Enhanced CD8+ T Cell Immune Responses and Protection Elicited against *Plasmodium berghei* Malaria by Prime Boost Immunization Regimens Using a Novel Attenuated Fowlpox Virus

Richard J. Anderson, Carolyn M. Hannan, Sarah C. Gilbert, Stephen M. Laidlaw, Eric G. Sheu, Simone Korten, Robert Sinden, Geoffrey A. Butcher, Michael A. Skinner and Adrian V. S. Hill

*J Immunol* 2004; 172:3094-3100; doi: 10.4049/jimmunol.172.5.3094

http://www.jimmunol.org/content/172/5/3094

---

**Why The JI?**

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average

---

**References** This article cites 43 articles, 15 of which you can access for free at: http://www.jimmunol.org/content/172/5/3094.full#ref-list-1

**Subscription** Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions** Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts

---

*The Journal of Immunology* is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852 Copyright © 2004 by The American Association of Immunologists All rights reserved. Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Enhanced CD8+ T Cell Immune Responses and Protection Elicited against Plasmodium berghei Malaria by Prime Boost Immunization Regimens Using a Novel Attenuated Fowlpox Virus

Richard J. Anderson,2* Carolyn M. Hannan,† Sarah C. Gilbert,* Stephen M. Laidlaw,‡ Eric G. Sheu,† Simone Korten,† Robert Sinden,§ Geoffrey A. Butcher,§ Michael A. Skinner,‡ and Adrian V. S. Hill*†

Sterile immunity can be provided against the pre-erythrocytic stages of malaria by IFN-γ-secreting CD8+ T cells that recognize parasite-infected hepatocytes. In this study, we have investigated the use of attenuated fowlpox virus (FPV) strains as recombinant vaccine vectors for eliciting CD8+ T cells against Plasmodium berghei. The gene encoding the P. berghei circumsporozoite (PbCS) protein was inserted into an FPV vaccine strain licensed for use in chickens, Webster’s FPV, and the novel FPV vaccine strain FP9 by homologous recombination. The novel FP9 strain proved more potent as a vaccine for eliciting CD8+ T cell responses against the PbCS Ag. Sequential immunization with rFP9 and recombinant modified vaccinia virus Ankara (MVA) encoding the PbCS protein, administered by clinically acceptable routes, elicited potent CD8+ T cell responses against the PbCS protein. This immunization regimen elicited substantial protection against a stringent liver-stage challenge with P. berghei and was more immunogenic and protective than DNA/MVA prime/boost immunization. However, further improvement was not achieved by sequential (triple) immunization with a DNA vaccine, FP9, and MVA.


1 Address correspondence and reprint requests to Dr. Richard J. Anderson at the current address: Oxxon Pharmaccines Ltd., 2nd Floor, Flory House, Robert Robinson Avenue, Oxford Science Park, Oxford, OX4 4JP, U.K. E-mail address: randerson@oxxonpharmaccines.com

2 Abbreviations used in this paper: ADV, adenovirus; VV, vaccinia virus; MVA, modified VV Ankara; FPV, fowlpox virus; PbCS, Plasmodium berghei circumsporozoite; CEF, chick embryo fibroblast; FPW, Webster’s FPV strain M; i.d., intradermal; SFC, spot-forming cell.
prime/boost immunization regimens in primates and is a promising candidate vaccine in clinical trials against HAG.

In this study, we sought to determine whether FPV could elicit CD8\(^+\) T cell responses against the \textit{P. berghei} circumsporozoite (PbCS) protein and protective immunity against \textit{P. berghei} sporozoite challenge. Toward this goal, we inserted the gene encoding the PbCS protein into a commercially available FPV vaccine strain licensed for use in chickens and into a novel attenuated strain, FPV vaccine strain FP9. The full genomic DNA sequence of FP9 has recently been obtained, making this strain attractive as a fully defined Ag delivery system (44). The immune response elicited by both recombinant vaccines was compared and the protective efficacy elicited by rFP9 (FP9PbCSP) administered in prime/boost immunization regimens was evaluated.

