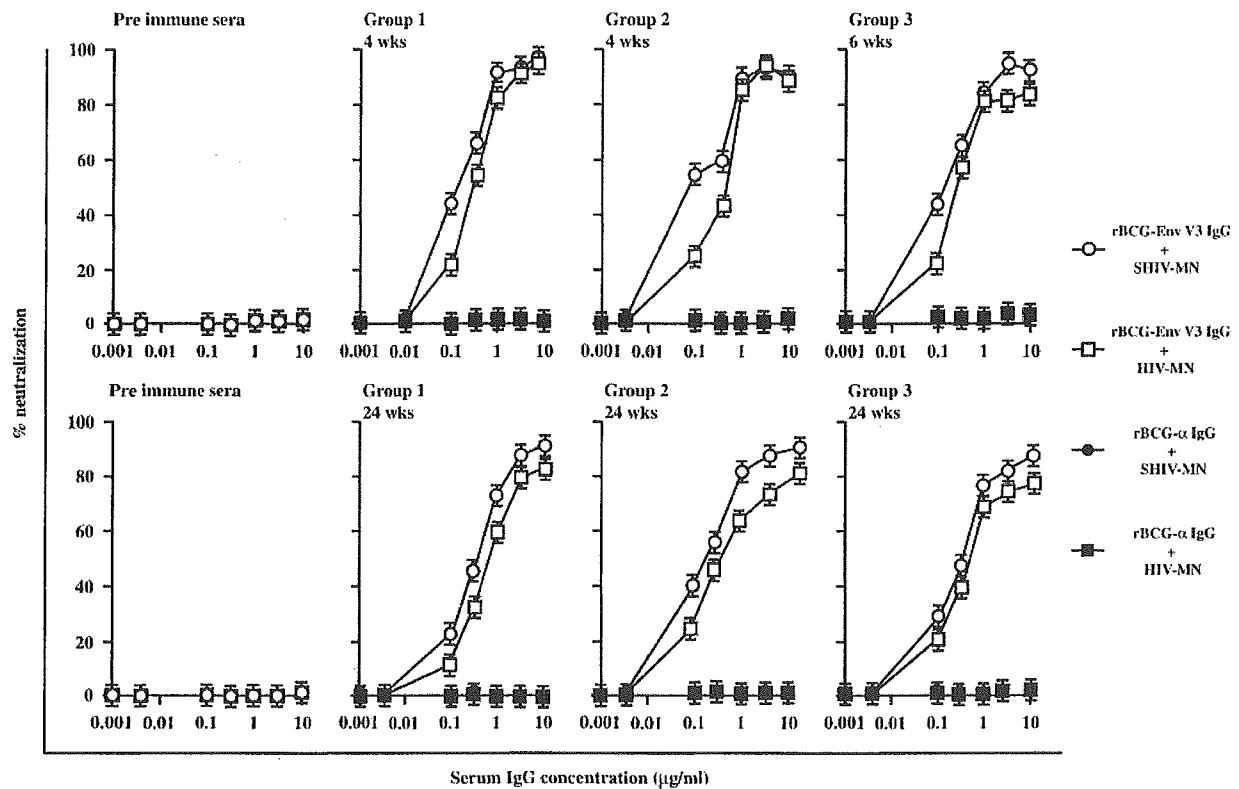


FIG. 3. HIV-1-specific neutralization antibody responses in macaques vaccinated with rBCG Env V3. Analysis of in vitro neutralization of SHIV-MN by anti-rBCG-HIV-1 antibodies using M8166 cell-based virus neutralization assays. Serum IgG was purified from preimmune or immune sera of macaques inoculated with rBCG Env V3 at the indicated times. The results are expressed as the means \pm SD of four independent assays.

TABLE 1. 50% neutralization calculated on the basis of neutralization curves^a

Serum sample	Neutralizing activity (μg)						
	BZ167/X4	MNp/X4	SF2/X4	SF33/X4	SF33/R5	V1313/R5	CI2/X4
Group 1	6.5	50	7	>50	>50	>50	10
Group 2	5	50	4	>50	>50	>50	5
Group 3	7	50	6.5	>50	>50	>50	15
Pre immunization sera of groups 1, 2, and 3	>50	>50	>50	>50	>50	>50	>50

^a The neutralization assays with the various viruses were carried out in GHOST cells expressing either CXCR4 (X4) or CCR5 (R5) as indicated in Fig. 4. BZ167, MNp, SF2, SF33, and CI-2 are HIV-1 clade B viruses. V1313 is an HIV-1 clade A virus.

SHIV challenge (120 and 110 SFC/10⁶ PBMC at 24 weeks p.i., respectively) (Table 2). In contrast, <100 SFC/10⁶ PBMC were observed in other immunized animals, and <20 SFC/10⁶ PBMC were observed in controls. Thus, the V3 region antigen in the rBCG Env V3 proved unable to induce significant levels of virus-specific T-cell responses in immunized animals.

Challenge with low-dose SHIV-MN. The first group of eight macaques (R-01 through R-08), consisting of five animals that received rBCG Env V3 and three that received control rBCG α -antigen, were intravenously challenged with low-dose SHIV-MN (20 TCID₅₀) at 24 weeks p.i. The cell-associated virus load was measured in PBMC cocultures, and proviral copy numbers were estimated by DNA PCR using primary PBMC genomic DNA. The level of plasma viremia in each macaque was quantified by competitive reverse transcription-

PCR to assess infection and virus replication for 16 weeks after virus challenge (Table 3).

Control macaques vaccinated with the vector alone (R-06 through R-08) were positive in all three viral-load assays 2 weeks after SHIV-MN challenge and remained positive for a follow-up period of 10 weeks. Because only low levels of viral RNA (<10⁴ RNA copies/ml) were transiently detected 2 weeks postchallenge, all three assays (virus isolation, plasma RNA, and proviral DNA) were used for virus detection. Using these criteria, we observed that all three parameters remained negative after low-dose SHIV-MN challenge in three of five macaques vaccinated with rBCG Env V3 (R-02, R-04, and R-05). However, macaque R-01 was transiently positive in all three assays for virus infection at 4 weeks. Another macaque immunized with rBCG Env V3 (R-03) exhibited a sharp in-

