Table 1. Total Viable Aerobic and Anaerobic Counts of the Whole Stomach and the Oral Mucosa of Antibiotic-Treated and Nontreated $TCR^{-/-}$ Mice (or mean CFU ± SD of 3 mice)

	Untreated	Treated
Aerobes		
Stomach (10^3 CFU/a whole stomach)		
Brain-heart infusion agar	159.3 ± 67.2	1.1 ± 0.7
Chocolate agar	167.0 ± 98.7	1.0 ± 0.6
Blood agar	80.3 ± 25.5	1.0 ± 0.5
Oral mucosa (CFU/swab)		
Brain-heart infusion agar	1969 ± 309	35 ± 36
Anaerobes		
Stomach (10^3 CFU/a whole stomach)	305.6 ± 119.7	1.2 ± 0.4
Oral mucosa (CFU/swab)	1444 ± 287	39 ± 30

induced by neonatal thymectomy was not dependent on a microflora.[51] Autoimmune gastritis was seen in germ-free mice with similar severity of inflammation, and autoantibody levels were comparable to those seen in conventional mice. In contrast, as shown in Figure 7, our gastritis model was dependent, in a significant way, on the microflora. Further, no anti-parietal cell autoantibody could be detected. Besides a requirement for a microflora and the absence of autoantibody, our model is different from autoimmune gastritis in several important ways. For example, although neonatal thymectomy caused gastritis as well as autoimmune oophoritis, orchitis, thyroiditis, pancreatitis, and prostatitis,[37,52] colitis has not been described previously. In contrast, the colon was a major organ affected by RB^{Hi} T cell transfer in SCID/$RAG2^{-/-}$/$TCR^{-/-}$ recipients, which requires the presence of a microflora. Further, in autoimmune gastritis, lesions are limited to the gastric corpus, and parietal cell destruction was the main histological feature. On the other hand, our model showed more severe inflammatory changes in the antral mucosa. Since other autoimmune disease models such as spontaneous gastritis in MRL-lpr mice were also independent of a microflora or infection,[37,52] it seems that different subsets of T regulatory cells are affected in autoimmune models and RB^{Hi} T cell-induced colitis/gastritis models. Thus, our model is quite distinct from autoimmune gastritis reported previously.

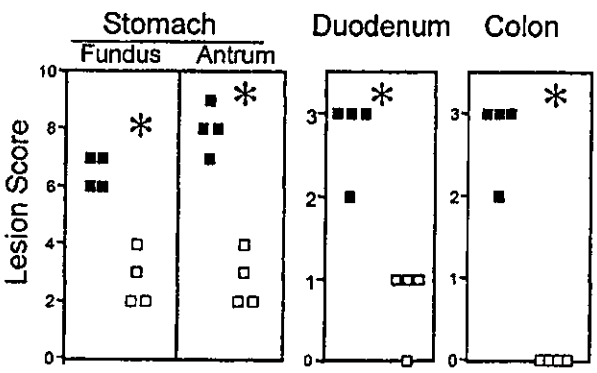

Figure 7. Treatment of recipient mice with antibiotics. A preparation of $IL\text{-}4^{-/-}$ RB^{Hi} T cells was transferred to eight $TCR^{-/-}$ mice (including two sets of male littermates in each experimental group). Four of these mice were given a combination of antibiotics in their drinking water (**blank squares**), and after 10 weeks histological scores were compared with control mice without treatment (**solid squares**).

