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Immunogenicity and Protective Efficacy of DNA Vaccines Expressing Rhesus Cytomegalovirus Glycoprotein B, Phosphoprotein 65-2, and Viral Interleukin-10 in Rhesus Macaques▽

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Rhesus cytomegalovirus (RhCMV) infection of macaques exhibits strong similarities to human CMV (HCMV) persistence and pathogenesis. The immunogenicity of DNA vaccines encoding three RhCMV proteins (a truncated version of glycoprotein B lacking the transmembrane region and endodomain [gBΔTM], phosphoprotein 65-2 [pp65-2], and viral interleukin-10 [vIL-10]) was evaluated in rhesus macaques. Two groups of monkeys (four per group) were genetically immunized four times with a mixture of either pp65-2 and gBΔTM or pp65-2, vIL-10, and gBΔTM. The vaccinees developed anti-gB and anti-pp65-2 antibodies in addition to pp65-2 cellular responses after the second booster immunization, with rapid responses observed with subsequent DNA injections. Weak vIL-10 immune responses were detected in two of the four immunized animals. Neutralizing antibodies were detected in seven monkeys, although titers were weak compared to those observed in naturally infected animals. The immunized monkeys and naïve controls were challenged intravenously with 10^5 PFU of RhCMV. Anamnestic binding and neutralizing antibody responses were observed 1 week postchallenge in the vaccinees. DNA vaccination-induced immune responses significantly decreased peak viral loads in the immunized animals compared to those in the controls. No difference in peak viral loads was observed between the pp65-2/gBΔTM DNA- and pp65-2/vIL-10/gBΔTM-vaccinated groups. Antibody responses to nonvaccine antigens were lower postchallenge in both vaccine groups than in the controls, suggesting long-term control of RhCMV protein expression. These data demonstrated that DNA vaccines targeting the RhCMV homologues of HCMV gB and pp65 altered the course of acute and persistent RhCMV infection in a primate host.

Human cytomegalovirus (HCMV) infection is usually asymptomatic in immunocompetent people. However, it may cause severe and, sometimes, fatal disease in immunologically immature or immunocompromised individuals, such as transplant recipients, human immunodeficiency virus (HIV)-infected patients, and developing fetuses (4, 63). The clinical significance of HCMV has increased due to the increased number of organ allograft transplant recipients and HIV-infected individuals. Furthermore, HCMV is the leading infectious cause for birth defects in newborns. Development of a safe and effective HCMV vaccine is thus required for those people at risk for HCMV infection and disease and has been placed in the top priority by the Institute of Medicine for the clinical and economic benefit that a vaccine would produce (64).

Studies of HCMV immunity are important for the development of vaccines. Although the nature of protective immune responses to HCMV infection is incompletely defined, clinical observations in immunocompromised humans and congenital infection have pointed to the importance of neutralizing antibody and cytotoxic T-lymphocyte (CTL) responses in controlling HCMV disease. Neutralizing antibodies appear to be critical for limiting the incidence and severity of HCMV congenital infection following primary and nonprimary maternal infection (10, 25) and the severity of HCMV disease in transplant patients and HIV-infected patients (1, 2, 10). Likewise, the recovery of HCMV-specific $CD8^+$ CTL response in organ recipients correlates to the protection from HCMV disease after transplantation (36, 54–56). In addition, passive transfer of ex vivo-expanded $CD8^+$ HCMV-specific CTL clones from seropositive donors reconstitutes cellular immunity in seronegative bone marrow recipients and prevents the onset of viremia and HCMV-related disease (68). Investigations into HCMV antigens that induce protective immunity have shown that glycoprotein B (gB) is the immunodominant target for neutralizing antibodies (13, 43) and lower matrix phosphoprotein 65 (pp65) is the principal target for HCMV-specific CTL responses after natural infection (11, 28, 45, 70). Studies of gB- and pp65-based DNA vaccines in the murine and guinea pig CMV models have demonstrated the ability of gB and pp65 to stimulate CTL and neutralizing antibody responses that confer protection against CMV infection and disease (46–48, 59, 60, 71). However, since results obtained in mice do not always predict the responses in humans (42, 69), experimental studies in nonhuman primates can provide insight into the efficacy of vaccine strategies prior to commencement of clinical trials in humans.

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Rhesus CMV (RhCMV) infection in rhesus macaques provides a relevant model for HCMV with regard to their similarities in genomic organization, pathogenesis, persistence, and immunity (3, 5, 7–9, 14–16, 18, 29, 32, 34, 35, 37, 38, 50, 52, 57, 61, 62, 65–67, 72, 73). Our recent studies demonstrate that RhCMV gB and pp65-2 (one form of RhCMV pp65) resemble their HCMV counterparts in the induction of humoral and cellular responses after natural RhCMV infection (72, 73), indicating the potential of gB- and pp65-2-based vaccines for RhCMV infection. In addition, HCMV and RhCMV possess several immunomodulating proteins, such as viral interleukin-10 (vIL-10), to evade the host immune responses (16, 38, 62). RhCMV vIL-10 elicits weak antibody and cellular responses in infected macaques (34, 38; Y, Yue, A. Kaur, M. Eberhardt, N. Kassis, S. S. Zhou, and P. Barry, unpublished observation). Since HCMV vIL-10 has potent immunosuppressive effects on human lymphoid cells in vitro (16, 62), vIL-10 may play a central role in modulating host immune responses in vivo. Thus, vIL-10 and the other immunomodulating proteins of HCMV may represent a novel class of vaccine antigens. In this study, RhCMV gB, pp65-2, and vIL-10 DNA vaccines were investigated for their immunogenicity and protective efficacy against RhCMV infection in rhesus macaques.

MATERIALS AND METHODS

DNA plasmids. The expression plasmid pND (41), encoding a truncated version of gB (pND/gBΔTM) with a deletion of the transmembrane (TM) region and the carboxyl portion of the protein downstream of TM, has been previously described (73). The RhCMV pp65-2 gene was amplified from the genome of RhCMV 68-1 and then cloned into TOPO-TA vector. The expression plasmid pND/pp65-2 was constructed by subcloning the pp65-2 gene from a recombinant vaccinia virus transfer plasmid, pAbT4587/pp65 (34), into the pND mammalian expression vector. pND/vIL-10 was generated by subcloning the cDNA of RhCMV IL-10 (p183-3 #3) (38) from the TOPO-TA vector into pND. The pND vector places open reading frames under the transcriptional control of the immediate-early promoter/enhancer of HCMV and the bovine growth hormone polyadenylation signal (41).

RhCMV phosphoprotein 150 (pp150) was engineered into pND in three stages. A complementary oligonucleotide pair, corresponding to the 22 carboxy-terminal base pairs of pp150 (AY186194), was cloned into the SalI restriction endonuclease site of pND. The complementary oligonucleotide pair was engineered to contain single-stranded overhangs compatible with the extension produced by SalI digestion. The 3′ overhang was constructed such that ligation would eliminate the SalI site, whereas the 5′ overhang would maintain the SalI site after ligation. The 22 bases of the oligonucleotide included an XhoI site found within the pp150 gene. Finally, a HindIII site was included in the design between the XhoI site of pp150 and the SalI site, as follows: 4-bp SalI extension-HindIII-XhoI-pp150 segment (16 bp)-SalI-compatible extension. This generated plasmid pND/Xho #12. The amino-terminal 543 bp of pp150 was amplified with the primer pair 5′-TGGAGTGACGCTCAAGATGAAGCTTAAG-3′ and 5′-TATCTCGTGTCGACTCCGGTTTCTGTGGCTGACAATCATGAG-3′, which incorporated a HindIII site found within pp150 and a novel SalI site into the primer design. The amplicon was cloned into the Sal and HindIII sites of pND/Xho #12, generating the plasmid p257-1-5.1. Finally, the XhoI fragment from the plasmid Sal 1–57ΔHindIII was cloned into the HindIII-Xho sites of p257-1-5.1. Sal 1–57ΔHindIII is a plasmid containing a restriction fragment of RhCMV 68-1 (29) that encompasses the entire pp150 open reading frame of RhCMV strain 68-1.

TABLE 1. RhCMV DNA immunization in BALB/c mice

Group	Plasmid(s)	Inoculation dates (wk)	No. antibody positive/ no. immunized
1	pND/gBΔTM	0, 3	5/5
2	pND/pp65-2	0, 3, 12	3/4
3	pND/vIL-10	0, 3, 12	4/4
4	pND/pp65-2 + pND vector	0, 3, 12	4/4
5	pND/pp65-2 + pND/vIL-10	0, 3, 12	5/5 for both pp65-2 and vIL-10
6	pND vector	0, 3, 12	0/3

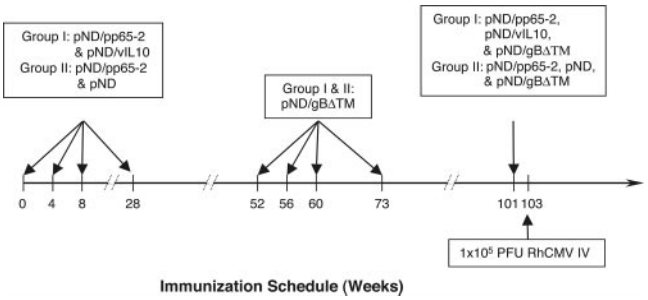

FIG. 1. Immunization groups and schedule for DNA vaccination in macaques in relation to the primary immunization at week 0 and i.v. challenge with 1×10^5 PFU of the 68-1 strain of RhCMV at week 103. pND vector DNA was used for group II monkeys to equalize with group I monkeys the total amount of DNA used per immunization.

Expression plasmids were purified with Endofree plasmid kits (QIAGEN). DNA was resuspended in phosphate-buffered saline (PBS) buffer (Invitrogen) at a concentration of 1 mg/ml and stored at −20°C. The ability of each plasmid to express the encoded protein was confirmed in vitro by detecting specific immunoreactive bands of the predictive molecular weights on immunoblots of transiently transfected 293T cells (human embryo kidney cells expressing adenovirus E1 and simian virus 40 T antigen) (23, 67, 73) with RhCMV-seropositive serum or rabbit anti-RhCMV IL-10 polyclonal antibodies (38).