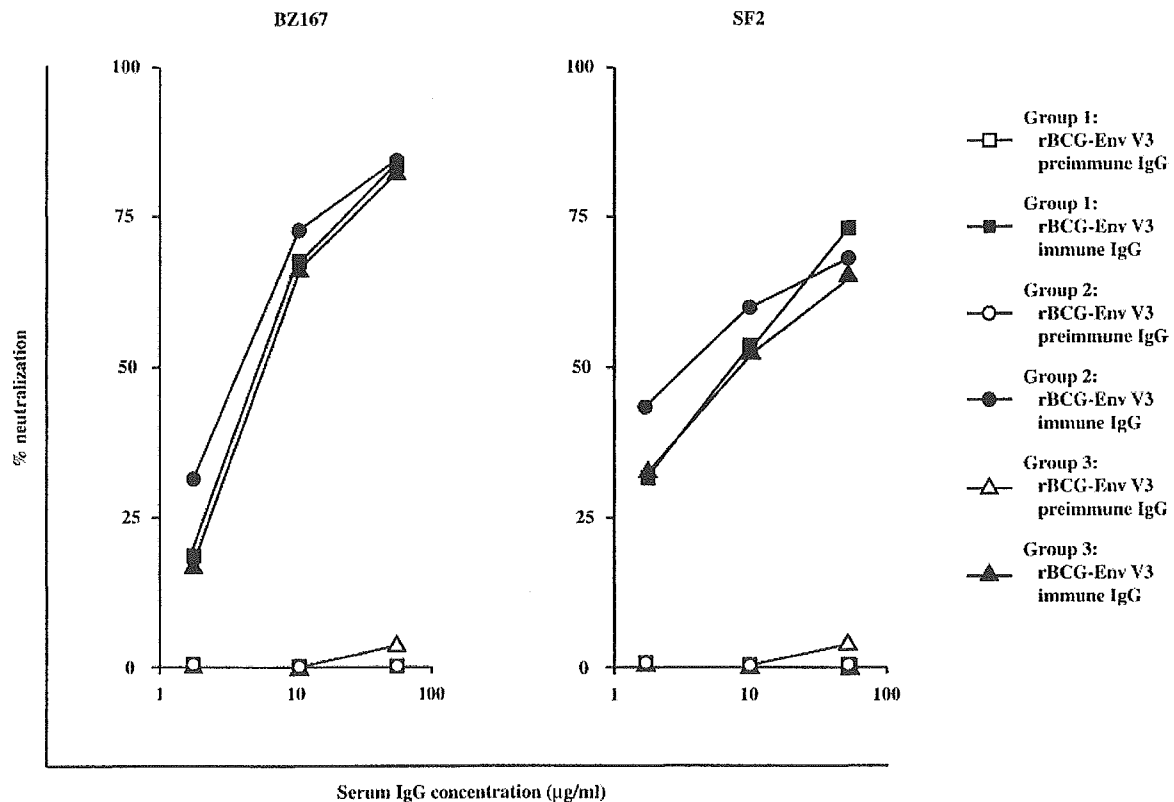


FIG. 4. Neutralization of HIV-1_{BZ167} and HIV-1_{SF2} in GHOST-X4 cells by immune sera from macaques vaccinated with rBCG Env V3. Dilutions of immune sera (closed symbols) and preimmune sera (open symbols) were tested in duplicate, and the percent neutralization was calculated using the mean value. The dose-response curves represent the means of three independent assays.

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MN      CTRPNYNKRKRIHI  GPGRAFYTTKNIIGTIRQAHC
BZ167  -----NKA-R--R-  ----T---G- -V-D---Y-
SF2    ---LSN-T--C-PL  ----V--A-DI- -D-----
CI2    ---SN-T-R-----  -----RQ-R-D-----
MNp    -----N-R-T-----

89.6P  -----N-T-E-LS-  -----ARR----D-----
SF33   -----N-R-R--TS  ---KVL---GE---D--K-Y-
VI131  -----N-T-QSV--  ---Q---A-GDV--D-----
IIIB   -----N-T---KS-QR-----V-IGK- -NM-----
    
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FIG. 5. Alignment of the amino acid sequences of HIV-1 Env V3 from primary and laboratory isolates. The spaces indicate amino acid deletions; dashes indicate homology. The V3 motif of a neutralization-sensitive HIV-1 strain is enclosed in the shaded rectangle (37).

crease in viral load following challenge, and the levels remained high until the animal was sacrificed. These results demonstrate that vaccination with rBCG Env V3 can induce protective immunity in rhesus macaques against a low-dose challenge with SHIV-MN.

Challenge with high-dose SHIV-MN. The second group of eight macaques (R-09 through R-16) was similarly challenged with a higher dose (200 TCID₅₀) of SHIV-MN by intravenous inoculation at 24 weeks p.i. (Fig. 6). Measurements of the viral loads in PBMC and plasma indicated that all the macaques were infected by the high-dose SHIV-MN challenge. However, the level of viremia during the acute phase of viral infection

was reduced by 1 to 2 log units in macaques immunized with rBCG Env V3 compared with controls (from 10⁶ to 10⁷, to <10⁵ to 10⁴ RNA copies/ml) (Fig. 6A). The control macaques developed a transient decrease in CD4⁺-T-cell counts that rebounded to normal levels ~3 weeks postchallenge (Fig. 6B). In contrast, macaques vaccinated with rBCG Env V3 had little or no change in CD4⁺-T-cell numbers.

Despite the low levels of V3 peptide-specific IFN-γ ELISPOT activities noted for animals R-09 and R-10 above (Table 2), these animals exhibited a plasma viral load and a rate of CD4⁺-cell loss after SHIV challenge that was comparable to those seen in the immunized animals designated R-11, -12, and -13. Thus, immunization with rBCG Env V3 generated even low levels of T-cell responses in only 2 animals out of 5 in this group and out of a total of 15 immunized animals. No evidence of higher virus-specific IFN-γ ELISPOT activity was demonstrated in samples obtained 0, 4, or 6 and 24 weeks after vaccination (Table 2), suggesting that few significant cellular anti-SHIV responses were generated and that those few did not affect virus control in this macaque population.

Challenge with pathogenic SHIV-89.6PD. The third group of macaques (R-17 through R-24) was challenged with pathogenic SHIV-89.6PD (20 TCID₅₀) 24 weeks postinoculation. The effects of vaccination with rBCG Env V3 on immune induction against the pathogenic virus were followed for 32 weeks, and the macaques were then autopsied. As shown in

TABLE 2. SHIV-MN-specific serum IgG neutralization titers and Env V3-specific ELISPOT responses^a

Monkey no.	Immunogen	IC ₅₀ of neutralization serum IgG (μg/ml) ^b			V3-specific IFN-γ SFCs/10 ⁶ cells ^c		
		0 week	4 or 6 weeks ^d	24 weeks ^e	0 week	4 or 6 weeks	24 weeks
R-01	rBCG Env V3	>50	0.5	0.6	<20	30	20
R-02	rBCG Env V3	>50	0.3	0.4	<20	40	40
R-03	rBCG Env V3	>50	0.5	0.6	<20	40	30
R-04	rBCG-Env V3	>50	0.2	0.3	<20	20	40
R-05	rBCG-Env V3	>50	0.08	0.3	<20	30	80
R-06	rBCG-α	>50	>50	>50	<20	<20	<20
R-07	rBCG-α	>50	>50	>50	<20	<20	<20
R-08	rBCG-α	>50	>50	>50	<20	<20	<20
R-09	rBCG-Env V3	>50	0.04	0.3	<20	180	120
R-10	rBCG-Env V3	>50	0.1	0.2	<20	160	110
R-11	rBCG-Env V3	>50	0.05	0.2	<20	20	30
R-12	rBCG-Env V3	>50	0.03	0.4	<20	60	20
R-13	rBCG-Env V3	>50	0.02	0.4	<20	30	30
R-14	rBCG-α	>50	>50	>50	<20	<20	<20
R-15	rBCG-α	>50	>50	>50	<20	<20	<20
R-16	rBCG-α	>50	>50	>50	<20	<20	<20
R-17	rBCG-Env V3	>50	0.2	0.6	<20	40	90
R-18	rBCG-Env V3	>50	0.3	0.3	<20	50	60
R-19	rBCG-Env V3	>50	0.3	0.4	<20	40	30
R-20	rBCG-Env V3	>50	0.5	0.7	<20	20	50
R-21	rBCG-Env V3	>50	0.4	0.5	<20	20	40
R-22	rBCG-α	>50	>50	>50	<20	<20	<20
R-23	rBCG-α	>50	>50	>50	<20	<20	<20
R-24	rBCG-α	>50	>50	>50	<20	<20	<20