$TCR^{-/-}$ recipients of wt or $IL\text{-}4^{-/-}$ T cells developed gastroduodenitis as well as colitis; however, no obvious changes in the jejunum or ileum were seen. This anatomical localization suggests the possible involvement of lumenal foreign antigens in the development of this type of inflammation. Colitis induced in SCID or $RAG2^{-/-}$ mice by adoptive transfer of RB^{Hi} T cells does not occur in the absence of an indigenous flora.[10,11] Through extensive testing for *Helicobacter spp.*, no pathogenic strains (including *Helicobacter* spp.) were detected in mice, which developed gastritis and duodenitis. In addition, neutrophil infiltration, which generally indicates bacterial infection, was not seen in the stomach or the duodenum in our model. On the other hand, $TCR^{-/-}$ recipients of $IL\text{-}4^{-/-}$ RB^{Hi} T cells developed a milder form of gastritis when they were treated with antibiotics. Of note, colitis was totally blocked in this group of mice. These results indicate that gastritis was partially dependent on an indigenous microflora, while colitis essentially required its presence. We speculate that orally ingested microbes or indigenous microflora in upper GI tract, in addition to food antigens, which have not been fully degradated in the stomach or duodenum, may play a similar role in this type of inflammation.

Another novel aspect of our model is the use of $TCR^{-/-}$ mice, as opposed to SCID or $RAG^{-/-}$ mice. Thus, $TCR^{-/-}$ mice lack T cells but have a fully responsive B cell repertoire. Indeed, after adoptive transfer of RB^{Hi} T cells, $TCR^{-/-}$ mice exhibit increased plasma IgG levels, and AFCs were seen in the mucosal tissues. The fact that transfer of RB^{Hi} T cells resulted in colitis and duodenitis but not gastritis in SCID mice clearly suggests the involvement of B cells in the pathogenesis of gastritis. In this regard, a different colitis model has also shown that B cells play protective roles from inflammation.[53] However, no AFCs were seen in the inflamed stomach tissues themselves, although small B cell aggregates were detected. Further, mucosal IgA production and IgA AFCs were actually lower in $TCR^{-/-}$ recipients of $IL\text{-}4^{-/-}$ RB^{Hi} T cells when compared with recipients of wt RB^{Hi} T cells, despite the more significant gastritis which characterize $IL\text{-}4^{-/-}$ RB^{Hi} T cell recipients. On the other hand, plasma IgG levels in $IL\text{-}4^{-/-}$ RB^{Hi} T cell recipients were comparable to recipients of wt RB^{Hi} T cells. The role of B cells and Ab production in our model certainly needs to be further investigated. We speculate that the presence of B cells and antibody production increases the sensitivity of

T cells to be triggered into an inflammatory expansion as well as the activation of macrophage-type cells. Our results suggest that $TCR^{-/-}$ recipients were more sensitive than SCID recipients and the former mice would be able to fully respond to the foreign antigens in the upper GI tract. To increase this sensitivity, T/B cell interactions are likely involved, although this interaction may not necessarily occur in the local mucosa but could occur in any lymphoid tissue, such as spleen or mesenteric lymph nodes. In the case of *H. pylori* infection, it is known that the presence of anti-*H. pylori* Abs are not required for the exclusion of bacteria,[53] but rather the Abs are actually involved in the pathogenesis of gastritis in humans[54] and mice due to antigenic mimicry.[55] Although we could not detect autoantibodies against the gastric parietal cells or H^+/K^+-ATPase, the presence of elevated Ig levels of particular idiotypes could play a role in the gastritis. In this regard, for our model of gastritis in the absence of infection or immunization, one could implicate molecular mimicry between any bacterial LPS and host blood group determinants[56–59] as an attractive hypothesis, although there is no direct evidence that this phenomenon actually occurs.