Animal protocols. All animal protocols were approved in advance by the Institutional Animal Care and Use Committee of the University of California, Davis (UC Davis). UC Davis is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. All animals were serologically negative for RhCMV prior to immunization or inoculation.

DNA immunization of BALB/c mice. To test the immunogenicity of the pND/gBΔTM, pND/pp65-2, and pND/vIL-10 plasmids in mice, female BALB/c mice, 6 to 8 weeks old, were purchased from Charles River (Wilmington, MA) and randomly divided into six groups of four mice per group. Mice were injected with 26-gauge needles (Becton Dickinson, Franklin Lakes, NJ) intradermally (i.d.) with 10 μg of plasmid and intramuscularly (i.m.) with 50 μg of plasmid at weeks 0, 3, and 12, except for mice that were immunized twice with pND/gBΔTM at weeks 0 and 3 (Table 1). Blood was routinely collected via the lateral saphenous vein and processed for the analysis of antibody responses (41).

DNA immunization and RhCMV inoculation of rhesus macaques. Twelve healthy colony-bred juvenile rhesus macaques (*Macaca mulatta*), ranging in age from 8 months to 2 years 8 months, from the California National Primate Research Center were grouped into four monkeys per group. Two groups were immunized with both 150 μg (i.m.) and 50 μg (i.d.) of each DNA plasmid according to published protocols (41). The third group was used as the control. The i.m. immunization was given in the triceps muscles, and i.d. vaccination was given in multiple sites on the abdomen. The DNA vaccination and RhCMV inoculation regimens are detailed in Fig. 1. Briefly, the two groups of animals were immunized with either a mixture of the pND/pp65-2 and pND/vIL-10 plasmids (group I) or a mixture of pND/pp65-2 plasmid and pND vector (group II) at weeks 0, 4, 8, 28, and 101. All animals were immunized with pND/gBΔTM plasmid at weeks 52, 56, 60, 73, and 101. Two weeks after the last boost (week 103), both groups of immunized animals and a third group of unimmunized control animals were intravenously (i.v.) inoculated with 1×10^5 PFU of the pathogenic 68-1 strain of RhCMV (9, 15, 18, 37, 66). This variant has been determined to contain a naturally occurring frameshift mutation within the UL36 (viral inhibitor of caspase-8-induced apoptosis [vICA]) open reading frame (44). Blood samples (EDTA anticoagulant) and oral and genital swabs were routinely collected and processed for antibody and cellular response assessment and viral DNA detection (61).

DNA extraction. DNA was extracted from oral and genital swab samples and plasma samples using a QIAmp blood kit according to a previously published protocol (32). Each sample was processed according to the manufacturer's in-

structions. The final elution volume was 200 μl. Extracted DNA was stored at −80°C until real-time PCR analysis was performed.

Real-time PCR. RhCMV DNA copies in plasma and oral and genital swabs were detected by a previously described real-time PCR assay for RhCMV 68-1 (61). Primers and probe sequences for real-time PCR were designed to the gB gene of RhCMV. Probe was labeled with TET at the 5′ end as the reporter dye and 6-carboxytetramethylrhodamine (TAMRA) at the 3′ end as the quencher dye (Applied Biosystems, Foster City, CA). Real-time PCR was performed with an ABI prism 7700 sequence detection system. Each PCR (12.5-μl volume in 1× Taqman universal PCR master mixture; Applied Biosystems) contained 17.5 pmol of each primer, 2.5 pmol probe, and 5 μl sample DNA. A standard curve was generated by using a 10-fold serial dilution of a gB plasmid containing 10^6 to 10^0 copies per 5 μl. All samples were run in triplicate. Results were reported as average copies per ml. The limit of RhCMV DNA detection was between 200 and 2,000 RhCMV genomes per ml (i.e., plasma and mucosal fluids).

Enzyme-linked immunosorbent assays. RhCMV, vIL-10, gBΔTM, pp65-2, and pp150 cell extracts were prepared from RhCMV-infected rhesus dermal fibroblasts or 293T (23) cells transiently transfected with pND/vIL-10, pND/gBΔTM, pND/pp65-2, or pND/pp150, following previously published protocols (72, 73). Antibodies to RhCMV, gBΔTM, vIL-10, pp65-2, and pp150 were assayed by antigen-specific enzyme-linked immunosorbent assay following previously described protocols (72, 73). Briefly, 96-well microplates (Immulon 4 HBX; Dynex Technologies, Inc.) were coated overnight at 4°C with specific antigens (0.5 μg/well for pp65-2 and pp150 antigens, 0.25 μg/well for RhCMV and gBΔ antigens, and 0.30 μg/well for vIL-10 antigen) and corresponding cell control extracts in 100 μl of coating buffer (0.05 M carbonate/bicarbonate buffer, pH 9.6 [Sigma], for pp65-2 and pp150; Hanks buffered salt solution–0.375% bicarbonate buffer [GIBCO] for RhCMV, vIL-10, and gBΔ). The following day, each plate was washed six times with PBS–0.05% Tween 20 (PBS-T; wash buffer) and blocked with 300 μl/well of PBS containing 1% bovine serum albumin (BSA) for 2 h at room temperature. After washing six times, plasma/serum samples, diluted in 1% BSA–PBS-T (dilution buffer), were added to duplicate wells (100 μl/well) and incubated for 2 h at room temperature. The plates were washed six times with PBS-T. Peroxidase-conjugated goat anti-monkey immunoglobulin G (KPL, Inc.), diluted to empirically determined optimal concentrations in 1% BSA–PBS-T, was added for 1 h. The plates were subsequently washed again with PBS-T and then incubated with 100 μl/well of tetramethylbenzidine liquid substrate (Sigma) for 30 min for color development. The reaction was terminated by addition of 0.5 M H_2SO_4 (50 μl/well), and the absorbance was recorded spectrophotometrically at a wavelength of 450 nm. For each sample, the A_{450} for control antigen-coated well was subtracted from that obtained for the antigen-coated well. A sample was considered positive if the net absorbance was 0.1 or greater. Titers of RhCMV- and gB-specific antibodies were calculated as the reciprocal endpoint dilution at which the net absorbance value was >0.1.

Neutralization assays. Neutralizing activity of monkey plasma was measured by a plaque reduction assay in the presence of guinea pig complement. Fifty microliters of serially diluted, heat-inactivated (56°C, 30 min) monkey plasma was mixed with 100 μl of RhCMV strain 68-1 containing ~50 PFU and 50 μl of diluted guinea pig complement (final dilution, 1:10; Sigma) and incubated at 37°C for 1 h. The mixtures (total volume 200 μl) were added subsequently to monolayers of Telo-RF cells (17) in 24-well plates, which had been seeded the day before at a density of 5×10^4 cells/well. After a 2-h incubation, the virus-plasma mixture was removed, and the cells were washed with Dulbecco's modified Eagle's medium (DMEM; GIBCO). The cells were overlaid with 0.5% SeaPlaque agarose (Cambrex Bio Science Rockland, Inc.) containing DMEM, 2% fetal calf serum, and 100 μg/ml of G418 (Invitrogen Corp). The viral plaques were counted 6 to 7 days later. Each plasma dilution was tested in triplicate. Results were recorded as the average number of plaques per well. A neutralizing titer was defined as the reciprocal dilution of plasma that reduced the number of plaques by 50% relative to virus-only control wells.

IFN-γ ELISPOT assay. Cryopreserved peripheral blood mononuclear cells (PBMC) were thawed and tested for their ability to secrete gamma interferon (IFN-γ) during in vitro restimulation with either sonicated RhCMV antigen preparation or overlapping peptide pools (15-mers overlapping by 11 amino acids) representing the entire amino acid sequence of either pp65-2 or vIL-10 using a previously described enzyme-linked immunospot (ELISPOT) protocol (33). Spots were counted using an automatic reader system (KS ELISPOT; Carl Zeiss, Inc., Thornwood, NY) and recorded as the number of spot-forming cells (SFC) per million PBMC. A sample was considered T-cell response-positive if the number of SFC was greater than or equal to 50 per million PBMC after subtraction of background spots in negative control cells.

ICS. Intracellular cytokine staining (ICS) was performed as follows. Detection of antigen-specific-$CD4^+$ and $CD8^+$ lymphocytes was performed as previously described (26, 33). Briefly, cryopreserved PBMC were thawed and stimulated for 6 h with the peptide pool or medium in the presence of cross-linked antibodies to CD28 and CD49d (clones 28.2 and 9F10, respectively; BD Biosciences) at the final concentration of 10 μg/ml. Brefeldin A at 10 μg/ml was added to the culture for the final 4 h of stimulation. After stimulation, the cells were surface stained with conjugated antibodies to CD3 and CD8 or CD3, CD4, and CD8 for 30 min at 4°C. Subsequently, the cells were fixed and permeabilized with successive incubations with fluorescence-activated cell sorter (FACS) lysing solution and permeabilizing solution (BD Biosciences). Permeabilized cells were then incubated with conjugated anti-IFN-γ with or without anti-CD69 monoclonal antibodies for 30 min at 4°C, washed, and kept overnight at 4°C in 2% paraformaldehyde. Two hundred thousand lymphocyte events were collected on a FACS Calibur flow cytometry system (BD Biosciences), and the data were analyzed with CELLQuest software (BD Biosciences).

Statistics. After a $\log_{10}$ transformation, groups were compared with one-way analysis of variance (ANOVA). Postlog comparisons among mean values were analyzed in SAS using the PROC GLM procedure. Both t (least significant difference) and Tukey's Studentized range (highly significant difference [HSD]) tests ($\alpha = 0.05$; df = 9) are presented in Table 4.