^a Animals were inoculated with either rBCG Env V3 or the vector control. Blood samples were obtained at 0, 4, or 6 and 24 weeks p.i., and antibody inhibitory concentration and the V3-specific IFN-γ ELISPOT activity were compared.

^b The IC₅₀ was derived from the data in Fig. 2 based on neutralization dose-response curves similarly obtained from Fig. 3.

^c Freshly isolated PBMC were assessed for their ability to produce IFN-γ in response to HIV-1_{MN} Env V3 peptide.

^d Mean IC₅₀s: R-01 to R-05, 0.32; R-09 to R-13, 0.05; R-17 to R-21, 0.35.

^e Mean IC₅₀s: R-01 to R-05, 0.44; R-09 to R-13, 0.30; R-17 to R-21, 0.50.

TABLE 3. Comparison of low-dose SHIV-MN infections in macaques vaccinated with either rBCG Env V3 or rBCG- α (control)

Monkey	Immunogen (10 mg)	Efficacy analysis	Results ^a							
			0 ^b	2	4	6	8	10	12	16
R-01	rBCG Env V3	Virus isolation	<1	<1	2	<1	<1	<1	<1	<1
		Provirus by PCR	<500	<500	>500	<500	<500	<500	<500	<500
		Plasma viral load	<500	<500	20,000	<500	<500	<500	<500	<500
R-02	rBCG Env V3	Virus isolation	<1	<1	<1	<1	<1	<1	<1	<1
		Provirus by PCR	<500	<500	<500	<500	<500	<500	<500	<500
		Plasma viral load	<500	<500	<500	<500	<500	<500	<500	<500
R-03	rBCG Env V3	Virus isolation	<1	32	<1	<1	2	<1	ND	ND
		Provirus by PCR	<500	>500	<500	<500	>500	<500	ND	ND
		Plasma viral load	<500	310,000	<500	<500	20,000	<500	ND	<500
R-04	rBCG Env V3	Virus isolation	<1	<1	<1	<1	<1	<1	ND	ND
		Provirus by PCR	<500	<500	<500	<500	<500	<500	ND	ND
		Plasma viral load	<500	<500	<500	<500	<500	<500	<500	<500
R-05	rBCG Env V3	Virus isolation	<1	<1	<1	<1	<1	<1	<1	ND
		Provirus by PCR	<500	<500	<500	<500	<500	<500	<500	ND
		Plasma viral load	<500	<500	<500	<500	<500	<500	<500	<500
R-06	rBCG- α	Virus isolation	<1	32	16	<1	2	2	<1	1<
		Provirus by PCR	<500	>500	>500	>500	>500	>500	>500	500
		Plasma viral load	<500	300,000	50,000	20,000	20,000	20,000	20,000	20,000
R-07	rBCG- α	Virus isolation	<1	2	32	<1	<1	<1	<1	ND
		Provirus by PCR	<500	>500	>500	>500	>500	<500	<500	ND
		Plasma viral load	<500	27,000	310,000	350,000	25,000	<500	<500	<500
R-08	rBCG- α	Virus isolation	<1	32	16	<1	2	2	ND	ND
		Provirus by PCR	<500	>500	>500	>500	>500	>500	ND	ND
		Plasma viral load	<500	300,000	50,000	<500	20,000	20,000	ND	<500

^a Viral loads were determined by either limiting dilution of PBMC or competitive PCR for HIV-1 Env V3 genes, and the results are expressed as the number of infected cells per million PBMC and virus copies per milliliter of blood. Nested PCR for HIV-MN Env V3 was used in all macaques to detect the provirus genome. Naïve macaques were injected intravenously with 20 TCID₅₀ of SHIV-MN and used as controls for SHIV infection. The results are expressed as the mean of three different assays; <1, <500, and <500 were the detection limits of virus isolation, proviral copy number, and plasma viral load, respectively. ND, not determined.

^b Weeks after challenge.

Fig. 7, high levels of plasma viremia were detected in the control macaques, with a viral set point of $\sim 10^6$ RNA copies/ml, accompanied by an abrupt decline in CD4⁺-T-cell counts. Prior vaccination with rBCG Env V3 appeared to have no positive effect on the viral load and CD4⁺-T-cell counts compared with the control animals.

Association of in vitro neutralization antibody responses following rBCG Env V3 immunization with control of viremia after SHIV challenge. Of the macaques challenged with low doses of homologous SHIV-MN (group 1), the three virus-controlled macaques R-02, -04, and -05 (Table 1) had higher IC₅₀s of SHIV-MN-specific neutralizing antibodies as measured in M8166 cells at 24 weeks p.i. or on the day of challenge, with serum IgG concentrations of 0.4, 0.3, and 0.3 μ g/ml, respectively (Table 2). The IC₅₀s of the uncontrolled macaques R-01 and -03 (Table 1) were both 0.6 μ g/ml (Table 2).

When the challenge dose was increased 10-fold (Fig. 1), all five animals in group 2 had high neutralizing antibody titers with a mean IC₅₀ of 0.30 μ g/ml on the day of challenge (Table 2). These animals in group 2 showed partial protection against the same homologous virus challenge (Fig. 6). In contrast, no animals similarly immunized with rBCG elicited any in vivo protection against a low-dose, heterologous viral challenge with SHIV-89.6PD (Table 2 and Fig. 7).

In summary, the rBCG Env V3-elicited NAb response afforded some degree of protection against a homologous viral challenge. However, infection by the heterologous virus SHIV-89.6PD was not controlled by heterologous virus SHIV-MN- or HIV-1_{MN}-specific NAb generated by the recombinant HIV-1_{MN} Env V3-expressed BCG immunization.