There is now clear evidence for a central role of T cell-mediated immunity in gastric inflammation. In the RB^{Hi} T cell transfer model described here, Th1-type cytokine production was required for the induction of colitis and gastroduodenitis, since transfer of IFN-$\gamma^{-/-}$ RB^{Hi} T cells resulted in much milder gastroduodenal inflammation (Figure 1) and colitis.[17] On the other hand, it is well known that IL-4 suppresses cytokine gene expression induced by IFN-γ and IL-2 in murine peritoneal macrophages.[60,61] The severe tissue damage in recipients of IL-$4^{-/-}$ RB^{Hi} T cells was likely caused by enhanced IFN-γ and IL-2 production by IL-$4^{-/-}$ T cells due to their predisposition toward a Th1-phenotype. However, the levels of IFN-γ release by isolated T cells were comparable in these mice. These quantitative differences in Th1 cytokines may not fully explain the fact that the epithelial cell apoptosis and surface erosion was much more frequent in IL-$4^{-/-}$ than in wt RB^{Hi} T cell recipients. Since IL-4 is a cytokine that has pleiotropic effects on a variety of cell types, including epithelial cells and other non-hematopoietic cells, a lack of IL-4 production by infiltrating T cells may have an impact on tissue repair in addition to a cytokine imbalance. Gastritis induced by infection with pathogenic *H. pylori* in IL-$4^{-/-}$ mice was more severe than that in IFN-$\gamma^{-/-}$ mice.[62] In a rat acute gastric ulcer model, healing was accompanied by a rapid rise in tissue IL-4 levels.[63] IL-$4^{-/-}$ mice were more susceptible to the colitis induced by administration of trinitrobenzene sulfonic acid, and tended to develop focal but penetrating ulcers, which were not frequently seen in IFN-$\gamma^{-/-}$ mice.[64] It is also known that fibroblasts express the IL-4 receptor, and Th2-type cells activate lung fibroblasts with resultant increase in deposition of collagen and fibronectin.[65,66] In the airway or ileal epithelium, IL-4 induces mucin gene expression[67] and goblet cell metaplasia.[17,67] Thus, IL-4 may be significantly involved in the epithelial cell turnover and tissue protection required for the maintenance of the gastrointestinal tract architecture, in addition to its role as a mediator for allowing immunological homeostasis in the gut.

In summary, we have established a novel murine model for the upper gastrointestinal tract, which does not require pathogen infection or deliberate immunization. The inflammation was mediated by Th1-type immune responses restricted to a particular subset of T cells isolated from normal mice. This model also points to the significance of the host immune system in gastric lesions and should be of importance to help better understand the pathophysiology of chronic gastroduodenitis seen in *H. pylori* infection of humans.

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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 elicitations.

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 effectively 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 substrain 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 (NTRKSIHIGPGRAFYATGS), 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 μg/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 ($TCID_{50}$) 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 μl of peptide MN (DKRIHIGPGRAFYTT)/well in 50 mM carbonate buffer (pH 9.3) at 5 μg/ml overnight at 4°C. The wells were washed and treated with 5% nonfat milk in phosphate-buffered saline for 1 h at 37°C. Duplicate samples containing either control or test macaque serum at appropriate dilutions were then added at 100 μl/well, and the plates were incubated for 1 h at 37°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 μl/well for 1 h at 37°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 H_2SO_4.

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 μM V3 peptide and were then incubated for 16 h at 37°C in 5% CO_2 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°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 $TCID_{50}$ 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 10^6 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 $TCID_{50}$ per 10^6 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 SEI probe, 5′-GCAGAAGAAGAGGTAGTAATTAGAT-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 ± 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