RESULTS

Antibody responses in immunized mice. The immunogenicity of DNA plasmids was investigated in mice as a prelude to studies in macaques. Antigen-specific antibody responses were detected in 100% of the mice immunized with either pND/gBΔTM plasmid or pND/vIL-10, and seven of eight of the mice immunized with pND/pp65-2 after two or three immunizations (groups 2 and 4 combined, Table 1 and Fig. 2D [discussed below]). Coadministration of pND/pp65-2 and pND/vIL-10 induced both anti-pp65-2 and anti-vIL10 antibodies in all of the immunized mice. No antigen-specific antibodies were present in preimmunization serum samples or in samples obtained from mice immunized with control plasmid (pND) alone.

Antibody responses in immunized rhesus macaques. After demonstrating the immunogenicity of DNA plasmids in mice, two groups of RhCMV-seronegative rhesus monkeys (four monkeys per group) were immunized with a combination of expression plasmids for either pp65-2, vIL-10, and gBΔTM (group I) or pp65-2 and gBΔTM (group II), according to the staggered immunization schedule shown in Fig. 1. Animals were vaccinated with either pND/pp65-2 and pND/vIL-10 (group I) or pND/pp65-2 (group II) on weeks 0, 4, 8, and 28, and all animals were vaccinated with pND/gBΔTM beginning on week 52. Each animal was boosted one last time with the appropriate plasmid mixture on week 101 prior to viral challenge at week 103.

The kinetics and levels of antibody responses to pp65-2 were generally indistinguishable for the two groups of immunized monkeys (Fig. 2A). Half of the animals (four of eight) were antibody positive at the time of the third DNA injection (week 8), and seven had detectable antibodies to pp65-2 2 weeks later (week 10). Unlike DNA immunizations in mice with pND/pp65-2 (data not shown), antibody responses declined after peak responses observed at weeks 10 to 12. Rapid increases were observed in all seven of these animals within 2 weeks of the booster immunization at week 28. Peak responses following the fourth DNA immunization were higher than those following the week 8 treatment, consistent with an anamnestic immune response. A similar decline in responses following the week 28 immunization and anamnestic response following immunization at week 101 were observed in these same seven animals. The rapid increases in antibody levels observed at

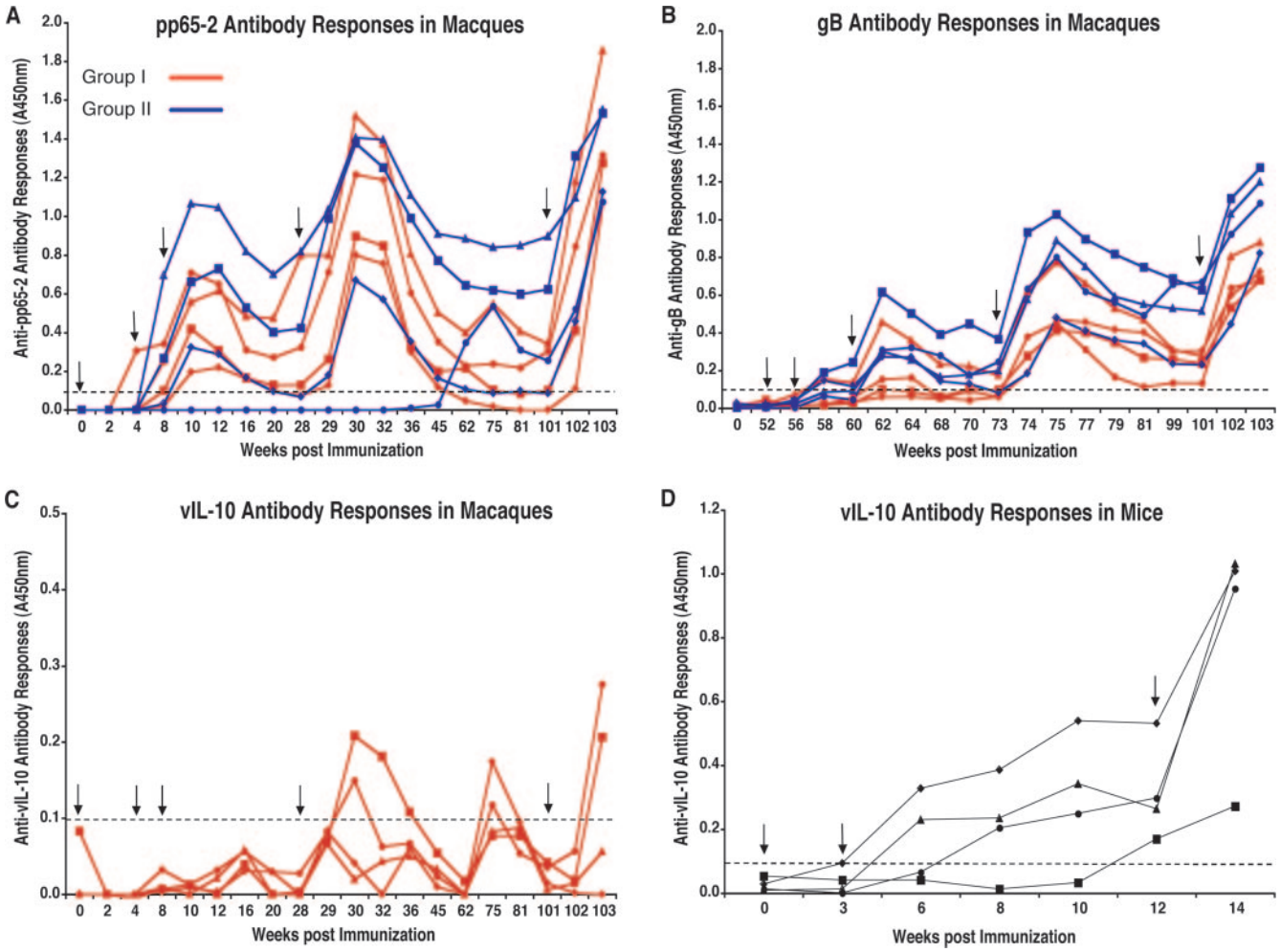

FIG. 2. Antibody responses to genetic immunization with pND/pp65-2, pND/gBΔTM, and pND/vIL-10 in macaques (A to C) and mice (D; vIL-10 only). Arrows indicate times of genetic immunization, and dashed lines indicate threshold for a positive antibody response.

week 103 demonstrated that DNA immunization elicited long-term memory responses in macaques. One animal in group I remained antibody negative until week 62. This animal also exhibited a vigorous increase in pp65-2 antibody levels following immunization at week 101.

Antibody responses to the truncated version of gB (gBΔTM) were similar to those observed for pp65-2 following the first immunization at week 52 (Fig. 2B). Six animals were demonstrably antibody positive 2 weeks after the third immunization (week 60), and 100% of the animals were positive following the fourth immunization (week 73). Each animal exhibited rapid increases in gB-specific responses within 2 weeks of the booster immunizations, with each succeeding immunization eliciting higher antibody levels. The gB antibody levels observed at week 103 were comparable to those detected in macaques naturally infected with RhCMV (73; data not shown). Since the RhCMV gB gene encodes the majority of neutralizing antibodies in RhCMV-infected macaques (73), plasma samples from the immunized animals were analyzed for neutralizing antibody titers. Low but detectable neutralizing activity was first observed at week 102 for seven animals and week 103 for all animals (Table 2). A 20-fold dilution of plasma achieved 43 to 99% neutralization of input virus, except for animal 5 (group II), which had essentially no activity at week 102. No neutralization was observed at dilutions of 1:50 or greater (data not shown). The levels of neutralizing activity were far lower than those observed in long-term-infected animals naturally exposed to RhCMV. A cross-sectional study of 21 female adult

TABLE 2. Neutralizing antibody activities in immunized monkeys after the last booster at week 101

Wk	% of plaque reduction in monkey[a]:							
	Group I				Group II			
	1	2	3	4	5	6	7	8
102	64.8*	55.6	88.0	92.5	2.99	67.92	61.19	94.0
103	81.3	50.5	91.2	98.7	42.86	76.92	94.51	98.9

[a] Neutralizing antibody activities in the plasma samples obtained from immunized monkeys were evaluated by complement-dependent plaque reduction. Plasma samples from each of the four monkeys in groups I and II were assayed at a 1:20 dilution for the ability to reduce the number of plaques compared to that in cells infected with virus alone. The numbers refer to the percentage of plaque reduction at the 1:20 dilution for samples obtained 1 (week 102) and 2 (week 103) weeks after the last DNA immunization at week 101.