DISCUSSION

First, our study demonstrates the potential of anti-Env V3 NAb induced by immunization of rhesus macaques with rBCG Env V3 to afford protection against homologous challenge with SHIV-MN but not against the heterologous SHIV-89.6PD. With the low-dose homologous SHIV-MN challenge (20 TCID₅₀), sterile protection was achieved in three of five immunized animals. These findings correlate well with our in vitro neutralization data for these animals. Protected animals showed higher levels of potent neutralization antibodies than did unprotected animals. Macaques serving as vector and naïve controls experienced high levels of replication of the SHIV-MN challenge virus. With a high-dose challenge, rBCG Env V3 vaccination was effective at reducing viremia during acute infection by ~ 100 -fold. The vaccine consisted of an rBCG vector that expresses a chimeric HIV-1 Env V3 region peptide and the α -antigen of *M. bovis*. The kinetics and magnitude of the HIV-1 Env V3-specific antibody responses elicited in macaques were comparable to those observed in our previous studies using guinea pigs vaccinated with rBCG Env V3 (9, 16).

Secondly, the levels of neutralizing antibodies generated after injection with a recombinant BCG vector-based vaccine expressing a chimeric protein of HIV-1 Env V3 peptide and α -antigen protein were maintained for at least 24 weeks p.i. with no diminishment in titer. A plausible explanation for the longevity of the neutralizing antibody titers after rBCG immunization is that the carrier protein, α -antigen (also known as MPT59 or antigen 85B), is derived from mycobacteria and has

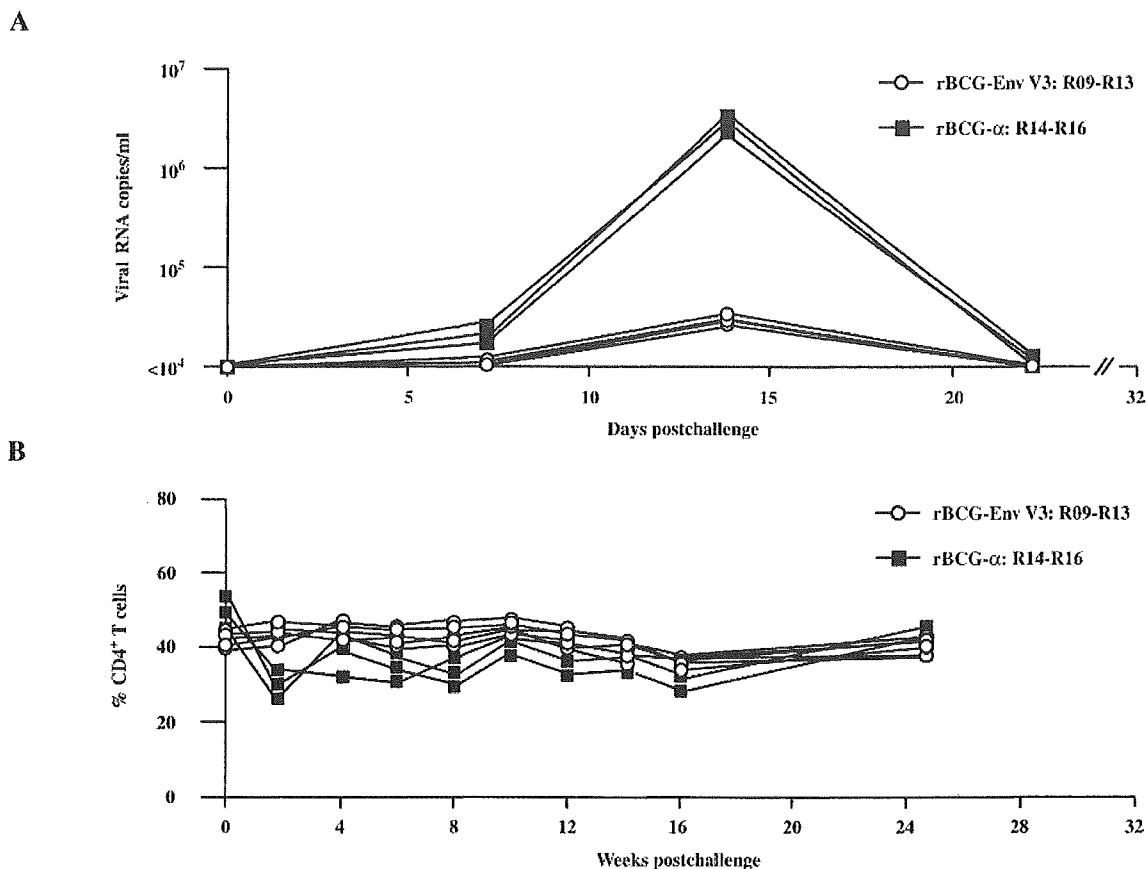


FIG. 6. Comparison of infection kinetics following high-dose (200 TCID₅₀) inoculation of SHIV-MN in macaques vaccinated with either rBCG Env V3 or rBCG vector control. (A) Viral RNA copy number per milliliter of serum. (B) CD4⁺-T-cell count as a percentage of total lymphocytes. The results in individual animals are expressed.

the ability to elicit potent Th1-type immune responses (24, 43). Our result is consistent with those of other groups, which have shown that BCG immunity is maintained for at least a few years and that the BCG bacillus is effective at increasing NAb responses (40). These characteristics might help to explain the long-lasting enhanced levels of NAb elicited by vaccination with rBCG Env V3.

The concentration of purified macaque IgG in serum was determined to be ~10 mg/ml. By this estimation, 0.5 mg corresponds to a serum dilution of 1:1 in virus neutralization assays. The IC₅₀ and IC₉₀ values for neutralization of SHIV-MN were 10³ to 10⁴ and 166, respectively (similar values were obtained for neutralization of HIV-1_{MN}). These neutralization titers suggest that antibody responses generated de novo may contribute to a degree of protection against SHIV-MN. The observed relationship of the NAb titer and viral protection is consistent with results obtained by repeated immunization with SHIV-89.6 C4-V3 peptides in guinea pigs and rhesus macaques (6, 27). In this case, NAb titers to homologous SHIV-89.6 were ~10³ greater than those against heterologous HIV-1_{MN}, while responses to HIV-1 R5 viruses were weak or absent. This suggests that the protection mediated by a C4-V3 peptide vaccine against SHIV-89.6 may be type (or strain) specific. Thus, we assume that the NAb generated by

SHIV-89.6 C4-V3 peptide immunization (6) would not mediate protection against a heterologous SHIV-MN challenge.