Materials and Methods

FPV vectors

The derivation of FP9 by plaque purification of an attenuated FPV, obtained from wild-type strain HP-1 by 438 passages in chick embryo fibroblasts (CEF) (31), has previously been described (32). Webster’s FPV strain M (FPW) was obtained from Salsbury Laboratories (Charles City, IA) (now Solvay Animal Health). FPV recombinants harboring the gene encoding the PbCS protein (csp) were constructed following established methods (33). Initially, the csp gene was ligated into the Smul cloning site of the fowlpox shuttle vector pFL29 (33), placing expression of this gene under the control of the VV P7.5 promoter. Recombinant viruses were prepared by in vitro recombination of the shuttle vector encoding csp with the FPV strains in primary cultures of CEF. For empty control viruses, FPV vaccine strains were recombined with pFL29 without an inserted Ag. Recombinant viruses were repeatedly plaque purified in CEF monolayers until homogenous.

Recombinant vaccines and immunizations

The pSG2PbCSP DNA vaccine consists of the pSG plasmid backbone (18) harboring a copy of the gene encoding the PbCS protein. Expression of the PbCS protein by this vector is driven in host eukaryotic cells by the CMV IE promoter/intron A and the bovine poly(A) transcription termination sequences. MVAPbCSP encoding the PbCS protein has been described previously (13). Viruses (2 \times 10\(^7\) PFU/ml) and DNA (1 mg/ml) were suspended in endotoxin-free PBS (Sigma-Aldrich, Gillingham, Dorset, U.K.) for immunization. Groups of three or four female BALB/c mice (6–8 wk old) were used in all experiments. Before i.m. or intradermal (i.d.) injection, animals were anesthetized with a 1:1:2 solution of Hypnorm (Janssen-Cilag, Saunderton, High Wycombe, U.K.), Hypnovel (midazolam; Roche, Welwyn Garden City, Hertfordshire, U.K.), and endotoxin-free water (Sigma-Aldrich). For DNA immunization, 50 \mu g of pSG2PbCSP was administered i.m. bilaterally into each musculus tibialis. Viruses were administered i.d. at a dose of 1 \times 10\(^7\) PFU bilaterally into the ears or i.v. into the lateral tail vein.

Ex vivo ELISPOT assays

Ex vivo IFN-\gamma assays were conducted as previously described (10, 13). Peptide-pulsed target cells were prepared by pulsing naive splenocytes with the H-2K\(^d\)-restricted test peptide Pb9 (SYIPSAEKI) or an irrelevant peptide-pulsed target cells were prepared by pulsing naive splenocytes with the H-2K\(^d\)-restricted test peptide Pb9 (SYIPSAEKI) or an irrelevant peptide-pulsed target cells were prepared by pulsing naive splenocytes with the H-2K\(^d\)-restricted test peptide Pb9 (SYIPSAEKI) or an irrelevant peptide-pulsed target cells were prepared by pulsing naive splenocytes with the H-2K\(^d\)-restricted test peptide Pb9 (SYIPSAEKI) or an irrelevant peptide-pulsed target cells were prepared by pulsing naive splenocytes with the H-2K\(^d\)-restricted test peptide Pb9 (SYIPSAEKI) or an irrelevant peptide-pulsed target cells were prepared by pulsing naive splenocytes with the H-2K\(^d\)-restricted test peptide Pb9 (SYIPSAEKI) or an irrelevant peptide. Target cells were washed three times with PBS and applied to test or control wells (5 \times 10\(^5\) cells/well) as appropriate. Spots were counted using an ELISPOT counter (Autonimmun Diagnostika, Strassberg, Germany).

Isolation of blood and liver lymphocytes

Mice were bled from the lateral tail vein directly into an Eppendorf tube containing 200 \mu l of PBS with 10 mM EDTA and 2.5 U/ml heparin. Red cells were lysed by the addition of ACK lysis buffer, and lymphocytes were obtained by centrifugation. Livers were obtained by centrifugation. Mice were bled from the lateral tail vein directly into an Eppendorf tube containing 200 \mu l of PBS with 10 mM EDTA and 2.5 U/ml heparin. Red cells were lysed by the addition of ACK lysis buffer, and lymphocytes were obtained by centrifugation. Livers were obtained by centrifugation. Mice were bled from the lateral tail vein directly into an Eppendorf tube containing 200 \mu l of PBS with 10 mM EDTA and 2.5 U/ml heparin. Red cells were lysed by the addition of ACK lysis buffer, and lymphocytes were obtained by centrifugation. Livers were obtained by centrifugation. Mice were bled from the lateral tail vein directly into an Eppendorf tube containing 200 \mu l of PBS with 10 mM EDTA and 2.5 U/ml heparin. Red cells were lysed by the addition of ACK lysis buffer, and lymphocytes were obtained by centrifugation. Livers were obtained by centrifugation.