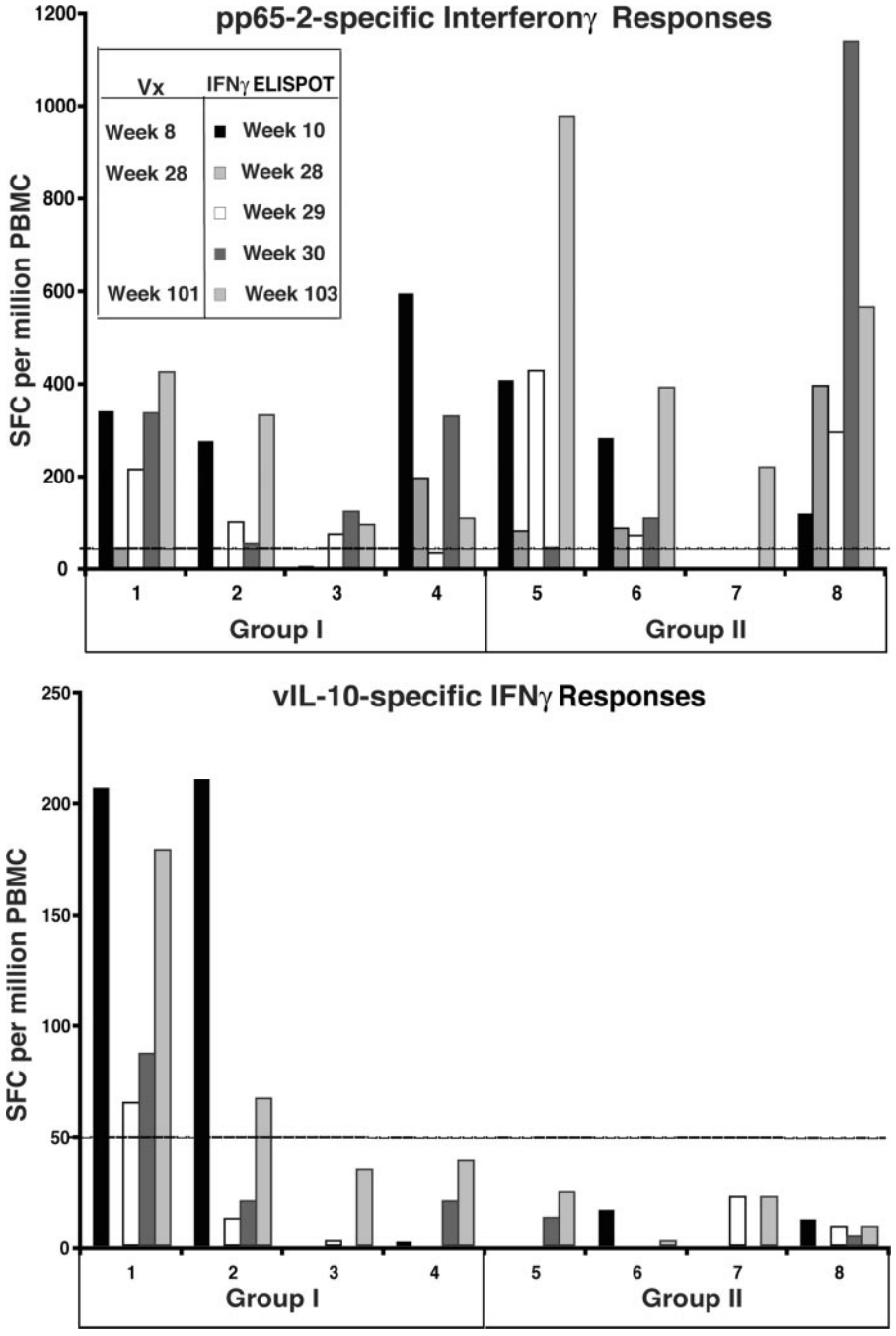

FIG. 3. Cellular responses to genetic immunization. Cellular immune responses were quantified by an IFN-γ ELISPOT to overlapping peptide pools for RhCMVpp65-2 (top) and vIL-10 (bottom) at weeks 10, 28, 29, 30, and 103. The animals were immunized at weeks 0, 4, 8, 28, and 101 with pND/pp65-2 and pND/vIL-10 (group I) or pND/pp65-2 and pND vector (group II).

macaques demonstrated a range of 50% neutralizing titers of 354 to >800 (9; Yue et al., unpublished observations). The absence of robust neutralizing titers in DNA-immunized macaques was similar to findings in DNA-immunized mice (48).

Immunization of group I monkeys with pND/vIL-10 stimulated a weak response in just two animal (Fig. 2C). In contrast, immunization of mice with the same plasmid construct elicited anti-vIL-10 antibody responses in 100% of vaccinated mice (Table 1 and Fig. 2D).

Cellular responses in immunized rhesus macaques. Cellular responses to pp65-2 and vIL-10 were assessed by IFN-γ ELISPOT using a pool of overlapping peptides for each protein as the antigen (Fig. 3). Three of the four monkeys in each immunization group had detectable pp65-2 responses (>50 SFC per 10^6 PBMC) by week 10, 2 weeks after the third immunization (week 8). Seven of eight monkeys had positive responses 1 to 2 weeks after the fourth immunization at week 28. The same monkey in group II (no. 7) that had a delayed

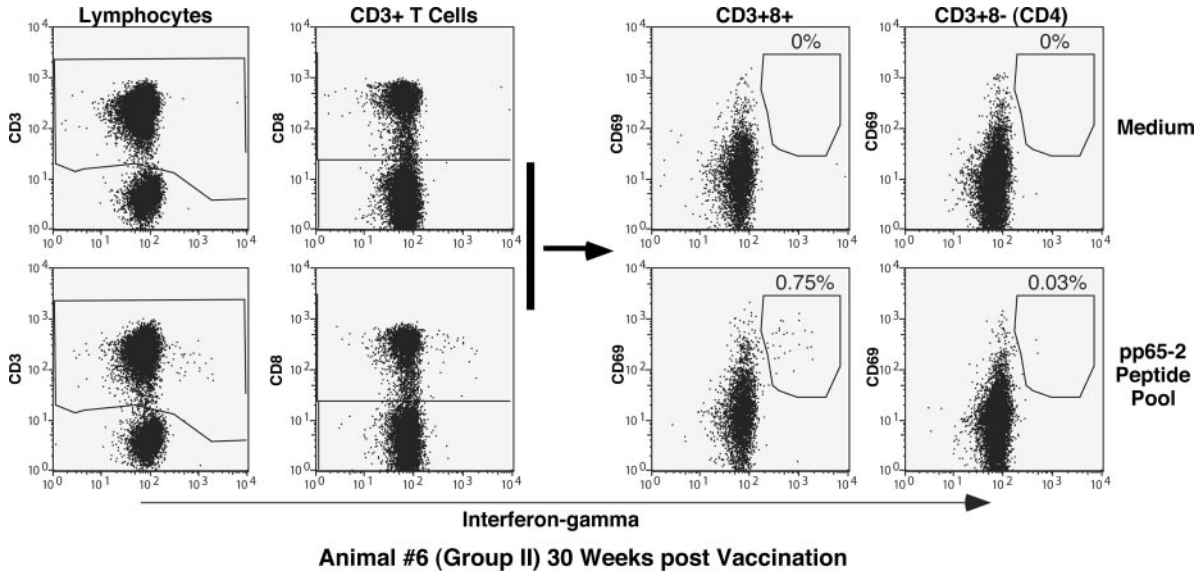

FIG. 4. Induction of RhCMV pp65-2-specific $CD8^+$ T lymphocytes following vaccination. Representative analysis of intracellular cytokine staining assay on pp65-2-stimulated (bottom panel) and unstimulated (top panel) PBMC from one rhesus macaque (no. 6) at week 30 postvaccination. Results of four-color flow cytometry using CD3, CD8, CD69, and IFN-γ are shown. Lymphocytes gated on $CD3^+$ T cells were further gated on $CD8^+$ or $CD4^+$ ($CD8^-$) T lymphocytes. Numbers on the $CD8^+$ and $CD4^+$ T-lymphocyte plots denote the percentage of the respective population coexpressing CD69 and IFN-γ after a 6-h stimulation period. Frequencies greater than 0.08% after subtraction of background obtained with unstimulated cells were considered pp65-2 specific.

antibody response (Fig. 2A) also had a delayed cellular response to pp65-2 peptides; antigen-specific T-cells only were detected at week 103 (Fig. 3). There was a trend for booster immunizations to increase the IFN-γ ELISPOT responses, although postimmunization responses were not always higher than the responses observed with the preceding boost. However, peak pp65-2-specific ELISPOT responses were observed at week 103 for five monkeys, 2 weeks after the booster at week 101 (Fig. 3). Since the previous pp65-2 immunization was performed at week 28, the responses observed at week 103 were consistent with the presence of long-lived memory cells, similar to that observed for the antibody responses (Fig. 2A). The mean for peak SFC responses for group II monkeys was higher than that observed for group I (684 and 374, respectively). In addition, the mean peak pp65-2-specific peak SFC responses for both groups were higher than those in long-term-infected macaques (mean, 326; range, 0 to 2616) (72). Cellular responses to vIL-10 were weaker in magnitude and detected in only two of the four monkeys comprising group I (Fig. 3). Although vIL-10-specific cells were detected at the time of immunization at week 103, peak responses were observed at week 10 in both animals, and the frequencies (220 and 202, respectively) were comparable to those observed in naturally infected animals (mean, 166; range, <50 to 543) (A. Kaur, unpublished observations). No cellular responses to vIL-10 were observed for group II animals.

In order to ascertain whether the cellular immune responses were mediated by $CD4^+$ or $CD8^+$ T lymphocytes, intracellular cytokine staining was performed with available cryopreserved PBMC for two group I monkeys and one group II monkey (weeks 28, 29, and/or 30). In all instances tested, pp65-2-specific ELISPOT responses were mediated by $CD8^+$ T lymphocytes (Fig. 4 [data from one monkey]).

Plasma viral loads and virus shedding in rhesus macaques postchallenge. Two weeks after the last booster at week 101, the eight immunized monkeys and four RhCMV-seronegative controls were challenged by i.v. inoculation of RhCMV. To evaluate whether DNA immunization conferred protection against infection, the RhCMV genome copy numbers in plasma were quantified by real-time PCR. The kinetics of DNA detection were similar for both vaccine treatment groups and control monkeys (Fig. 5). Viral DNA loads in plasma rapidly increased 5 days after challenge and reached primary peak levels on day 7 in all the immunized animals and most (three of four) control animals. The peak RhCMV copy number in plasma was detected 10 days postinoculation in one of the controls. Whereas the timings of RhCMV DNA detection in the plasma were nearly identical among the treatment groups, the peak magnitude of RhCMV in the plasma was significantly reduced in the vaccinees compared to controls (summarized in Table 3). The ranges of peak viral DNA loads in groups I (pp65-2/vIL-10/gBΔ) and II (pp65-2/gBΔ) were 7.5×10^3 to 4.2×10^5 and 1.4×10^4 to 2.0×10^5 RhCMV genome equivalents per ml of plasma, respectively. In contrast, the range of peak viral loads for the control group was 5.7×10^5 to 3.7×10^6 genome equivalents per ml of plasma. The mean peak RhCMV copy numbers in the two vaccine groups were ~1.5 logs lower than that of the control group. No statistical differences in peak RhCMV copy numbers were noted between the two vaccine groups.