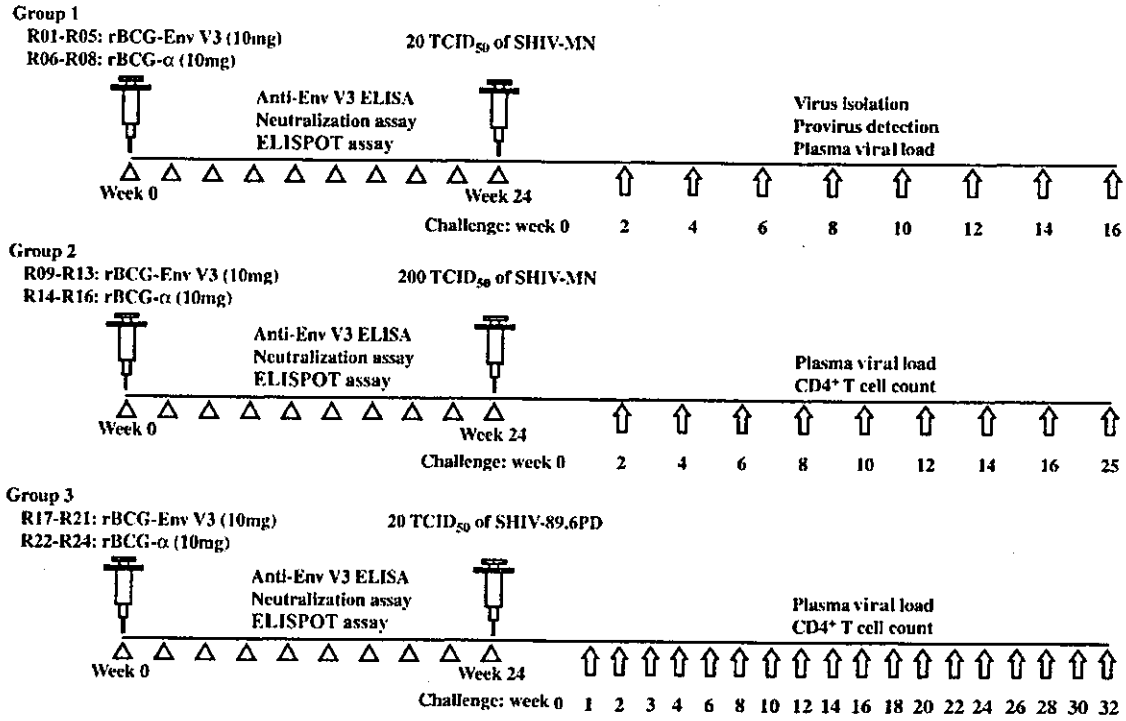

FIG. 1. Schematic representation of the experimental protocol for immunization of rhesus macaques with rBCG Env V3 and challenge with either SHIV-MN or SHIV-89.6PD. A total of 24 macaques were assigned to either the rBCG Env V3 vaccine or rBCG vector control group. The animals each received a single 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 $TCID_{50}$) or SHIV-89.6PD (20 $TCID_{50}$) (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 naïve 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 (IC_{50}) of 0.05 to 0.5 μg of IgG/ml was measured against SHIV-MN, and a mean IC_{90} of ~3.0 μg 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 IC_{50} values of 5 to 7, 4 to 7, and 5 to 15 μg/ml, respectively. By comparison, neutralization of HIV-$1_{MNp/X4}$ required ~10-fold more serum IgG, with a mean IC_{50} of 50 μg/ml. Three additional isolates, HIV-$1_{SF33/X4}$, HIV-$1_{SF33/R5}$, and the clade A isolate HIV-$1_{VI313/R5}$,

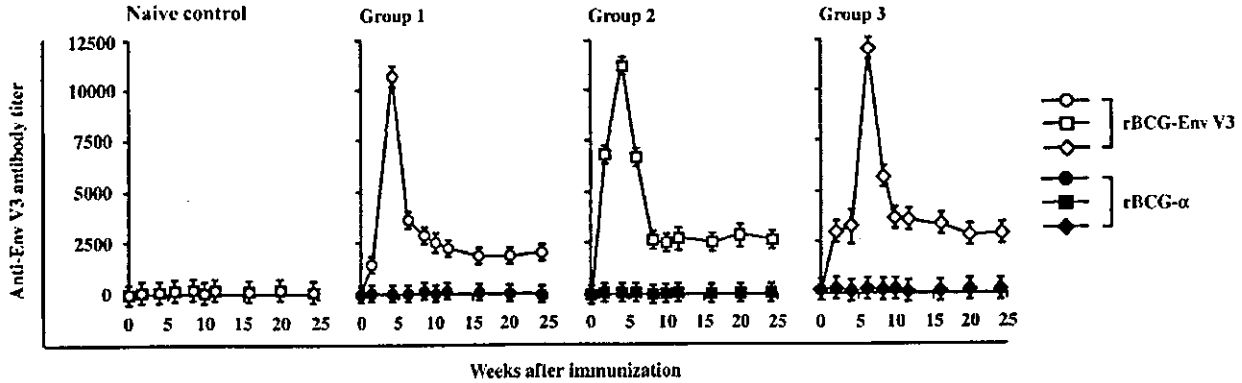

FIG. 2. Serum anti-V3 antibody titers determined by peptide-based ELISA. Preimmune and immune sera from macaques inoculated with rBCG Env V3 were collected and stored at −80°C until they were used. Sera from naïve macaques were used as controls. Data using preimmune sera were within the control levels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

were not neutralized with serum IgG concentrations up to 50 μg/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