H-2K\(^d\) Pb9 tetramer staining and FACS analysis

The K\(_p\)-Pb9 tetramer was constructed and purified as described previously (34). For tetramer staining, isolated splenocytes, blood lymphocytes, or liver lymphocytes were washed in FACS buffer (PBS, 2% FCS, 2 mM EDTA), and 1 \times 10\(^6\) cells were incubated with B220-FITC (Caltag-Medsystems, Tucson, U.K.), CD8\(^\text{R}6\)-Tricolor (Caltag-Medsystems), and K\(_p\) Pb9 tetramer for 20–30 min at 37°C. After incubation, the cells were washed two to three times in FACS buffer, fixed in PBS/2% paraformaldehyde, and analyzed on a FACS Calibur (BD Biosciences, Oxford, U.K.) using CellQuest software.

\textit{P. berghei} challenge

Challenge was conducted with \textit{P. berghei} (ANKA strain; clone 234) sporozoites as previously described (13, 14). In brief, 10 mice per group of mice were challenged by i.v. injection in the tail vein with sporozoites dissected from the salivary glands of infected female \textit{Anopheles stephensi} mosquitoes and homogenized in RPMI 1640 medium (Sigma-Aldrich). Infection was determined by the presence of ring forms in Giemsa-stained blood smears taken 7–14 days after challenge, and animals were sacrificed on first confirmation of parasitemia. Extensive titration of the \textit{P. berghei} challenge strain revealed that 500 sporozoites were sufficient to establish 100% infection of groups of five BALB/c mice (data not shown). Thus, animals were challenged with 2000 sporozoites to provide a stringent liver-stage challenge.

Statistical analysis

Statistical analysis was performed using SPSS for Windows, version 10 (SPSS, Chicago, IL). Unpaired Student’s \(t\) tests were performed to detect significant differences in the mean frequencies of CD8\(^+\) T cells between groups. Differences between groups in the sporozoite challenge assay were determined using a \(\chi^2\) test. A value of \(p \leq 0.05\) was considered significant in all cases.

Results

Comparison of rFPV vaccine strains

rFPV vaccine strains FP9 and FPW were compared for their capacity to elicit CD8\(^+\) T cell responses against the PbCS protein. The PbCS protein contains an H-2K\(^d\)-restricted 9-aa peptide epitope (Pb9), which can induce a protective CD8\(^+\) T cell response against liver-stage \textit{P. berghei} infection (4, 13). Immunization with a single dose of FP9 harboring the gene encoding the PbCS protein (FP9PbCSP) elicited a significantly (\(p < 0.006\)) higher frequency of IFN-\(\gamma\)-secreting cells in murine splenocytes against the Pb9
cross-reactive antiviral T cells against MVAPbCSP and vice versa. Importantly, immunization with empty virus vectors did not elicit IFN-γ-secreting T cell responses against P9, indicating that the viruses induced a specific response against the recombinant Ag.

Comparison of FP9PbCSP with MVAPbCSP

Direct comparison of FP9PbCSP with other recombinant vaccines given by clinically acceptable routes showed that the frequency of IFN-γ CD8+ T cells elicited by FP9PbCSP was significantly (p = 0.012) higher than that elicited by the DNA-vaccine encoding the PbCS protein (pSG2PbCSP), although significantly (p = 0.006) lower than that elicited by rMVA encoding the same Ag (MVAPbCSP) (Fig. 2A). Interestingly, splenocytes from mice immunized with FP9PbCSP did not produce IFN-γ when exposed to naive cells infected with MVA, but did when exposed to cells infected with FP9 (Fig. 2B). Conversely, MVAPbCSP immune splenocytes recognize naive cells infected with MVA, but not FP9 (Fig. 2B). These results indicated that FP9PbCSP elicited IFN-γ-secreting CD8+ T cell responses against the PbCS protein without eliciting cross-reactive antiviral T cells against MVAPbCSP and vice versa.

