Supporting Information

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SI Text

Anti-Human IgG Antibodies in Transduced Mice. Virus-vectored gene therapy treatments can induce the development of an immune response against the transgene product that sometimes negatively impacts efficacy (1). Expression of human antibodies in humans by vectored immunoprophylaxis (VIP) is not expected to induce an immune response; however, human IgG (hIgG) expression in mice might elicit an immune response that could impact antibody levels or protection from challenge. Therefore, we measured antibodies that recognized the adeno-associated virus (AAV) encoded hIgG in our mice by ELISA. To detect anti-human Fc antibodies, plates were coated with 1 μg per well hIgG (Human Reference Serum; Bethyl). Serum dilutions started at 1:100, and plates were incubated with an horseradish peroxidase-conjugated anti-mouse IgG secondary. Anti-human kappa light chain antibody was used as a positive control. Among all transduced mice, 58% developed no detectable anti-hIgG, 38% developed anti-hIgG with low titer (1:300 or less), and six mice (4%) developed titers of 1:900 (Fig. S5*A*). Anti-hIgG levels tended to increase over time, but among mice that produced hIgG for an extended period (24 wk), 63% of mice still had no detectable levels of hIgG antibodies (Fig. S5*B*). Additionally, mice with very high hIgG levels did not seem to be more likely to develop anti-hIgG antibodies, and no statistically significant correlation between hIgG concentration and anti-hIgG antibody was observed. Finally, the presence of anti-hIgG antibody titers did not appear to impact protection from sporozoite challenge either by i.v. injection or mosquito bite (Fig. 2*A*). This is likely to be due to the high concentrations of mAb expressed and the modest anti-hIgG titers present.

In an initial experiment to assess pathology induced by persistent high-level hIgG expression, groups of five mice were transduced with 2A10-AAV or mock-transduced. Twenty-six weeks posttransduction, mice were killed and submitted for autopsy to identify gross pathology that might be due to hIgG expression or an immune reaction against hIgG. No changes attributable to hIgG production were seen, and, in particular, no evidence for deposition of antibody–antigen complexes was found either in lung or kidney.

Although lack of an antibody response to hIgG expression in immune-competent mice is surprising, similar immune tolerance has been noted in studies of expression of mAb by VIP and of other proteins by other AAV2/8 vectors (2–4). Recently, it was demonstrated that AAV2/8 did not effectively transduce and activate antigen presenting cells, resulting in reduced inflammatory signaling and consequent failure to prime a functional T-cell response (4). The lack of a robust antibody response against hIgG seen in this study may be the result of this mechanism. Additionally, transduction with AAV2/8 did not stimulate major histocompatibility complex class I expression on myocytes (4). This would inhibit recognition of AAV2/8 by transgene-specific cytotoxic T cells and thus may contribute to the failure of cytotoxic T cells to eliminate transduced cells, permitting long-lived transgene expression.

1. Manno CS, et al. (2006) Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat Med* 12(3):342–347.
2. Balazs AB, Bloom JD, Hong CM, Rao DS, Baltimore D (2013) Broad protection against influenza infection by vectored immunoprophylaxis in mice. *Nat Biotechnol* 31(7): 647–652.
3. Balazs AB, et al. (2012) Antibody-based protection against HIV infection by vectored immunoprophylaxis. *Nature* 481(7379):81–84.
4. Mays LE, et al. (2014) AAV8 induces tolerance in murine muscle as a result of poor APC transduction, T cell exhaustion, and minimal MHCI upregulation on target cells. *Mol Ther* 22(1):28–41.

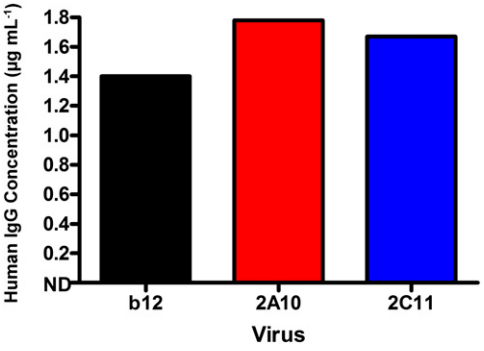

Fig. S1. VIP vectors express mAb 2A10 and 2C11 in vitro. Vectors 2A10-AAV, 2A11-AAV, or b12-AAV [2.5×10^{10} genocme copies (GC)] were used to infect 5×10^5 293T cells. Culture supernatant was assayed for hIgG antibody by ELISA at 6 d postinfection.

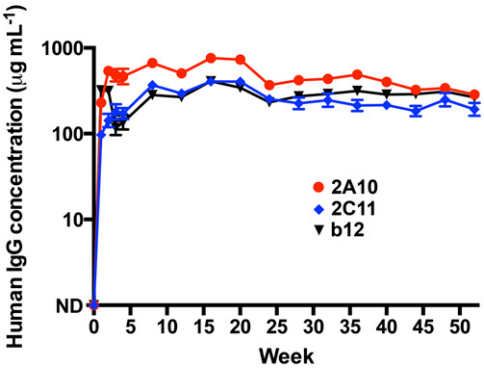

Fig. S2. Durability of VIP-mediated circumsporozoite protein (CSP) mAb expression. Ten mice per group were transduced with b12-, 2A10- or 2C11-AAV, and hIgG antibody expression in mouse sera was monitored by ELISA. Plot shows the mean and SE of the 10 mice in each group.