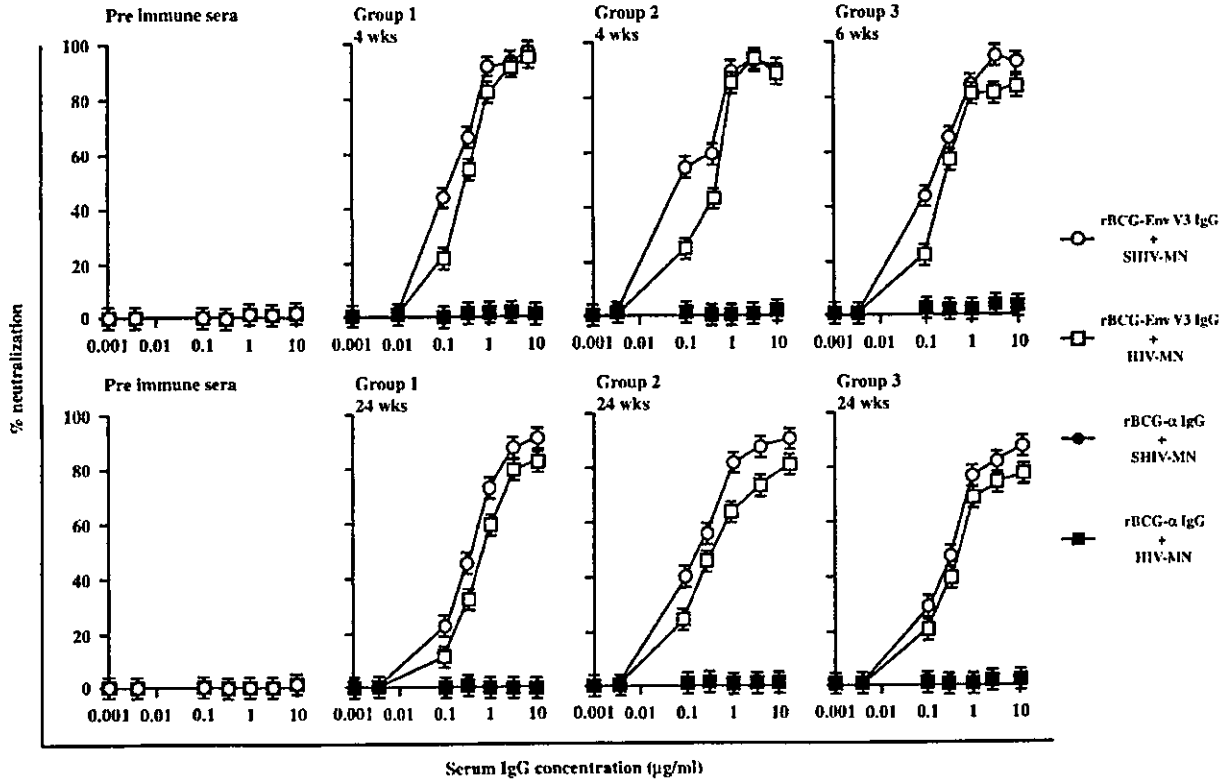

FIG. 3. HIV-1-specific neutralization antibody responses in macaques vaccinated with rBCG Env V3. Analysis of in vitro neutralization of SHIV-MN by anti-rBCG-HIV-1 antibodies using MS166 cell-based virus neutralization assays. Serum IgG was purified from preimmune or immune sera of macaques inoculated with rBCG Env V3 at the indicated times. The results are expressed as the means ± 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	C12/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. VI313 is an HIV-1 clade A virus.

