vector expressing an HIV-1 immunogen suggest that preexisting antibodies to this strain of Ad might reduce the magnitude of cellular immune response induced by more than threefold (24,25). However, in both of these clinical trials, vaccine-induced immune responses were detected, even in subjects who were seropositive for Ad5 before vaccination, and the use of higher doses of the Ad5 vaccine helped to overcome this preexisting anti-vector immunity. The utilization of less-prevalent human strains, or simian strains to which there is little human exposure, may circumvent this problem (26). The use of heterologous adenoviral subtypes from different serotypes may also help circumvent the problem of anti-vector immunity (27). Preexisting immunity against smallpox does not appear to be a problem for the use of MVA as a vector, perhaps in part because of the long time interval between smallpox vaccination and the current studies with MVA. The same viral vectors are currently being used for the development of vaccines against multiple pathogens; this may limit the number of times the same vector is utilized. There is evidence from clinical trials that anti-vector immunity against MVA is present at three weeks but lasts less than one year (28,29). Further work is needed to define more precisely the nature and length of anti-vector immunity for these promising boosting vectors.

CLINICAL TRIALS

Following the success of prime-boost immunization strategies in preclinical models, many regimens have been evaluated in small-scale clinical trials. The main outcome measures used in these clinical trials are safety and immunogenicity. These data are essential in proceeding with the clinical evaluation of these strategies; however, the precise immunological correlates of protection are not clearly defined for these pathogens. For this reason, even small-scale clinical studies that evaluate efficacy, such as those conducted with vaccines against malaria and cancer, are invaluable in evaluating the potential utility of prime-boost strategies in humans, and in identifying potential correlates of protection.

Malaria

Since 1999, there have been a series of clinical trials evaluating the use of several vectors expressing pre-erythrocytic antigens from *P. falciparum*, both alone and in combination in heterologous prime-boost regimens. These trials initially evaluated the safety and comparative immunogenicity of different heterologous prime-boost regimens. The protective efficacy of the most immunogenic regimens was subsequently evaluated using sporozoite challenge (30). The early trials used plasmid DNA as a priming agent and boosted with a recombinant MVA expressing the same antigen. The antigen used in these studies was the pre-erythrocytic protein antigen, thrombospondin-related adhesion protein (TRAP), which was fused to a polyepitope string of 14 $CD8^+$ T-cell epitopes (multiple epitopes, ME) from six different pre-erythrocytic *P. falciparum* antigens (ME-TRAP). A range of DNA doses from 0.5 to 2.0 mg were evaluated in these clinical trials, and DNA ME-TRAP was also delivered using a needleless ballistic ''gene gun'' device. Recombinant MVA ME-TRAP was delivered intradermally at doses of 3×10^7 to 1.5×10^8 pfu. In total, 150 vaccinees received these vaccines and the safety profile was good for both vaccines (31). The results of these early trials demonstrated that 5- to 10-fold higher immunogenicity was seen in the heterologous prime-boost regimes than in the homologous boosting regimes (30). These were predominantly $CD4^+$ T cells, with fewer $CD8^+$ T cells and little or no antibody induced. Two or three priming immunizations followed by an MVA boost intradermally at a short interval were particularly immunogenic. Lengthening the interval between the final DNA and the MVA immunization from three to eight weeks appeared to reduce immunogenicity slightly. Importantly, these immunogenic regimes were found to confer partial protection after sporozoite challenge manifest as a delay in time to parasitemia. The protective efficacy of this DNA prime-MVA boost was subsequently evaluated in a phase IIb efficacy trial in Gambian adults (32). This trial demonstrated that although vaccination with two doses of DNA ME-TRAP followed by a single boost with MVA ME-TRAP was safe and highly immunogenic for effector T-cell induction, it did not significantly reduce the *P. falciparum* infection rate in a semi-immune adult African population. This is unlikely to have been caused by TRAP strain variation in field parasites as T-cell responses were broad and largely cross-reactive between strains.

Preclinical studies aimed at improving both immunogenicity and protective efficacy demonstrated better immunogenicity and efficacy in mice when a second viral vector, recombinant fowlpox (strain FP9), was used to prime and MVA used to boost (33). Using the same insert as in the previous studies, a clinical trial with FP9 ME-TRAP prime-MVA ME-TRAP was subsequently conducted in U.K. adults. This vaccination regimen was found to be safe and well tolerated (34). Although the maximal ELISPOT level induced was lower than with the optimized DNA-MVA regime, a higher proportion of $CD8^+$ T cells were induced with the FP9-MVA regimes (35). The protective efficacy of this regime was subsequently evaluated by sporozoite challenge, and in a small number of individuals complete sterile protection was induced that lasted for up to 20 months (36). Interestingly, protection at 20 months was associated with persisting memory but not effector T-cell responses. Importantly, for both DNA-MVA and FP9-MVA the protective efficacy of various immunization regimes correlated with the magnitude of induced immune responses (30,36,37) (Fig. 2), supporting the strategy of maximizing durable T-cell immunogenicity to develop more effective liver-stage vaccines against *P. falciparum* malaria. Using the percentage reduction in liver-stage parasites as a measurement of efficacy, the FP9-MVA regime produced a 92% reduction in parasite burden compared with an 80% reduction with DNA-MVA regimes (38). This FP-MVA prime-boost regimen was then evaluated in a phase I trial in Kenyan children and immunogenicity was lower than in U.K. adult vaccinees (39). This clinical study also investigated the effects of anti-vector immunity and found that partial cross-reactive immunity induced by administration of the first poxviral vector reduced the immunogenicity of the second, boosting vector (39). This study intriguingly suggests that priming with a lower initial dose limits this anti-vector immunity and paradoxically stimulates stronger cellular immunity after boosting than full dose priming. Further work on anti-vector immunity showed that alternating vector regimes (e.g., MVA/FP9/MVA or FP9/MVA/FP9) induced higher levels of memory T cells, as measured in a cultured ELISPOT assay than heterologous prime-boost regimens such as FP9/MVA or FP9/FP9/MVA (40). A phase IIb field trial was conducted in Kenyan children to evaluate the protective efficacy of FP9 ME-TRAP prime-MVA ME-TRAP boost regime. The immunogenicity results seen in this trial were disappointingly low, probably explaining the