The present study suggests that the vaccine-elicited antibodies directed against the HIV-1 Env V3 peptide can in some cases confer a degree of neutralization against primary isolates of HIV-1 (26). Following vaccination of rhesus macaques with rBCG Env V3, both binding and NAb responses against this novel construct were clearly evident. At the time of SHIV challenge, immune sera from the vaccinated macaques efficiently neutralized a homologous, type-specific TCLA HIV-1 strain (HIV-1_{MN}) and a related SHIV strain (SHIV-MN) with IC₉₀ values of <5 μg/ml. Controls, including preimmune sera and sera from macaques vaccinated with rBCG vector alone, had no neutralizing activity in assays using GHOST cells expressing either CCR5 or CXCR4 or in M8166 cells. Immune sera from macaques vaccinated with rBCG Env V3 were able to neutralize several primary HIV-1 X4 isolates (HIV-1_{BZ167}, HIV-1_{SF2}, and HIV-1_{CI2}); however, neutralization of an X4-R5 dual-tropic strain (HIV-1_{MNP}) was weak. No neutralization of HIV-1 R5 isolates and primary HIV-1 isolates from different clades was observed. These findings were confirmed in an independent international neutralization trial (conducted by Simon Beddows and Jonathan Weber, Imperial College School of Medicine, Medical Research Council, London, En-

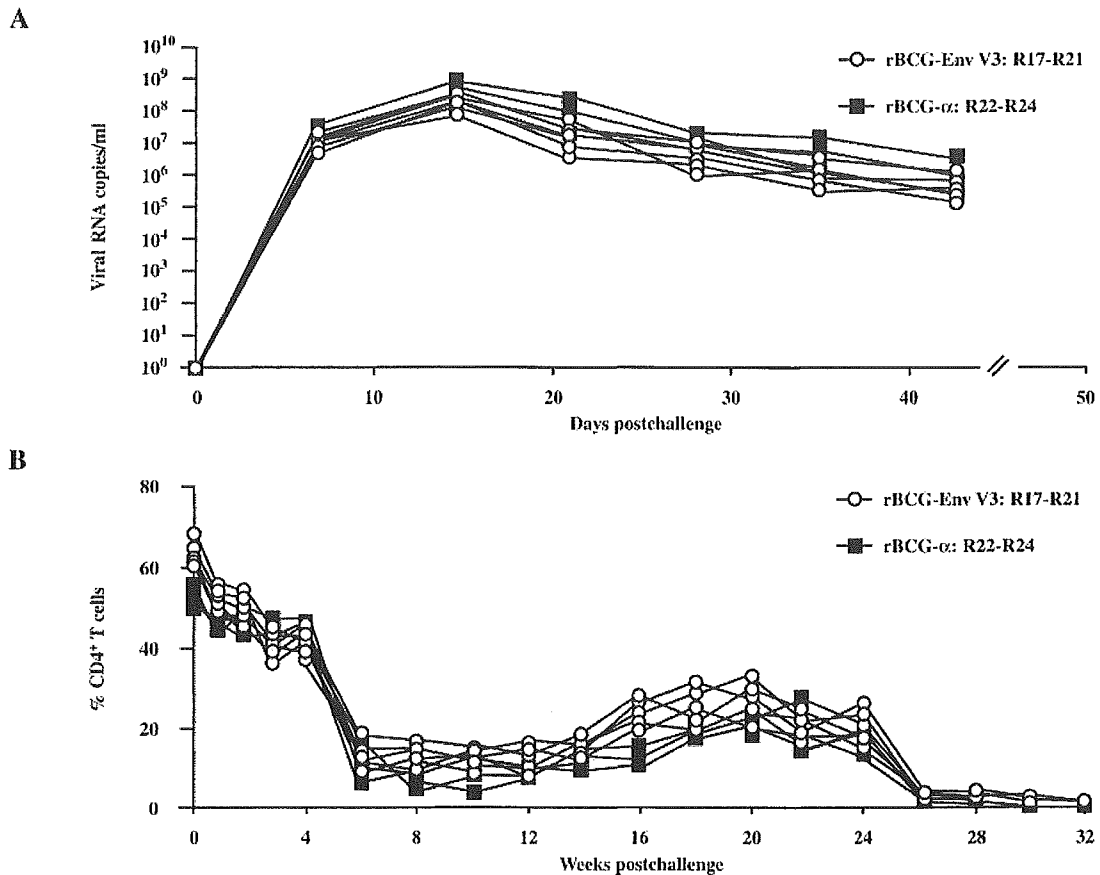


FIG. 7. Comparison of infection kinetics following challenge with pathogenic SHIV-89.6PD in macaques vaccinated with either rBCG Env V3 or rBCG vector control. (A) Plasma viral-RNA copy numbers per milliliter. (B) CD4⁺-T-cell count as a percentage of total lymphocytes. The results in individual animals are expressed.

gland, and Pia Scott and Eva-Maria Fenyo at Microbiology and Tumorbiology Center, Karolinska Institute, Stockholm, Sweden). Preliminary results from this study have had been summarized and reported (11). Despite similarities in the V3 sequence motif, neutralization of the TCLA strain HIV-1_{MN} was found to be 10- to 50-fold more sensitive than neutralization of primary HIV-1 isolates, such as HIV-1_{CI2}, HIV-1_{MNP}, or HIV-1_{JR-CSF} (11). A reasonable explanation for the relative insensitivity of primary HIV-1 isolates—particularly primary HIV-1 R5 isolates—to neutralization is the presence of cryptic or occluded sites within the virus-associated V3 region (13, 53).

In the Japanese consensus HIV-1 Env V3 expressed in the rBCG construct, the core V3 motif of the neutralization epitope is IHIGPGRAF (39). Although the consensus sequence of the V3 loop differs from the MN-V3 sequence in five amino acid positions, the neutralization epitope of the tip V3 region in the Japanese consensus is identical to that of MN-V3. Some substitutions of amino acids at certain positions within this motif (for example, H to R and A to T in the core motif in BZ167) are tolerated, suggesting that NAbS generated by immunization with rBCG Env V3 are not strictly type specific. Immune sera from macaques vaccinated with rBCG Env V3 were able to neutralize primary HIV-1 X4 and some HIV-1 X4-R5 dual-tropic isolates, suggesting that the antigenic struc-

ture of the chimeric V3 peptide mimics to some extent that of the virus-associated V3 region. Indeed, the chimeric V3- α -antigen protein is estimated to be 38 kDa and contains four cysteine residues, suggesting the possible formation of a new loop structure in the V3 portion of the protein. With regard to the heterologous SHIV-89.6PD challenge in macaques vaccinated with rBCG Env V3, NAbS specific for SHIV-89.6PD were not generated efficiently (IC_{50} , >50 μ g of immune serum IgG/ml) and did not provide any protection against the SHIV-89.6PD challenge. The V3 neutralization site of SHIV-89.6PD may differ in sequence or structure or both from that of SHIV-MN or other viral strains, including some of the HIV-1 isolates, making it unrecognizable to antibodies. Such a difference could account for the poor cross-neutralization activity against SHIV-89.6PD.

Thus, our data from the SHIV-macaque models show that the in vitro neutralization titers generated in rBCG-immunized animals correlate with protection. Although a present goal of HIV-1 vaccine development is to reduce the viral set point by eliciting high levels of virus-specific cellular immune responses, induction of cross-reactive NAbS may also contribute to control virus replication in the course of HIV-1 infection and may therefore be useful in the context of a preventive vaccine. Furthermore, although the choice of HIV Env V3 and the

autologous challenge virus SHIV-MN are unlikely to provide information that predicts efficacy in humans, the results presented here demonstrate that recombinant BCG vectors have the potential to deliver a more appropriate immunogen for desirable immune elicitation.