SHIV challenge (120 and 110 SFC/10^6 PBMC at 24 weeks p.i., respectively) (Table 2). In contrast, <100 SFC/10^6 PBMC were observed in other immunized animals, and <20 SFC/10^6 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 $TCID_{50}$) 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 (<10^4 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-

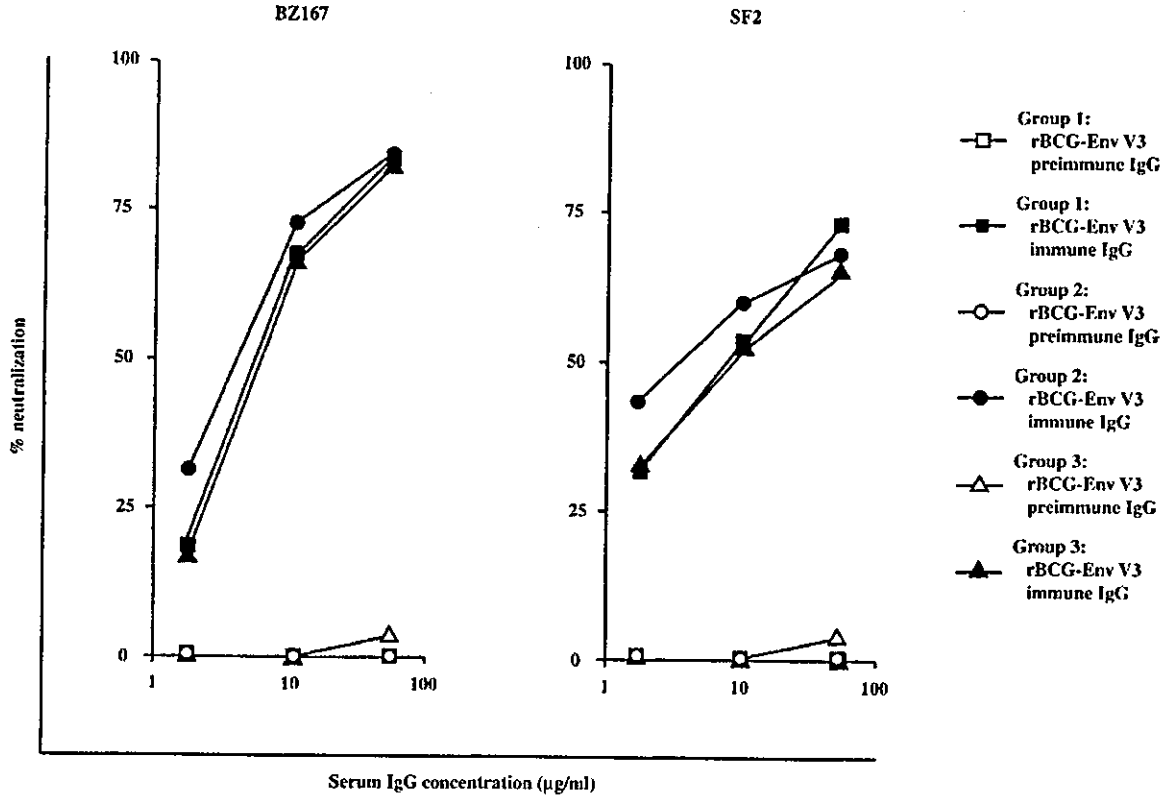

FIG. 4. Neutralization of HIV-1_{BZ167} and HIV-1_{SF2} in GHOST-X4 cells by immune sera from macaques vaccinated with rBCG Env V3. Dilutions of immune sera (closed symbols) and preimmune sera (open symbols) were tested in duplicate, and the percent neutralization was calculated using the mean value. 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 $TCID_{50}$) 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 10^6 to 10^7, to $<10^5$ to 10^4 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 $TCID_{50}$) 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 IC_{50} 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 IC_{50}s: R-01 to R-05, 0.32; R-09 to R-13, 0.05; R-17 to R-21, 0.35.
[e] Mean IC_{50}s: 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 $TCID_{50}$ 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 ~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 IC_{50}s 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 μg/ml, respectively (Table 2). The IC_{50}s of the uncontrolled macaques R-01 and -03 (Table 1) were both 0.6 μg/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 IC_{50} of 0.30 μg/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 $HIV\text{-}1_{MN}$-specific NAb generated by the recombinant $HIV\text{-}1_{MN}$ 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 $TCID_{50}$), 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