RhCMV DNA became undetectable in the plasma of group I animals on days +14 ($n = 1$), +28 ($n = 1$), and +42 ($n = 2$) and group II animals on days +28 ($n = 2$) and +35 ($n = 2$). For control monkeys, RhCMV DNA became undetectable on days +28 ($n = 2$), +35 ($n = 1$), and +42 ($n = 1$), although one animal was positive one additional time at day +54. Plasma

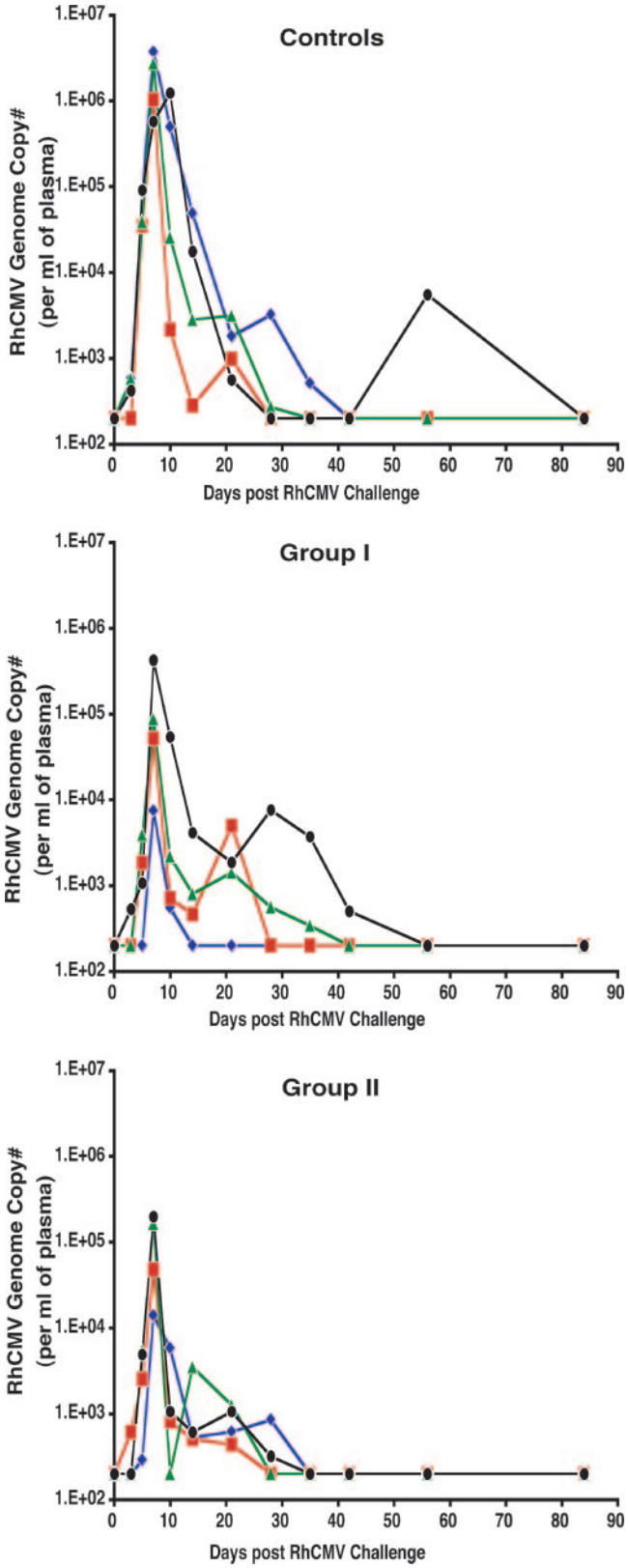

FIG. 5. RhCMV copy numbers in plasma for group I, group II, and the controls. All samples beyond day 84 were negative for RhCMV DNA and are not shown.

TABLE 3. Viral and immune parameters of control and vaccinated monkeys postchallenge

Parameter (*P*)	Mean result	Group	Statistical comparison by[a]:	
			t test	Tukey's HSD test
Peak RhCMV copy no./ml of plasma (0.0055)	6.23	C	A	A
	4.83	II	B	B
	4.79	I	B	B
gB antibody peak titer (<0.0001)	5.01	II	A	A
	4.86	I	A	A
	3.58	C	B	B
gB antibody titer at 24 wk (0.1661)	3.43	II	A	A
	3.43	I	A	A
	3.05	C	A	A
Neutralizing antibody peak titer (0.1086)	3.50	II	A	A
	3.49	I	A	A
	3.10	C	A	A
Neutralizing antibody titer at 24 wk (0.0272)	3.06	C	A	A
	2.70	II	AB	AB
	2.26	I	B	B
RhCMV antibody peak titer (0.0110)	4.86	I	A	A
	4.71	II	A	A
	3.96	C	B	B
RhCMV antibody titer at 24 wk (0.1250)	3.66	C	A	A
	3.43	II	AB	A
	3.13	I	B	A
pp65-2 peak ELISPOT response (0.0013)	3.04	II	A	A
	2.78	I	A	A
	2.14	C	B	B
pp65-2 ELISPOT response at 24 wk (0.0310)	2.33	II	A	A
	2.19	I	A	AB
	1.18	C	B	B
RhCMV peak ELISPOT response (0.3036)	2.58	II	A	A
	2.48	I	A	A
	2.19	C	A	A
RhCMV ELISPOT response at 24 wk (0.1137)	2.23	I	A	A
	1.36	C	A	A
	1.11	II	A	A

[a] *P* values are from one-way ANOVA on log-transformed data. Mean values with no common letters differ significantly at the $\alpha = 0.05$ level.

samples for all monkeys were negative for RhCMV after day +54.

HCMV virus shedding in oral and genital secretions is an important route of transmission for HCMV infection. RhCMV DNA is also frequently found in oral and genital swab samples in healthy rhesus monkeys (32). To evaluate whether the DNA vaccines restricted virus shedding, oral and genital swab samples were collected from the day of virus inoculation up to 144 days following inoculation and analyzed for the presence of RhCMV DNA. Most animals became DNA positive in oral and/or genital swabs by 7 days post-RhCMV challenge, and all were positive by 10 days (Fig. 6). This time frame was contemporaneous with peak plasma loads (Fig. 5). The detection of RhCMV at mucosal surfaces confirmed previous reports de-

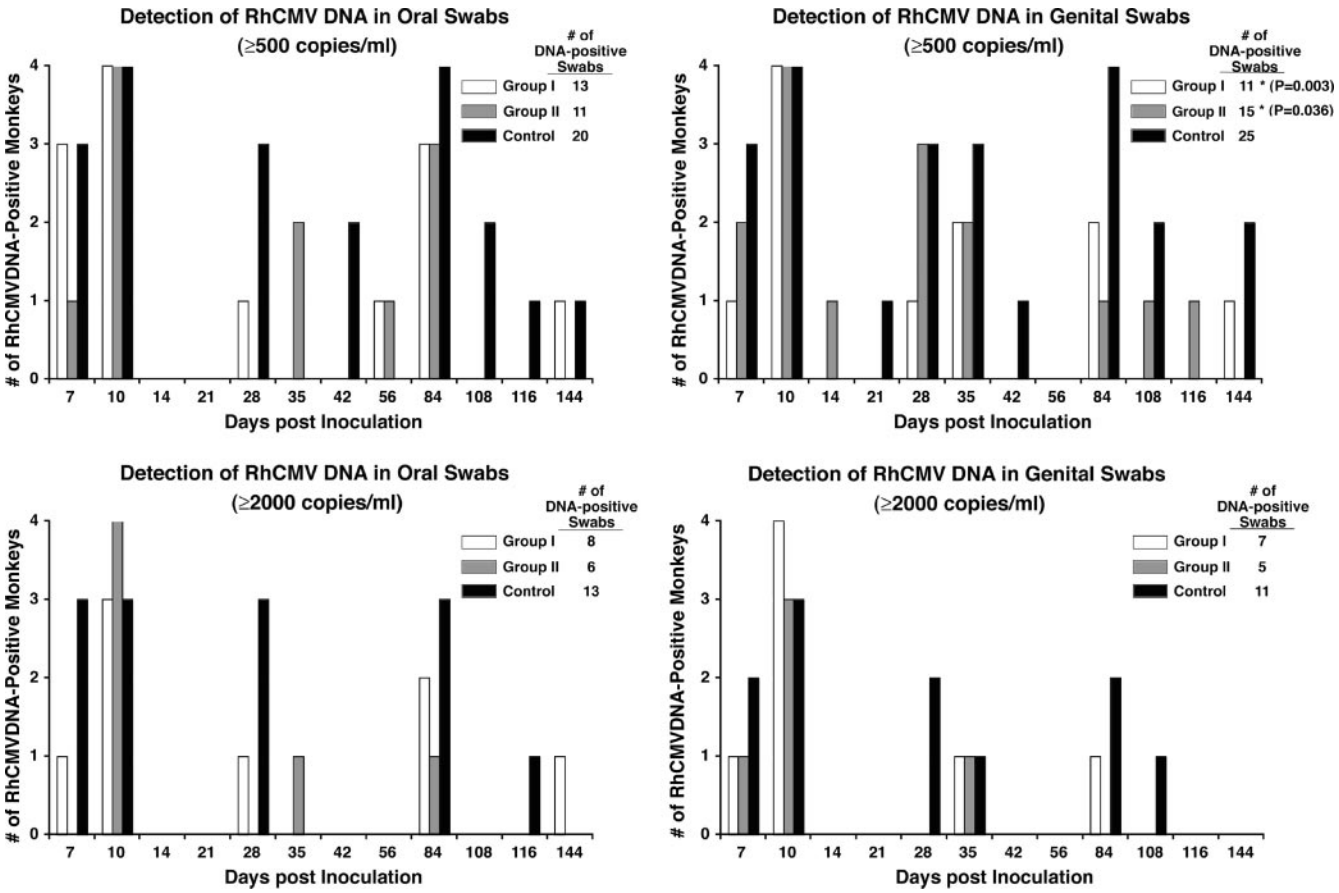

FIG. 6. Detection of RhCMV DNA in oral and genital swabs using a cutoff threshold of either 500 or 2,000 copies/ml. Values that were statistically different (two-tailed chi-square test) from the controls are indicated by an asterisk.

scribing the rapid viral dissemination to distal locations following intravenous inoculation (37). Following the burst of RhCMV shedding at both mucosal sites by 10 days, RhCMV DNA was sporadically detected thereafter. To assess whether the frequencies of shedding were different between the treatment groups, the following was done. The number of DNA-positive samples was summed for each treatment group over the 24 weeks of observation using two thresholds for whether a sample was considered positive (500 and 2,000 copies/ml of mucosal fluid) (Fig. 6). These two thresholds were chosen because the limit of RhCMV genome detection was consistently between 1 and 10 copies per 5 μl of plasmid standard (200 to 2,000 copies/ml) in every real-time PCR assay (data not shown). The data using either threshold indicated that both vaccination treatments resulted in a reduction in the frequency of RhCMV detection at both the oral and genital mucosa. Differences between controls and either treatment group either achieved or approached statistical significance using the lower threshold of 500 copies/ml. None of the reductions in the frequency of RhCMV DNA detection in the two vaccine groups was significantly lower than the frequency of DNA detection in the control group using the detection threshold of 2,000 copies per ml.