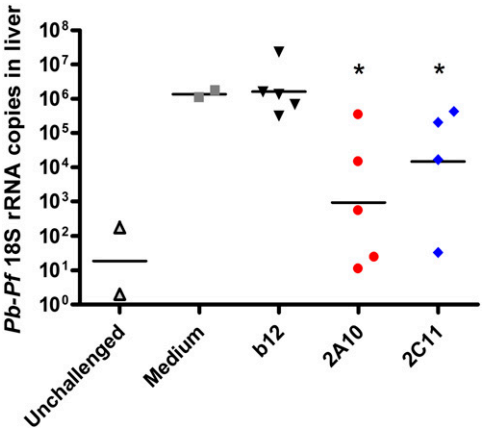

Fig. S3. Both VIP-encoded CSP antibodies reduce liver burden. Mice were challenged i.v. with 2×10^4 *Pb*/*Pf* sporozoites isolated from infected *Anopheles stephensi* mosquitoes 8 wk posttransduction with b12-AAV, 2A10-AAV, 2C11-AAV, or mock transduction. Liver parasite burdens were assessed 40–42 h postchallenge by qRT-PCR measurement of *Plasmodium berghei* 18S rRNA in liver homogenates. Plotted are values for individual mice ($n = 2$ or 5) and the geometric mean of each group. Unchallenged mice did not receive sporozoites ($n = 4$). Asterisks indicate mean rRNA levels significantly different ($P < 0.01$) from b12 control mice by two-tailed *t* test.

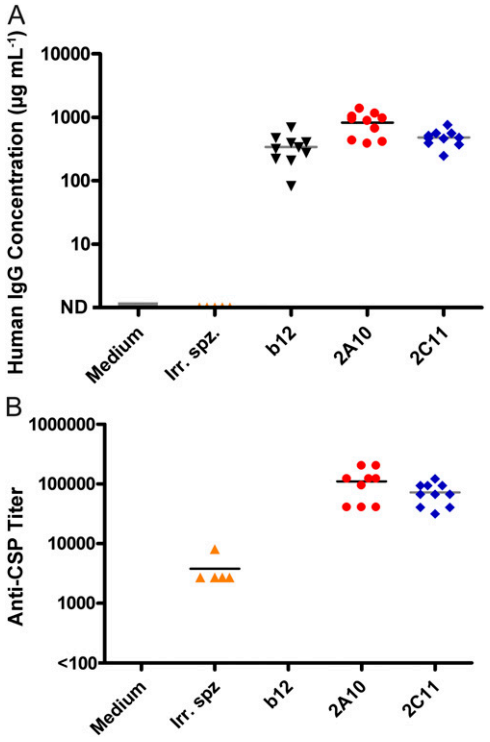

Fig. S4. HIgG and anti-CSP antibodies in mice before mosquito bite challenge. Mouse sera were assayed for hIgG (*A*) and anti-CSP antibodies (*B*) before the mosquito bite challenge shown in Fig. 3 at 11 wk posttransduction with 2A10-, 2C11-, or b12-AAV ($n = 10$ per group). Additional control mice ($n = 5$ per group) received either medium alone or three i.v. injections of 1×10^5 to 1.5×10^5 irradiated *Pb/Pf* sporozoites. Plotted are values for individual mice ($n = 5$ or 10) and the mean of each group. CSP-reactive antibody in sporozoite-immunized mice is murine and is detected by the CSP, but not the hIgG ELISAs.

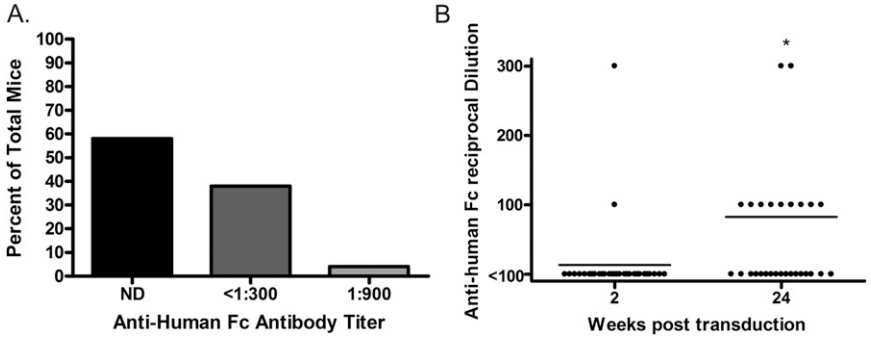

Fig. S5. Minimal antitransgene immune response to hIgG Fc in transduced mice. (*A*) Anti-human Fc antibodies were measured by ELISA for all transduced mice used in this study at the time of challenge or sacrifice ($n = 164$). Shown are the percent of total mice that had no detectable antibodies (ND; $n = 95$), a titer of less than or equal to 1:300 ($n = 63$), or a titer of 1:900 ($n = 6$). (*B*) Anti-human Fc antibodies were measured by ELISA at weeks 2 and 24 in b12-, 2A10-, and 2C11-AAV– transduced mice. At week 2, 93% of mice (28 out of 30) had no detectable levels of antitransgene antibodies (reciprocal titer less than 1:100). At week 24, 63% of mice (19 out of 30) had no detectable levels of antibodies against the transgene, and none had a titer greater than 1:300. Asterisks indicate anti-human Fc antibody levels significantly different ($P < 0.01$) from week 2 by a two-tailed *t* test ($n = 30$).