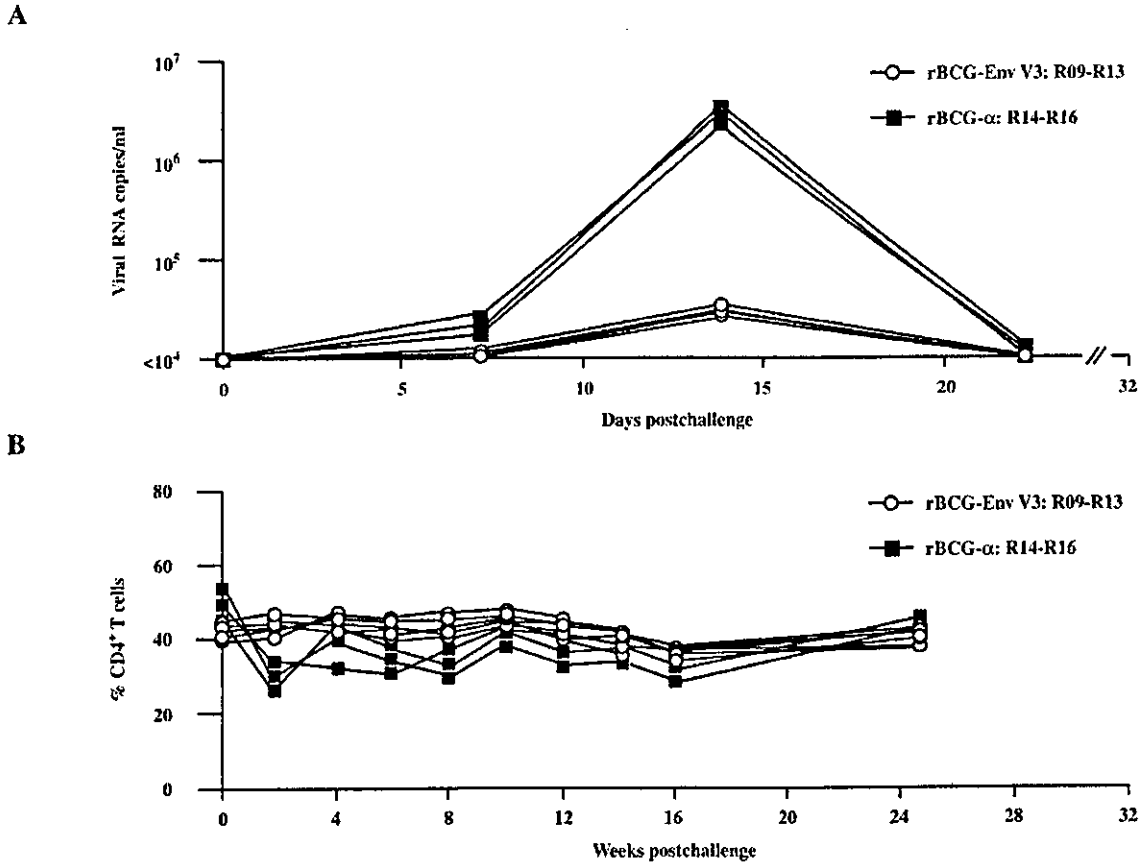

FIG. 6. Comparison of infection kinetics following high-dose (200 $TCID_{50}$) inoculation of SHIV-MN in macaques vaccinated with either rBCG Env V3 or rBCG vector control. (A) Viral RNA copy number per milliliter of serum. (B) $CD4^+$-T-cell count as a percentage of total lymphocytes. The results in 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 IC_{50} and IC_{90} values for neutralization of SHIV-MN were 10^3 to 10^4 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 ~10^3 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 IC_{90} 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-