Humoral immune responses post RhCMV challenge. To determine whether decreased viral loads observed in the immunized monkeys were associated with specific immunological parameters, humoral and cellular immune responses were prospectively evaluated in the vaccinees and controls. RhCMV challenge stimulated immediate and profound increases in gB-specific, neutralizing, and RhCMV-binding antibody titers by 2 weeks postinfection (Fig. 7). gB-specific titers increased 32- to 256-fold in the first 2 weeks to peak titers that ranged from 51,200 to 204,800 for both vaccine groups. gB titers exhibited a constant decline over the next 13 to 22 weeks. The rapid rise and subsequent decline in gB titers stood in contrast to the gB responses in the control monkeys. In the latter group, gB titers were first detected 4 to 5 weeks post-RhCMV inoculation, and the titers continued to rise to a relatively stable plateau titer between 6 and 15 weeks postchallenge. The gB response in the experimentally inoculated control animals recapitulated the responses observed in naturally exposed monkeys (73). The mean peak gB titer observed in the control group was significantly less than the means of both vaccine treatment groups (Table 3). The end-point gB titers at 24 weeks postinoculation ranged from 800 to 1,600 (group I), 800 to 6,400 (group II), and 1,600 to 6,400 (control). Although gB titers remained slightly higher at 24 weeks postchallenge in both of the vaccine groups than in the controls, the differences were not statistically significant.

The neutralizing and RhCMV-binding antibody titers were

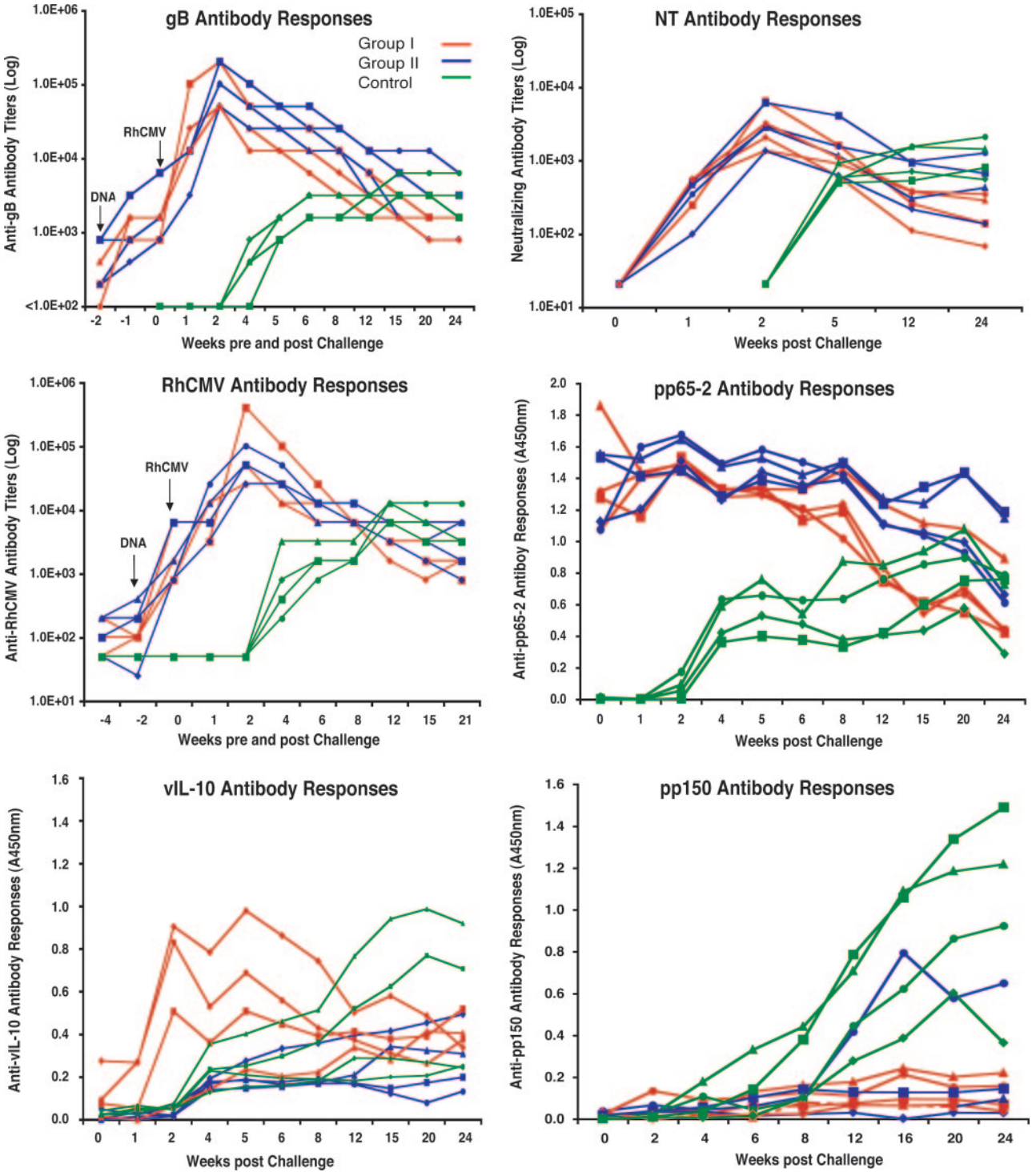

FIG. 7. Antibody responses post-RhCMV challenge. Antibody responses (gB, neutralizing, RhCMV binding, pp65-2, vIL-10, and pp150) are presented relative to the number of weeks postchallenge (week 0). End-point titers were determined for gB, neutralizing, and RhCMV-binding antibodies. Antibody responses to pp65-2, vIL-10, and pp150 were measured spectrophotometrically at 450 nm.

similar to the gB responses both in terms of the magnitude and kinetics of the increase, as well as in the steady decline in titer after 2 weeks postinoculation (Fig. 7). The increases in neutralizing antibody titers in the vaccinees were particularly striking since the 50% neutralizing titers at the time of inoculation were low. The peak neutralizing titers, observed at 2 weeks postinoculation, ranged from 2,263 to 7,335 for group I and 1,463 to 3,570 for group II. The mean peak neutralizing titers for the control monkeys (range, 750 to 2,313), observed either 12 or 24 weeks after inoculation with RhCMV, were lower than

TABLE 4. Long-term pp65-2 cellular responses postchallenge

Vaccinated group and monkey	Wk post-RhCMV challenge	% pp65-2-specific T lymphocytes[a]	
		$CD8^+$	$CD4^+$
I			
1	16	0.05	0.02
	24	0.13	0.14
2	16	0.13	0.02
	24	0.05	0
3	16	0.05	0.04
	24	0.15	0.04
4	16	0.01	0.02
	24	0.04	0
II			
5	16	0.53	0
	24	0.30	0.03
6	16	0.45	0.02
	24	0.34	0.07
7	16	0.06	0
	24	0.18	0.05
8	16	0.01	0
	24	0.07	0.01
Control			
9	16	0	0
	24	0.03	0.01
10	16	0	0
	20	0	0.01
11	16	0	0
	20	0	0
12	16	0.08	0.01
	24	0	0

[a] Frequency of $CD8^+$ and $CD4^+$ T lymphocytes responding to pp65-2 stimulation. Values are shown after subtraction of background with unstimulated PBMC. Values of >0.08% were considered positive.

those of both vaccine groups, although the difference was not statistically significant (Table 3). In contrast, the mean neutralizing titer of the control group was higher than those of vaccine groups I and II at 24 weeks postchallenge. Only the mean titer for group I was significantly less than that of the controls. Total RhCMV-binding antibody responses were similar to the gB and neutralizing antibody responses (Fig. 7). The mean peak titer for the control monkeys was significantly less than the mean titers for both vaccine groups. The mean titer of group I at 24 weeks was significantly less than that in the controls only using the *t* test (based on least significant differences) but not with the more stringent Tukey's HSD method (Table 3).

Challenge infection with RhCMV did not appear to stimulate a large increase in pp65-2 antibody responses similar to that observed for gB (Fig. 7). Although end-point titers were not determined, the increases in absorbency were inconsistent with the 32 - to 256-fold increases observed for gB-specific antibody responses. Like gB, however, antibody responses to pp65-2 declined from the peak responses observed at 2 weeks postchallenge during the subsequent 22 weeks. The levels of pp65-2 antibody responses for group I animals at 24 weeks post-RhCMV challenge were generally lower than those observed for either group II or control monkeys.

RhCMV challenge stimulated rapid increases in vIL-10-specific antibody responses 2 weeks postchallenge in three animals of the group I cohort, and a marginally positive increase in a third animal of the same group (Fig. 7). Development of vIL-10 antibodies in group II vaccinees was contemporaneous with the responses in the control monkeys. All 12 animals had detectable vIL-10 antibodies at 24 weeks postchallenge, although the level of the responses in both vaccine groups tended to be at the lower range of responses observed in the control animals.