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わが国の HIV/AIDS 患者に合併する寄生虫症

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はじめに

わが国では、エイズおよび HIV 感染症に合併する寄生虫症は、その数が余り多くないために、患者

の医療における重要性の認識が低く、適切な診断・治療の体制整備が遅れている。エイズ指標疾患に入る寄生虫症は、トキソプラズマ脳症、クリプトスポリジウム症、イソスポラ症の 3 疾患で、患者の免疫

Parasitic Diseases Complicated with HIV/AIDS Patients in Japan

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力が低下してきた際の日和見原虫感染症である。さらにエイズおよび HIV 感染症に高率に合併する寄生虫症としては、赤痢アメーバ症、ランブル鞭毛虫症、糞線虫症などが知られている¹⁾。全国 360 余のエイズ拠点病院にアンケート用紙を配布し、エイズ/HIV 感染症患者に合併した上記寄生虫症の調査を行うなど、わが国における同合併症の疫学特性を把握するための研究を展開したので報告する。

赤痢アメーバ症

全国のエイズ拠点病院 (368 施設) にアンケート用紙を配布し、平成 15 年度に HIV 感染症患者に合併した赤痢アメーバ症の患者背景について調査を行った。

回収率は 223 施設 (61%) で、175 施設は HIV 感染症患者の診療経験があり (175/223 = 78%)、赤痢アメーバ症の診療経験のある施設は 28 施設 (28/223 = 13%)、症例数は 38 例であった。すべて男性症例で、平均年齢 37 歳、33 例は MSM (Men who have sex with men) による STD (Sexually transmitted disease) 感染と考えられた。CD4 数の平均は 306/ μ l、HIV ウイルス量の平均は 2.2×10^4 コピー/ml であった。

赤痢アメーバ症の病型は、腸炎 18 例 (18/33 = 55%)、肝膿瘍 13 例 (39%)、腸炎と肝膿瘍の合併 3 例、その他の部位の膿瘍 2 例であった。

すべての症例でメトロニダゾールが治療薬として用いられ、抗嚢子薬フロ酸ジロキサニド (フラミド[®]) が 16 例 (42%) で使用されていた。転帰としては完治 12 例、改善 24 例、不変 1 例、死亡 1 例 (アメーバ症によるものではなく原疾患である悪性リンパ腫によるもの) であった。

わが国のエイズ/HIV 感染症に合併する赤痢アメーバ症は、CD4 数の平均値から考えて患者の免疫状態は比較的良好であり²⁾、また海外旅行歴が少ないのに対し、梅毒や B 型肝炎などの合併が多かった。すなわち感染は、流行地における食事等を介した糞口感染としてではなく³⁾、また日和見感染としてでもなく⁴⁾、MSM に STD として感染していると考察できた。

トキソプラズマ症

全国のエイズ拠点病院 362 施設にアンケート調査を行った。回収率は 235 施設 (65%) で、169 施設は HIV 診療経験があり (169/235 = 72%)、トキソプラズマ症の診療経験のある施設は 29 (29/235 = 12%)、症例数は 55 例であった。これは全 5,147 例の HIV の 1.07% にあたる。男性 48 例、女性 6 例、不明 1 例。全症例の平均年齢は 41.2 歳 (2 例は年齢記載なし)。CD4 数の平均値は 44.3/ μ l であった。HIV ウイルス量は測定限界以下から最高では 8.8×10^5 /ml まで幅広く分布した。すなわち、ウイルス量 $< 10^3$ /ml : 6 例、同 $< 10^4$ /ml : 4 例、同 $< 10^5$ /ml : 13 例、同 $< 10^6$ /ml : 8 例。感染経路の検討では、非加熱血液製剤による HIV 感染者は 7 例 (12.7%)、同性間性交渉による者は 10 例 (18.2%)、異性間性交渉による者は 31 例 (56.4%)、その他 7 例 (12.7%) であった。

本調査結果は、対象施設をエイズ拠点病院と限定はしているものの、その症例数から信頼度は高いものと考えられた。また、調査期間を限定せず過去から現在までのすべての症例について回答を得たが、トキソプラズマ症合併例の HIV 感染経路の大半は性感染によるもの (74.6%) であることや、58% の症例で抗 HIV 療法が施行されていることを考慮すると、集められた症例のほとんどは最近 10 年程度に経験されたものが多いものと思われた。合併率は 1.07% であったが、一般には 2% 程度といわれていることから低い数値と思われるが、アンケート報告された 5000 例を超える HIV 症例が最近 10 年くらいの症例へのかたよりがあると過程すると、ST 合剤を用いたカリニ肺炎の予防がトキソプラズマ症の発症の予防につながっている可能性があると考えられた。それと同時に、抗 HIV 療法は 55 症例中の半数強に施行されているのみであることから、感染予防をさらに充実させればトキソプラズマ症の合併頻度をさらに低下させることができる可能性があると考えられた。

ジアルジア症

愛知県内の大型 10 病院 (500 床以上) にアンケー

ト調査を行ったが、ジアルジア症の報告はなかった。ジアルジア症は、やはり MSM に STD として感染することが想定される。名古屋医療センターでは、従来一般患者（HIV 陰性患者）からのジアルジア症の検出が続いていたが、最近報告がなくなっていることも考慮に値する。

国立国際医療センターでは、海外渡航歴の無い下痢を主訴とした患者に、HIV 感染を認めた 2 例を最近経験したが、ジアルジア症の重症化がエイズで起こることが疑われた。

一般的にエイズでの合併の報告がわが国で少ないことは、検査法の非徹底ならびに技術の低下による見逃しが予想される。近年直接蛍光抗体法による検出キットが市販の状況にあるが、その導入も今後は検討する必要がある。しかしながら、従来のギムザ染色等の検査法でも、体制の整った検査室では診断が可能であるので、同検査技術の熟練の必要性を強く認める。

イソスポラ症・クリプトスポリジウム症

全国で発症した AIDS 報告症例の集計（1985～2002）を行い、大阪医療センターの症例に関しては、過去約 7 年（1997.4.1.～2003.12.3）の実態を調査した。

全国集計の結果は、AIDS 患者 2,556 名（日本国籍 1,906 名、外国籍 650 名）の内、イソスポラ症の合併報告はなく、クリプトスポリジウム症の合併は計 9 件（内外国籍 8）であった。

大阪医療センターでは、HIV 感染者 524 例のうち、イソスポラの合併は認められず、クリプトスポリジウム症の合併は 3 例であった。3 例とも激しい下痢、体重の減少、CD4 数の顕著な減少があり、AIDS の病態として重篤であった。すべての症例で、クリプトスポリジウムの特異的な治療は行わず、抗 HIV 療法による免疫力回復で治癒した。2 例は、それぞれ赤痢アメーバおよびキャンピロバクターとの重複感染例であり、下痢の病態が特定しなかった。感染経路については、他の下痢起因微生物の感染経路を鑑みて、性的接触によるクリプトスポリジウム症の感染の可能性が考えられた。