Immunogenicity of prime/boost immunization regimens using FP9PbCSP

Administration of FP9PbCSP either as a priming or boosting agent in immunization regimens with MVAPbCSP was found to elicit significantly higher frequencies of P9-specific (p < 0.006) and IFN-γ-secreting (p < 0.002) CD8+ T cells than homologous prime/boost immunization regimens using the same viruses (Fig. 3). Moreover, this heterologous prime/boost immunization regimen also proved to be significantly more potent than immunization with DNA/MVAPbCSP in eliciting total Ag-specific CD8+ T cells (p < 0.03) and those that secrete IFN-γ (p < 0.006). Single-immunization experiments indicated that FP9PbCSP alone was less immunogenic than MVAPbCSP (Fig. 2), and the same trend was apparent when the viruses were used to boost the response primed by the DNA vaccine. FP9PbCSP boosted DNA vaccination to induce lower frequencies of total Ag-specific and IFN-γ-secreting CD8+ T cells than those induced by boosting DNA with MVAPbCSP, although the difference between these groups was not significant. Importantly, FP9PbCSP elicited significantly higher frequencies of Ag-specific IFN-γ-secreting CD8+ T cells than FPWPbCSP when used as a priming (p = 0.022) or boosting (p = 0.022) agent in combination with MVAPbCSP (Fig. 4), thus confirming the observation that the FP9 construct was more potent in eliciting CD8+ T cell responses against the PbCS protein than the FPW construct.

T cell response in spleen, blood, and liver

The distribution of T cells following prime/boost immunization was characterized by FACS analysis using a P9 H-2Kd tetramer (Fig. 5). Ag-specific CD8+ T cells were detected in blood and liver and spleen of animals following heterologous prime-boost immunization with all combinations of DNA, FP9PbCSP, and MVAPbCSP tested. Although the overall frequency of Ag-specific CD8+ T cells varied between compartments, the frequency of CD8+ T cells in the spleens and livers of immunized animals were generally quite similar and about half of those detected in the blood.

Protection against P. berghei challenge

To determine whether the enhanced CD8+ T cell responses observed in immunized animals were indicative of a protective immune response against liver-stage malaria, i.d. immunized mice were challenged with P. berghei sporozoites (Table I). The dose of P. berghei sporozoites administered in the challenge was determined in titration studies to be four times higher than that required to establish complete infection of groups of five BALB/c mice (data not shown), and thus provided a stringent P. berghei liver-stage challenge. Heterologous prime/boost immunization with the two poxviruses consistently elicited a significantly higher (p < 0.0001) level of protection in this stringent challenge model when compared with other immunization regimens. In contrast, priming with the DNA vaccines and subsequent boosting with the poxviruses elicited comparatively poor protection in this challenge model, which was not significantly higher than that elicited by homologous immunization with either of the viruses. Notably, FP9PbCSP induced significantly (p = 0.007) higher protection when used as a priming agent rather than a boosting agent.

Triple combination immunization

In an attempt to increase the protective immune response elicited against the PbCS protein, animals were immunized sequentially with three heterologous vaccines (Fig. 6). Sequential immunization with DNA, FP9PbCSP, and MVAPbCSP induced significantly (p < 0.002) higher CD8+ T cell responses in the spleens of mice when compared with prime/boost immunization with DNA/
FIGURE 3. FP9PbCSP can serve as both a priming and boosting agent in heterologous prime/boost immunization regimens. Mice were primed by i.m. immunization with a DNA vaccine (D) or i.d. with FP9PbCSP (F) or MVAPbCSP (M) and boosted i.d. 14 days later. The CD8\(^+\) T cell response in splenocytes was determined 14 days after the booster immunization. A, The percentage ± SEM of Pb9-specific CD8\(^+\) T cells determined by FACS using an H-2K\(^d\) tetramer. B, IFN-\(\gamma\) SFC per million ± SEM in splenocytes from four mice per group. Results are representative of four experiments in which the viruses were administered i.d. or i.v.

FP9PbCSP or DNA/MVAPbCSP. However, the T cell response elicited by triple sequential immunization with DNA/FP9PbCSP/MVAPbCSP was not significantly \((p = 1.000)\) higher than that elicited by prime/boost immunization with FP9PbCSP and MVAPbCSP. Despite this, animals given a triple sequential immunization were significantly \((p = 0.003,\) two-sided) more susceptible to stringent liver-stage challenge with \(P. berghei\) than those immunized with FP9PbCSP/MVAPbCSP (Table II).

Discussion

In this study, we have shown that rFPV vectors can be used to elicit CD8\(^+\) T cell responses against a malaria pre-erythrocytic Ag. Interestingly, we found that the FP9 strain was more effective in eliciting a response against the PbCS protein than the commercially available FPW vaccine strain. rFPV vaccine strains have been used to elicit immune responses against HIV (28, 30) and tumor Ags (24–27). FP9 expressing a model Ag has been used to infect dendritic cells leading to up-regulation of MHC and costimulatory molecules and recognition of the dendritic cells by a CD8\(^+\) T cell clone in vitro (35). In addition, FP9 encoding HIV epitopes has recently been shown to elicit CD8\(^+\) T cell responses against HIV Ags in mice (29). However, this is the first illustration that rFPV can be used to elicit a protective CD8\(^+\) T cell response in the malaria model, and that FP9 is more immunogenic than a similar attenuated FPV vaccine strain.