The preceding data (Fig. 7) were consistent with the interpretation that RhCMV challenge stimulated anamnestic antibody responses to gB, pp65-2, and vIL-10 in vaccinated animals. To determine how immunization and challenge affected the development of de novo antibody responses to a nonvaccine RhCMV antigen, vaccinated and control monkeys were evaluated for antibodies to RhCMV phosphoprotein 150 (pp150, UL32). pp150 is a strong immunogen in macaques naturally exposed to RhCMV (Yue et al., unpublished data). Whereas all four control animals developed moderate to strong pp150 antibody responses, only two monkeys in each vaccine group developed detectable responses (Fig. 7). Of these four pp150-positive animals in both vaccine treatment groups, three had pp150 antibody levels substantially lower than those of the controls or the fourth vaccinee. Similar results were also observed for another nonvaccine viral antigen, phosphoprotein 28 (pp28; UL99) (data not shown). Given the minimal or absent responses to the challenge-specific pp150 and pp28 proteins, the pattern of the RhCMV-binding antibody titers in the vaccinees to a total RhCMV antigen preparation (derived from infected cell extracts) indicated that the increase in titers was primarily the result of increased titers to RhCMV gB and, possibly, pp65.

Cellular immune responses post-RhCMV challenge. Challenge infection with RhCMV stimulated demonstrable increases in the frequency of pp65-2-specific T cells similar to the increases observed for pp65-2 antibody responses. All four of the vaccinees in group I and two of the animals in group II had two- to sevenfold increases in the frequency of IFN-γ-secreting T cells following stimulation with an overlapping peptide pool of pp65-2 either 2 or 6 weeks post-RhCMV challenge (Fig. 8). The peak responses were distributed equally between these two time points. The other two monkeys in group II exhibited nominal increases in the frequency of pp65-2-specific T cells postchallenge. In all cases, the frequency of IFN-γ-secreting cells declined from the peak values, although seven of the eight vaccinees comprising both groups remained positive after 24 weeks. The four control animals also developed low frequencies of pp65-2-specific T cells during the acute course of infection (+2 to +6 weeks), although the mean peak response of the control group was significantly less than those of both vaccine groups I and II (Table 3). Only one of the controls had a detectable response to pp65-2 at 24 weeks post-RhCMV. ICS analysis of PBMC from weeks +16 and +24 revealed that cellular responses to the pp65-2 peptide pool were exclusively derived from $CD8^+$ T cells in all but one instance (Table 4 and Fig. 9). $CD4^+$ responses were detected in just one animal (no. 1) at +24 weeks postchallenge. No pp65-2-specific $CD4^+$ or $CD8^+$ responses were detected in the control monkeys at +16 or 24 weeks post-RhCMV by the ICS assay.

Cellular responses to a sonicated extract of RhCMV-in-

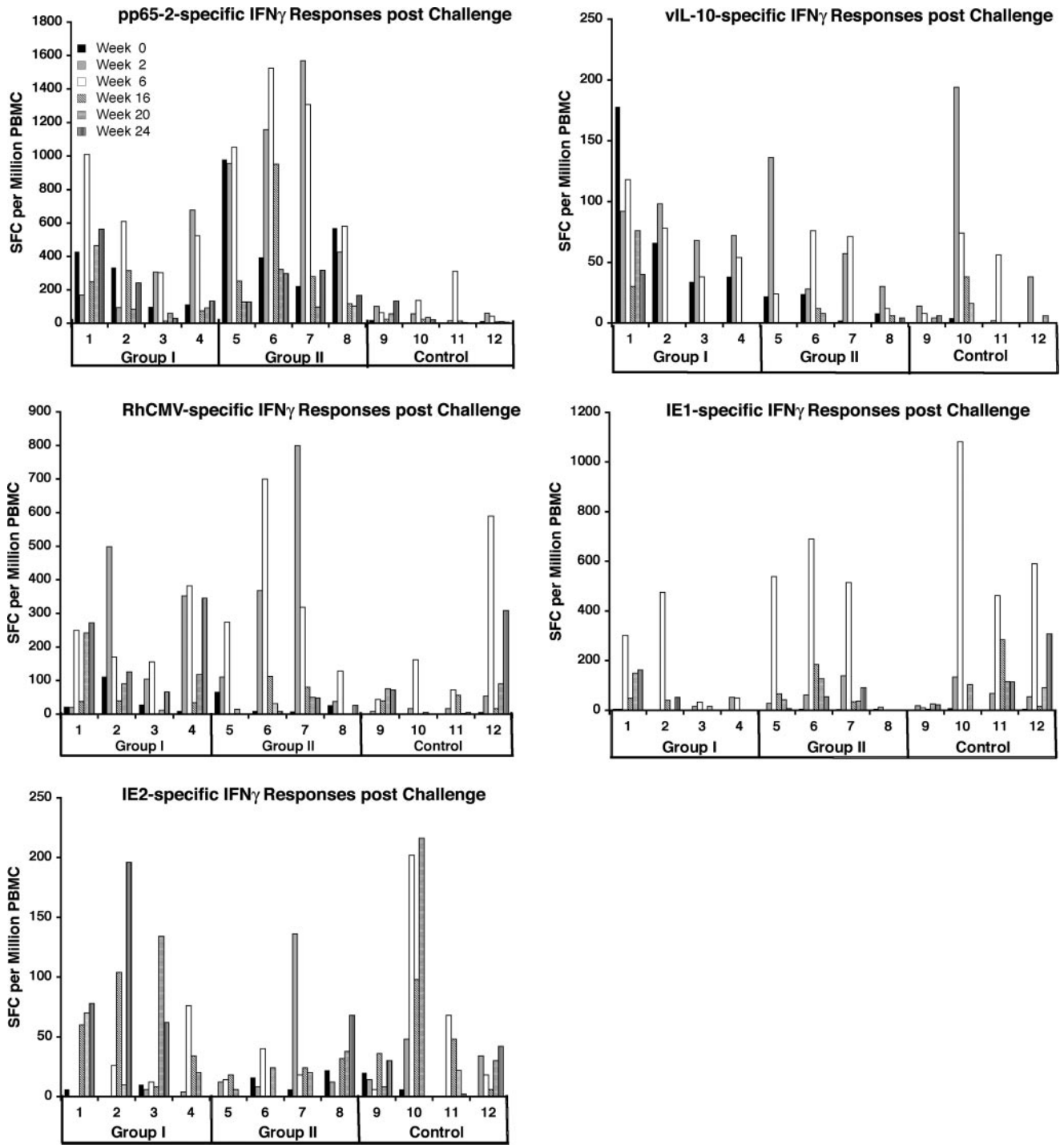

FIG. 8. Cellular responses to RhCMV post-RhCMV challenge. Cellular responses (IFN-γ) to pp65-2, vIl-10, RhCMV antigen, and immediate-early 1 and 2 regulatory proteins (IE1 and IE2, respectively) were quantified by ELISPOT for each treatment group relative to RhCMV challenge at week 0.

fected cells were observed within 2 weeks of RhCMV challenge for six of the eight vaccinees, versus one of four controls (Fig. 8). Since T-cell responses to pp65-2 were essentially $CD8^+$ mediated (Fig. 9), the presence of the anamnestic responses observed with sonicated extracts implies a memory response to another immunogen, most likely gB.

The effects of RhCMV infection on vIL-10 responses were less apparent. Although three of the four vIL-10-vaccinated monkeys in group I had increased frequencies of vIL-10-specific T cells 2 weeks postinfection, three of the non-vIL-10-vaccinated monkeys in group II and two of the control monkeys also developed vIL-10 responses by either 2 or 6 weeks