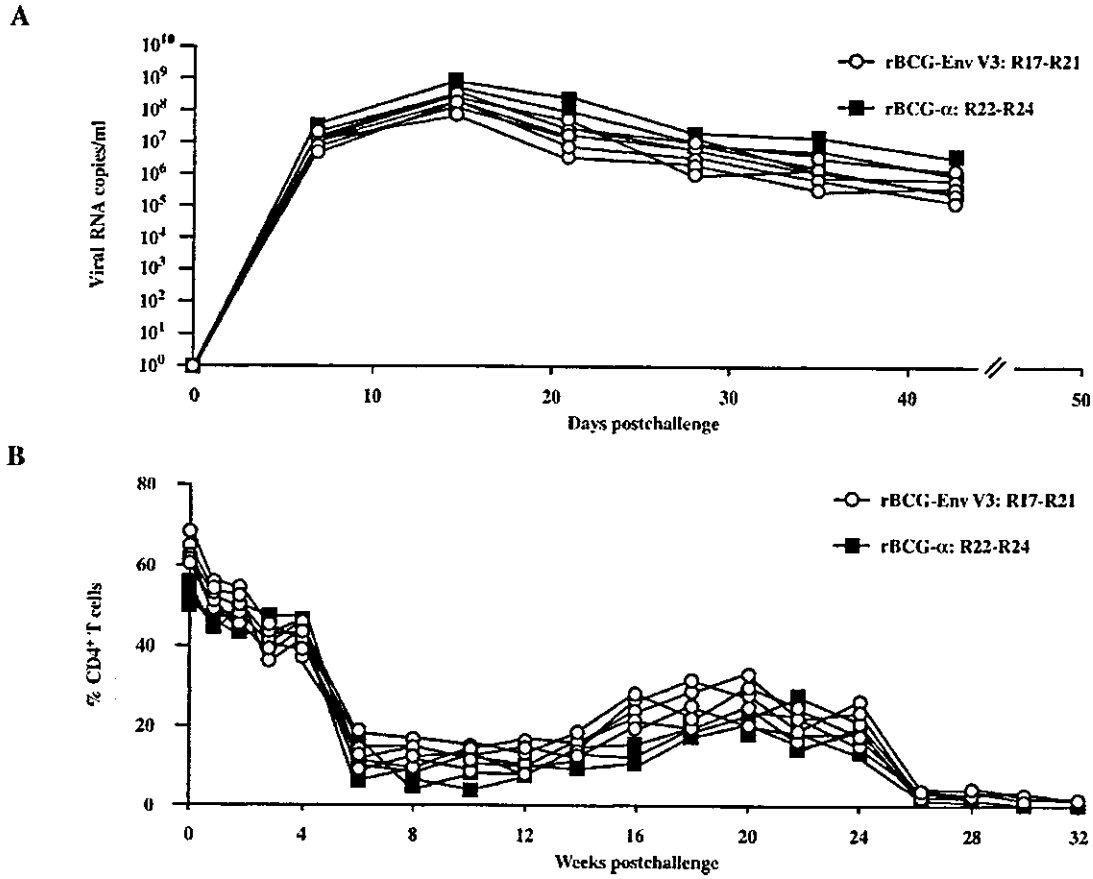

FIG. 7. Comparison of infection kinetics following challenge with pathogenic SHIV-89.6PD in macaques vaccinated with either rBCG Env V3 or rBCG vector control. (A) Plasma viral-RNA copy numbers per milliliter. (B) $CD4^+$-T-cell count as a percentage of total lymphocytes. The results in individual animals 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-$1_{JR\text{-}CSF}$ (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 structure 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 μg 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 elicitations.

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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/μl，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/μl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ずらかった。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）/μl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 EMERGENCY NOW

●救急に関連する新興・再興感染症

その他の感染症

源河いくみ※ Genka-Ikumi

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 antiretoroviral 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/μL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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