Previous studies have shown that attenuated rVVVs are more immunogenic delivery systems than their nonattenuated counterparts (36, 37). In addition, MVA, which was derived by serial passage from a nonattenuated VV, is reported to elicit more potent T cell responses against the PbCS Ag than the rationally attenuated NYVAC strain (13). FP9 was derived in a similar manner by 438 serial passages from a wild-type FPV (HP-1), which has rendered FP9 highly attenuated (31) and possibly introduced genetic deletions/modifications that enhance the immune response elicited by this vector. Comparison of the FP9 genome sequence with the published sequence of a pathogenic FPV reference strain (38) reveals several inserted as well as deleted sequences (data not shown). Such deletions/insertions may account for the enhanced capacity of FP9 to elicit CD8\(^+\) T cell responses over the FPW strain, and additional experiments to determine the genetic basis of these differences are presently underway. In addition, sequence analysis has shown that FP9 does not harbor an infectious copy of the avian reticuloendotheliosis provirus that has been found in several other attenuated FPV strains (39). Moreover, high yields of rFPV viruses have been obtained in initial clinical batch, good manufacturing practice manufacturing procedures (our unpublished observation). Thus, FP9 shows great promise as a candidate vector for human immunization.

FIGURE 5. Ag-specific CD8\(^+\) T cells detected in the spleen, liver, and blood of immunized mice. Pb9-specific CD8\(^+\) T cells were detected in isolated spleen, liver, and blood cells from mice by FACS analysis using a Pb9-H-2K\(^d\) tetramer. The mice were immunized and boosted as described in Fig. 3, and spleen, liver, and blood cells were isolated 14 days after the boost. Columns represent the percentage of Pb9-specific CD8\(^+\) T cells ± SEM for three animals per group.
Immune response primed by FP9PbCSP could be boosted by 15, 40. Accordingly, in this study, the protection in different strains of mice against cell responses against the circumsporozoite Ag, and complete protective agents in combination is therefore likely be due to their failure to induce cross-reactive CD8\(^+\) T cells against the viral Ags.

Protection against liver stage with \(P.\) \(berghei\) in mice is mediated by IFN-\(\gamma\)-secreting CD8\(^+\) T cells rather than cytotoxic CD8\(^+\) CTLs (2, 41). In addition, it has been suggested that intrahepatic Ag-specific T cells may be required for protection (42, 43). Accordingly, the frequency of Ag-specific IFN-\(\gamma\)-secreting T cells detected ex vivo in the spleens of mice following prime/boost immunization in this study was found to correlate well with protection against sporozoite challenge. Protection also correlated with the total frequency of Ag-specific CD8\(^+\) T cells measured in the blood, spleens, and livers of immunized animals using a Pf9 H-2K\(^d\) tetramer. Interestingly, the frequency of IFN-\(\gamma\)-secreting T cells measured in ELISPOT assays account for only ~10% percent of the total frequency of Ag-specific T cells measured in the spleen by the Pf9 H-2K\(^d\) tetramer (for example, 3000 spot-forming cells (SFC) per million splenocytes corresponds to ~1.5% Ag-specific CD8\(^+\) T cells). This difference may be the result of a consistent underestimation of the number of effectors cells in the IFN-\(\gamma\) ELISPOT assay, or that prime/boost immunization generates a number of Ag-specific CD8\(^+\) T cells that do not secrete IFN-\(\gamma\), but play a different role in the immune response.

Sequential immunization with FP9PbCSP and MVAPbCSP induced potent CD8\(^+\) T cell responses against the PbCs Ag and substantial protection against stringent \(P.\) \(berghei\) challenge. Intriguingly, sequential administration of DNA, FP9PbCSP, and MVAPbCSP elicited an equivalent frequency of Ag-specific CD8\(^+\) T cells against the PbCs Ag, but reduced protection. Brun-Romero et al. (15) have recently shown that boosting early (within 8 wk) after immunization with a high dose of rADV compromises protective immunity against \(P.\) \(yoelii\) sporozoite challenge. They have proposed that boosting of highly activated effector CD8\(^+\) T cells leads to activation-induced cell death, rather than an increase in the number of Ag-specific CD8\(^+\) T cells. Preliminary results in