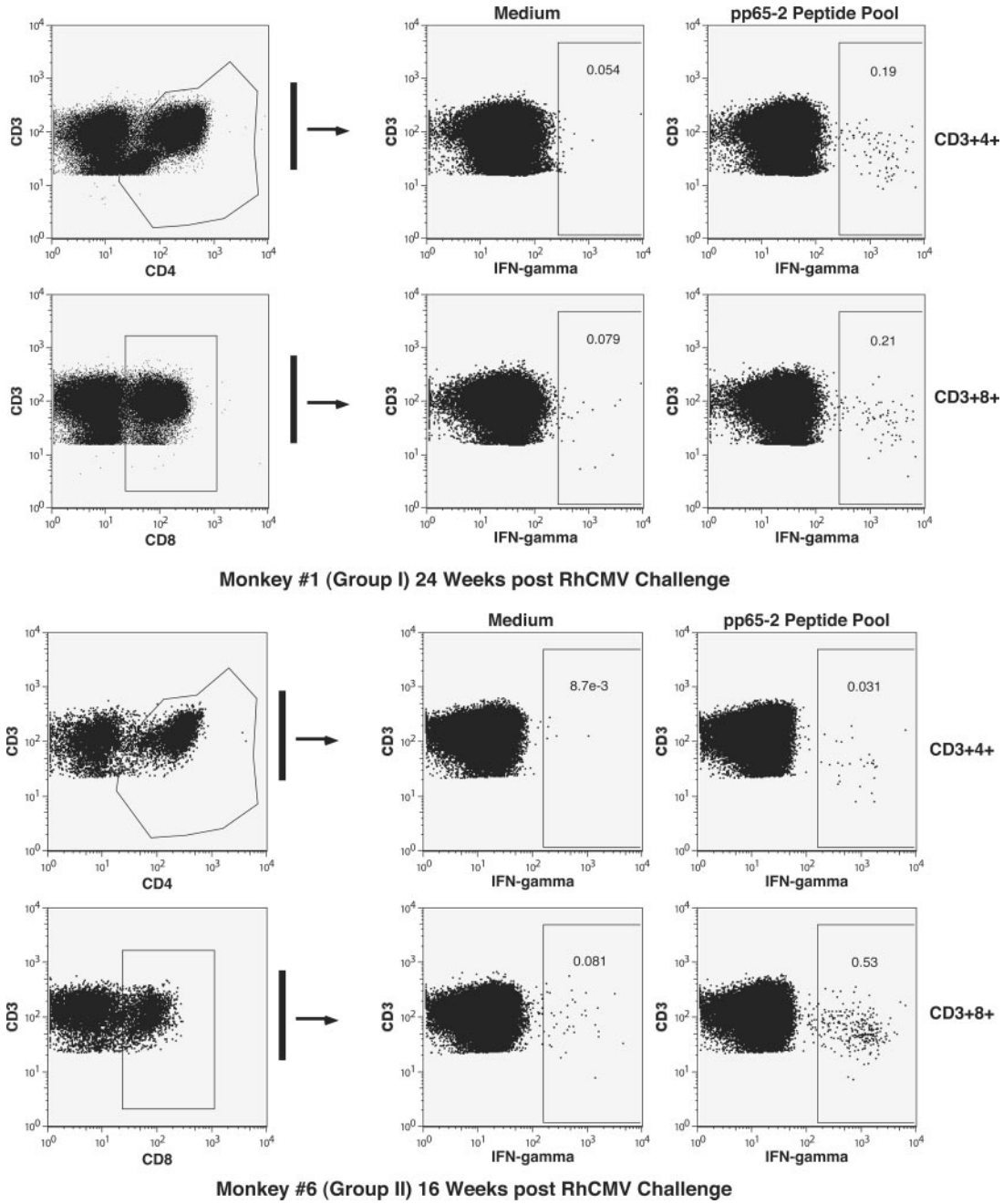

FIG. 9. RhCMV pp65-2-specific $CD4^+$ and $CD8^+$ T lymphocytes following RhCMV challenge. Shown are representative analyses of intracellular cytokine staining assay on pp65-2-stimulated and unstimulated (medium) PBMC from monkeys 1 (group I) and 6 (group II) at 24 and 16 weeks postchallenge, respectively.

postinfection. The cellular responses to vIL-10 were short-lived. Of the nine total monkeys that developed vIL-10 responses by 6 weeks, only one had a single positive response after week +6 (no. 1 at week +20; Fig. 8). The frequencies of vIL-10-specific cells in both vIL-10-vaccinated and unvaccinated monkeys were substantially lower than the pp65-2 frequencies observed in the monkeys of groups I and II.

Unlike the differences in humoral responses to the challenge-specific pp150 and pp28 structural antigens (Fig. 7; data not shown), there were no apparent differences between the treatment groups in the cellular responses to the immediate-early 1 and 2 regulatory proteins (Fig. 8). The majority of animals within each group developed IFN-γ responses during the period of observation with similar kinetics of detection (Fig. 8).

DISCUSSION

Genetic immunization of rhesus macaques stimulated antigen-specific immune responses that altered the virological parameters of infection following intravenous RhCMV chal-

lenge. The peak plasma viral loads (i.e., the infectious burden in plasma) were significantly reduced in both vaccine groups compared to the control group during primary infection. Vaccination also reduced the frequency of viral DNA detection at mucosal surfaces over the course of the study. Detection of RhCMV DNA represents the net difference between the rates of production and clearance of progeny virus. It is not known whether the decrease in plasma viral loads reflects a decrease in RhCMV production, an increase in clearance of virus, or a combination of both. Similarly, it is not known whether the decrease in the frequency of RhCMV DNA detection in mucosal fluids is also a function of a decreased viral colonization of mucosal sites. The pattern of antibody responses to the challenge antigen pp150 in seven of the eight vaccinees (Fig. 7) is consistent with the interpretation that there is minimal production of pp150 to generate and/or sustain a strong de novo antibody response. While this remains to be formally proven, RhCMV pp150 is normally highly immunogenic in experimentally inoculated and naturally infected macaques (Yue et al., unpublished observations). It is a relevant marker of challenge infection since antibody responses to HCMV pp150 have been used to monitor HCMV infection in gB-vaccinated humans (51).

The relative contributions of reduced production and increased clearance are vital issues to resolve for the design of an effective HCMV vaccine because the balance between the two determines the threshold of protective immunity a vaccine is designed to achieve. If the goal for a vaccine is prevention of a primary infection, vaccine-induced immunity has to block infection or absolutely minimize the production of progeny virus following exposure to virus at a mucosal surface. This is an exceedingly high threshold to achieve for a virus with a proven capacity to reinfect a previously immune host (12). Since DNA immunization against RhCMV gBΔTM, pp65-2 ± vIL-10, does not prevent primary infection against a high-titer i.v. challenge, this combination of immunogens delivered by this route does not achieve this particular threshold. Alternatively, a vaccine that does not prevent infection could sufficiently alter viral replication dynamics such that a protective level of protection is still achieved (6). One such scenario would include immunized pregnant women who are exposed to HCMV. A vaccine could be considered protective if there was no congenital infection and/or fetal disease despite demonstrable evidence of primary infection in the mother. Presumably, this would result from both a reduction in virus production and an increase in the clearance of progeny virus to restrict the potential for transplacental transmission. The rhesus macaque model is not yet amenable to evaluating protection from congenital infection (9). However, a vaccine could also be considered protective if it reduced the risk of horizontal transmission to at-risk individuals, a situation the rhesus model is especially suited to address.

Mathematical modeling of the rate of primary infection in humans suggests that a HCMV vaccine does not have to be 100% effective in preventing primary infection to have a measurable benefit for those susceptible to infection. Griffiths et al. prospectively evaluated the extent of primary infection in women during pregnancy and concluded that, "if the efficacy of candidate vaccine preparations was only 80 to 90% in preventing primary infection, CMV could be eradicated by immunization of 66 to 75%" (27). Theoretically, the same end could be achieved if the potential for horizontal transmission could be reduced by 80 to 90%. The observed reductions in the detection of RhCMV DNA in mucosal fluids of the vaccinated animals in this study suggest that DNA immunization alone offers a modest measure of protection. Naturally infected rhesus macaques can persistently shed high copy numbers of RhCMV in mucosal fluids years after primary infection (32), and ongoing studies with macaques are in progress to optimize strategies that reduce mucosal shedding following challenge.

DNA-based vaccines have demonstrated considerable antigenicity in both small animal and nonhuman primate models, and the results of this study add to the list of infectious agents for which the efficacy of DNA vaccination has been evaluated in macaques (30, 31, 40). However, promising results in these systems have not necessarily been recapitulated in human clinical trials, although DNA immunization remains a potential adjunct to other immunization modalities (21, 22). The results of this study highlight that further optimization of this immunization technique is required to achieve a higher threshold of efficacy in primates. Immune responses generated by DNA immunization in mice are poor predictors of the magnitude and duration of the immune responses in macaques, confirming previous studies (41). Whereas two or three immunizations in mice achieve long-term elevations in antibody responses in mice, cellular and humoral responses waned with time following each booster immunization in macaques. However, vigorous anamnestic responses were noted following each booster immunization, even when immunizations were 73 weeks apart, as was the case for pp65-2. Macaques that are infected with RhCMV following either natural exposure to the virus or experimental inoculation maintain persistently high antibody titers to many RhCMV antigens, indicating the persistent production of antigens in immunocompetent animals. One notable deficiency in the immune response stimulated by injection of plasmid expression vectors alone was the absence of robust neutralizing antibody titers, a key marker of protective immune responses to HCMV. This was unlikely the result of the RhCMVgBΔTM immunogen used in this study. The RhCMV gB gene encodes the majority, but not all, of the neutralizing epitopes in RhCMV-infected macaques (73). The truncated version of RhCMV gB (gBΔTM) generated neutralizing antibodies in mice (data not shown).

Other studies in macaques have demonstrated that DNA immunization can produce protective levels of neutralizing antibodies to rabies virus, hantavirus, and monkeypox (immunized against vaccinia virus) (30, 31, 39, 40), which may be explained, in part, by the use of gene gun delivery of DNA vaccines into professional antigen-presenting cells of the skin, allowing direct presentation of antigen to T cells (20, 39, 53). The RhCMV results reflect similar studies in mice in which DNA immunization alone does not stimulate detectable neutralizing antibodies (48). Neutralizing antibody titers in mice were detected only after two immunizations with formalin-inactivated MCMV. The rapid induction of high neutralizing antibody titers within 1 week of RhCMV challenge demonstrates that neutralizing antibodies were effectively primed by RhCMV gBΔTM and significantly boosted by challenge virus. This suggests that DNA immunization should be part of a prime-boost strategy in which DNA immunization is used to

prime the immune system for subsequent boosts with other strategies, such as inactivated virions, heterologous viral expression vectors, and/or immunity-enhancing molecules. Similar strategies have augmented protective immune responses against simian immunodeficiency virus and *Plasmodium falciparum* in macaques (19, 24, 49, 58). In addition, broadening of the vaccine antigen cocktail may enhance the level of protective immunity, as has been demonstrated for MCMV (48).

Inclusion of vIL-10 as an antigen for group I was an attempt to determine whether viral immunomodulatory proteins should be included as possible vaccine candidates. Since no demonstrable differences were noted in viral parameters of infection between groups I and II (gBΔ/pp65-2 ± vIL-10, respectively), the results might suggest at first glance that immunization against vIL-10 conferred no protective benefit. However, further evaluation will be required to determine whether such an immunization strategy is justified. Assessment of antibody and cellular responses following immunization in macaques with the vIL-10 expression plasmid indicated that minimal vIL-10-specific immunity was stimulated in only two animals of group I. In contrast, antibody responses were readily generated in mice. RhCMV challenge resulted in apparent recall responses in all four monkeys in group I, suggesting that a DNA prime/protein boost may be need to induce high levels of vIL-10-specific responses.

In summary, we have demonstrated that DNA vaccines containing virus encoding RhCMV pp65-2, vIL10, and gBΔTM antigens are immunogenic in rhesus macaques. Administration of these vaccines resulted in the generation of antigen-specific antibodies and cellular responses that altered the pattern of RhCMV infection. These results highlight the utility of the RhCMV model in assessing HCMV vaccine strategies and warrant further studies to improve the efficacy of DNA vaccines against HCMV.

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