イソスポラ症およびクリプトスポリジウム症は

AIDS 指標疾患に入る寄生虫症であり、免疫力の低下による日和見感染が起こりうる⁵⁾。しかしながら、わが国の合併症例はきわめて少なく、症例の蓄積が困難である。大阪医療センターにおけるクリプトスポリジウム症 3 例の報告はきわめて貴重であり、真に有効な治療薬が無いクリプトスポリジウム症に対して、補液による脱水と電解質バランスの補正や、適切な抗 HIV 療法による免疫力の回復を図る治療法の有効性が強く認識された。

糞線虫症

本症との合併に関するアンケート調査を、全国のエイズ拠点病院 364 施設に行った。回収率は 201 施設（55%）で、糞線虫症との合併例は 6 例〔日本人 2 例、外国人 3 例（アフリカ人、タイ人、ブラジル人）、国籍不明 1 例〕（男 5 例、女 1 例）で、合併時の平均年齢は 41 歳であった。地域としては、沖縄が 1 例、その他は東京、大阪などの大都市であった。HIV 感染経路は、同性間性交渉 2 例、異性間 2 例、両性間 2 例、不明 1 例であった。

糞線虫症合併時の CD4 は 3～793（平均 206）/ μ l で、かなりの幅が見られた。本 6 例のうち、播種性糞線虫症が認められたのは 1 例のみであった。

糞線虫症は、熱帯・亜熱帯に広く分布する線虫類の寄生虫症で、本邦では、沖縄や南西諸島に未だに多くの感染者が認められる。特に消耗性疾患、栄養不良、悪性リンパ腫や成人 T 細胞性白血病、ステロイド投与など免疫力が低下すると感染が進行することが知られており、エイズ患者における糞線虫症の合併の危険性が考えられている。しかしながら今回の調査研究では、本邦における症例では重症化の傾向は見られなかった。HIV 感染者における糞線虫症合併頻度はおよそ 0.1%以下であろうと考えられ、なかなか特異的な症例の蓄積ができないのが現状である。

おわりに

わが国におけるエイズ・HIV 感染症との寄生虫症の合併頻度やその重篤性は、諸外国の報告とはかなり異なり⁶⁾、診断・治療法の選択も、諸外国の例をそのまま適用できないことが分かった。また、合併

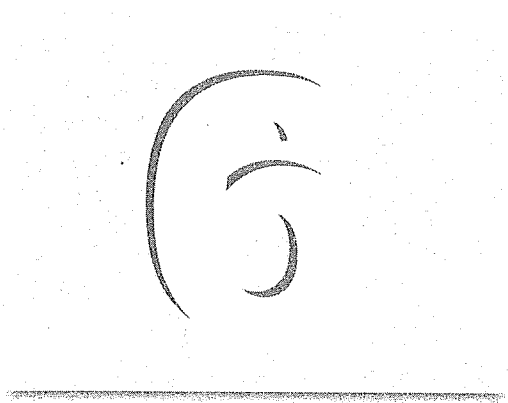
例に対する適切な治療薬の配備もわが国には十分でない。たとえば、アメーバ赤痢におけるフロ酸ジロキサニドや、糞線虫症におけるイベルメクチンの使用は制限された状況にある。

それぞれの合併寄生虫症には、その疫学および臨床的特徴があることから、今後わが国における有効な治療法の確立のために、個別の症例研究をかさねてゆく必要があるものと考えられた。

追記：本報告は、「平成 15 年度エイズ医療共同研究（15 公-6）」の成果による。

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●救急に関連する新興・再興感染症

その他の感染症

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HIV治療のコンセプト

HIV感染症を診断した場合には5類感染症として届出する義務があり、この届出に基づいてエイズ動向調査が行われている。それによると年次患者数は年々増加していて平成15年12月までの累積HIV患者は5,780人、AIDS患者は2,892人である(図1)。新規に診断される患者の中ではMSM (men who have sex with men)の増加が著しい。

HIV感染症の治療は、日和見感染の予防と治療、抗HIV薬を用いた抗ウイルス治療からなる。

それぞれの患者のCD4数に応じて日和見感染症の予防を開始しながら抗HIV薬の導入を行っていく。

現在、抗HIV薬は核酸系逆転写阻害薬、非核酸系逆転写阻害薬、プロテアーゼ阻害薬の3つのクラスがあり、17剤が認可されている。現在の治療は、これらを用いた強力な多剤併用療法 (highly active antiretroviral therapy ; HAART) が主流となっている。

HAART開始の適応は、すでにエイズを発症している症例、 $CD4 < 200/\mu L$ は絶対適応となるが症状が安定している場合は、 $CD4$ 数が

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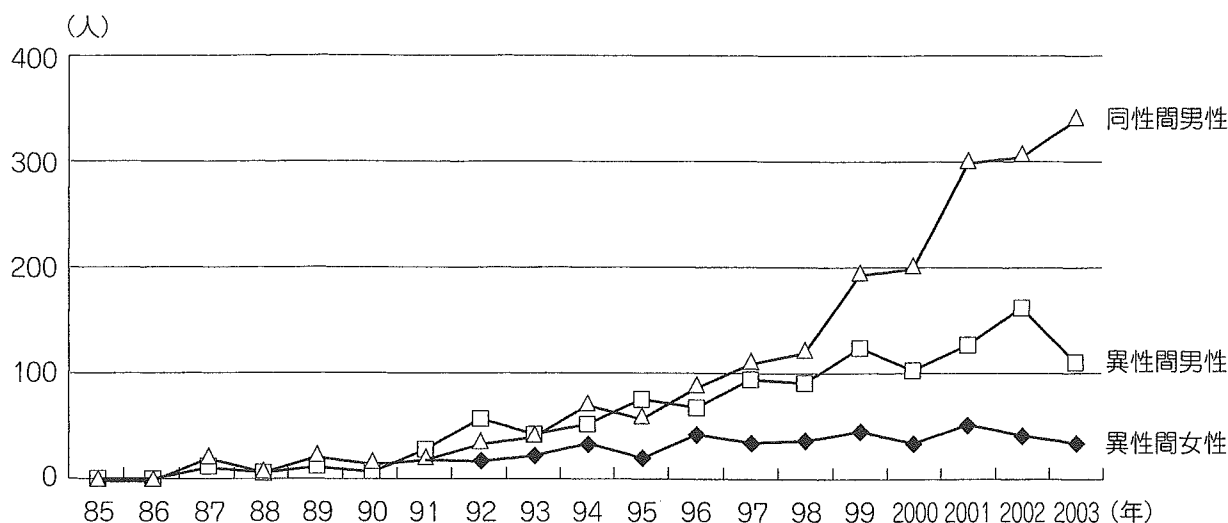