### Table I. Protection against stringent \(P.\) \(berghei\) challenge elicited by prime/boost immunization regimes

<table>
<thead>
<tr>
<th>Prime/Boost(^a)</th>
<th>Animals Protected(^b)</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D/F</td>
<td>5/40</td>
<td>12.5</td>
</tr>
<tr>
<td>D/M</td>
<td>7/40</td>
<td>17.5</td>
</tr>
<tr>
<td>F/M</td>
<td>27/40</td>
<td>67.5</td>
</tr>
<tr>
<td>M/F</td>
<td>15/40</td>
<td>37.5</td>
</tr>
<tr>
<td>F/F</td>
<td>3/19</td>
<td>15.7</td>
</tr>
<tr>
<td>M/M</td>
<td>3/19</td>
<td>15.0</td>
</tr>
</tbody>
</table>

\(^a\) Female BALB/c mice were immunized i.d. with FP9PbCSP (F) or MVAPbCSP (M), or i.m. with pSG2PbCSP (D), and boosted i.d. 14 days later.

\(^b\) Animals were challenged 14 days after the booster immunization. Results are cumulative for four experiments.

### Table II. Protection against stringent \(P.\) \(berghei\) challenge elicited by sequential immunization with two or three heterologous vectors

<table>
<thead>
<tr>
<th>Prime/Boost/Boost(^a)</th>
<th>Animals Protected(^b)</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D/F/M</td>
<td>3/20</td>
<td>15</td>
</tr>
<tr>
<td>–/F/M</td>
<td>12/20</td>
<td>60</td>
</tr>
<tr>
<td>D*/F*/M*</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td>–/F*/M*</td>
<td>0/10</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) Female BALB/c mice were immunized i.d. with FP9PbCSP (F) or MVAPbCSP (M), or i.m. with pSG2PbCSP (D), and boosted i.d. sequentially 14 days later. Control animals were immunized with empty constructs without recombinant Ags (DNA\(^a\), FP9\(^a\), MVA\(^a\)).

\(^b\) Animals were challenged 14 days after the booster immunization. Results are cumulative for two experiments.

### FIGURE 6. Triple-combination immunizations. Groups of mice were immunized at 14-day intervals with the vaccines shown. The first administration of the double combination was given on the same day as the second administration of the triple combination. The figure depicts the percentage ± SEM of Pf9-specific CD8\(^+\) T cells measured by FACS (A) and the percentage of IFN-\(\gamma\) SFC per million ± SEM (B) for three mice per group determined 14 days after the final immunization.
our laboratory indicate that 2 wk after prime/boost immunization with DNA/F9P9pBCSP, P9-specific T cells are predominantly of the activated CD62L–CD44+CD43+ phenotype (data not shown). Thus, administration of MVAPBCSP at this time point may have induced activation-induced cell death, thereby inhibiting rather than boosting the protective immune response.

Despite promising results using DNA/MVA to immunize rodents against *Plasmodium* sporozoite challenge, recent clinical studies have shown that a similar immunization regimen elicits only partial protection against a high-dose heterologous *P. falciparum* strain challenge in human volunteers to whom the MVA is administered i.d. (18). Optimal protection against sporozoite challenge in mice is only observed when MVA is administered i.v. (13). Although i.v. administration might be acceptable for therapeutic vaccination of humans, this route is not acceptable for widespread vaccination with a prophylactic vaccine. In this study, we have shown that a prime/boost immunization regimen using F9P and MVA will elic it a potent CD8+ T cell response against the PbCS Ag when the viruses are administered i.d. Importantly, this response was substantially more immunogenic than that elicited by priming with DNA and boosting i.d. with MVA, and led to markedly higher protection against a stringent *P. berghei* sporozoite challenge. Because both FPV and MVA are acceptable for clinical use, this work has provided the basis for clinical trials using recombinant FPV and MVA encoding Ags from *P. falciparum*. Preliminary results from these trials indicate that prime/boost immunization with these viruses will elicit sterile protective immunity in some volunteers (D. Webster, S. Dunachie, J. Vuola, T. Berthoud, S. Keating, S. Laidlaw, S. McConkey, I. Poulton, L. Andrews, R. Andersen, et al., manuscript in preparation), providing evidence that this vaccination strategy could be of value in humans.

**Acknowledgments**

We thank Jacqui Mendoza for supplying *P. berghei*-infected mosquitoes for the challenge studies.

**References**