図1 新規日本人HIV感染者の年次別報告数

350/ μ L以上では治療は開始しない。200から350/ μ Lの間はウイルス量や患者の状態に応じて治療を検討する。選択する薬剤はKey drugと呼ばれる強力な抗ウイルス薬であるプロテアーゼ阻害薬，または非核酸系逆転写阻害薬とそれを補助する2剤の核酸系逆転写阻害薬の3剤で治療を行う。最近，抗HIV薬によるリポジストロフィー（脂質代謝異常や脂肪分布異常）や乳酸アシドーシス（核酸系逆転写阻害薬により体内に乳酸が増加し肝不全，膵炎，末梢神経障害などを起こす）などの長期服用後の副作用が問題となっており，以前よりは早期に開始しない傾向になっている。月に数回の飲み忘れでも耐性を獲得する可能性があり，治療開始前に治療の効果，副作用，内服の重要性などを十分に説明し服薬のアドヒアランス（患者が治療方針決定に参加し自らの決定に従って服用し，それを続けていく姿勢）を維持していくことが重要である。

抗HIV療法のガイドラインは頻繁に改訂されているので最新の詳細な情報は<http://www.aidsinfo.nih.gov/>を参照いただきたい。

インフルエンザウイルスと抗ウイルス薬

インフルエンザウイルスは，A，B，C型に分類されヒトに流行するのはAとBである。A型は抗原性が変化しやすく，大変異を起こし数年から数十年ごとに世界的流行を起こす。B型は大変異を起こさない。潜伏期は1～5日で悪寒戦慄を伴う急激な発熱（38℃以上の高熱），頭痛，倦怠感，関節痛，筋肉痛などが突然現れ，咳・鼻汁などの上気道炎症状がこれに続き，約1週間の経過で軽快する。通常のかぜよりも症状が強いのが特徴である。高齢者や呼吸器，循環器疾患，糖尿病などの慢性疾患を持つ患者がインフルエンザに罹患すると原疾患が悪化するとともに，二次的な細菌性肺炎などの合併症を起こしやすく入院や死亡の危険が増加する。ま

た、小児では脳炎や脳症などの重症中枢神経系合併症が増加することが報告されている。インフルエンザ脳症の増加の原因として一部の非ステロイド系消炎鎮痛剤の使用との関連性が疑われているため、それらをインフルエンザ治療中には使用しない。また、アスピリンを投与するとまれにライ症候群を起こすことがありアスピリンの使用も原則禁忌である。診断は咽頭拭い液やうがい液からのウイルス分離や抗体検査であるが時間を要するため、ベッドサイドで迅速簡便に抗原診断が可能なキットが有用である。

治療はA型に対しては塩酸アマンタジンが使用されるがB型に無効である。最近開発されたノイラミダーゼ阻害薬(リン酸オセルタミビル、ザナミビル水和物)はA、B型とも有効で発症後48時間に投与すれば臨床症状が軽くなり、罹患期間を短くすることができる。ただし、1歳未満の乳児に対してのリン酸オセルタミビルの安全性が確立していないために1歳以上に使用する。アスピリンや非ステロイド系解熱薬の使用は禁忌で、解熱薬が必要なときはアセトアミノフェンを使用する。

予防にはインフルエンザワクチンの接種が有用でインフルエンザウイルスの感染やインフルエンザの発症を完全には防御できないが、重症化や合併症の発生を予防する効果は証明されている。特に高齢者、慢性呼吸器疾患、循環器疾患患者、免疫低下機能患者などのハイリスクグループ、医療従事者、慢性疾患患者の介護者、家族に背積極的な接種が奨められている。

輸入感染症で注目すべきもの

2001年9月の米国でのテロ事件の影響や2003年のSARSのアウトブレイクで海外旅行者は一時的に減ってしまったが、それまでは日本人の海外旅行者は年々増加し、2000年には1,819万人が海外に出ている。その主な旅行先は欧米や東南アジアが多くを占めているが、アフリカ、ラテンアメリカなどの熱帯、亜熱帯地域への渡航も増加している。旅行者や外国人の増加に伴い、海外で感染症に感染し、日本で発症するといった“輸入感染症”を診療する機会がさらに増えることが予想される。特に熱帯、亜熱帯地域からの帰国患者が発熱、下痢を主訴に来院されることが多い。

そのような患者の診療を行う場合は、渡航歴を聴取しその地域で流行している感染症をCDC (<http://www.cdc.gov/travel>) やWHO (<http://www.who.int/ith>) などの情報を参考に確認する。また、病歴を聴取するときは現地での活動状況や食物、水、湖や河に入ったか、カ、ダニ刺され、動物との接触歴、性交渉の有無などの感染のリスク、渡航前のワクチン接種やマラリア予防内服の有無を確認する。

発熱患者を診療するときは一般的な発熱の鑑別(急性上気道炎、肺炎、尿路感染症など)をするのはもちろんであるが、渡航地や潜伏期を参考に現地で流行している疾患を鑑別することが重要である。特に重要な発熱疾患はマラリア、腸チフスやデング熱、リケッチア疾患やレプトスピラ症である。マラリアとデング熱、腸チフ

スは症状（発熱，悪寒戦慄，頭痛）や検査異常（血小板減少，肝機能異常）が類似している。マラリアは治療が遅れると致命的になるのでマラリア流行地からの帰国患者はまず末梢血の塗抹検査，迅速診断キットを用いた抗原検査をまず行う。デング熱は，潜伏期が短いので，帰国後すぐに突発する発熱，頭痛，その後の皮疹と血小板減少が特徴である。デング出血熱になることはまれで大部分が保存的治療で回復する。腸チフスについては血液培養を繰り返し，患者状態が悪く腸チフスが疑わしいときは，血液培養の結果が出る前に治療を開始する。ダニの刺し口（Escar）を認める場合はリケッチア疾患を考えるが診断が難しいため，発熱の原因が分からずリケッチア疾患が除外できない場合はテトラサイクリンの投与を行うこともある。レプトスピラは河などでの水の接触歴があり，発熱，筋肉痛，頭痛，眼球結膜の充血などがある場合は疑う。

渡航者下痢症はどの地域でも罹患する可能性があり，必ずしも感染症が原因ではなく，食事や水や環境の変化などでも下痢をすることがある。軽症では保存的治療のみで回復する場合が多いが赤痢やコレラなどの細菌性腸炎やアメーバ性腸炎は重症化する可能性がある。発熱や血

便，テネズムスがあるときは赤痢やサルモネラ，アメーバ腸炎が，また水様性便が大量にある場合はコレラが疑われる。症状（発熱や血便がある場合）やバイタルサインの悪い場合は早期に抗菌薬（ニューキノロン剤など）を開始する。便の直接検査でアメーバの栄養体やシストを確認，あるいは抗アメーバ抗体が上昇している場合は赤痢アメーバの治療としてメトロニダゾールによる治療を行う。

抗マラリア薬などの日本で未認可の薬剤が必要な場合にはヒューマンサイエンス総合研究事業「熱帯病に対するオーファンドラッグ開発研究」班 <http://www.ims.u-tokyo.ac.jp/didai/orphan/index.html> を通じて入手することができる。

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