Malaria DNA vaccines in *Aotus* monkeys


In preparation for the development of DNA vaccines designed to produce protective antibodies against *Plasmodium falciparum* antigens (Ag), we conducted studies to optimize antibody responses in *Aotus* monkeys after immunization with the *P. yoelii* circumsporozoite (CSP) DNA vaccine. We demonstrate in *Aotus* monkeys that an intradermal route of immunization with a PyCSP plasmid DNA vaccine generates antibody responses equivalent to a multiple antigen peptide/adjunct based vaccine, and that these data support the use of the intradermal route for initial studies of the efficacy of DNA vaccines in inducing protective antibodies against *P. falciparum* antigens in *Aotus* monkeys. © 1997 Elsevier Science Ltd

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We are currently pursuing the development of a malaria DNA vaccine (reviewed in Hoffman). One of the critical issues in this development program is the reliability of immunogenicity data generated in mice for predicting outcomes in humans. It is our contention that it will be optimal to demonstrate immunogenicity in nonhuman primates prior to conducting human studies. Additionally, it would be beneficial to demonstrate protective efficacy of the DNA vaccine prior to human studies. A limitation to the demonstration of protective efficacy is the fact that *Plasmodium falciparum* and *Plasmodium vivax*, the major targets for human malaria vaccine development, only infect primates. Thus, optimal assessment of immunogenicity and protective efficacy of a human malaria DNA vaccine in an animal model prior to human trials can only be conducted in nonhuman primates.

We have therefore embarked on a series of experiments to assess immunogenicity, and when possible, protective efficacy of DNA vaccines in *Aotus* monkeys. The *Aotus* monkey has served as the most consistent animal host/model for the study of *P. falciparum*. The host/parasite relationship in this model is not perfect, however, despite its limitations, *Aotus* monkeys have been successfully used for decades in the development of anti-malarial drugs and, relatively recently, to study the efficacy of candidate anti-malaria vaccines.18,19

RESULTS AND DISCUSSION

Malaria DNA vaccines: optimization of dose and route in *Aotus*

We have previously reported that intramuscular (i.m.) immunization with a plasmid-based DNA vaccine encoding the gene for the *Plasmodium yoelii* circumsporozoite protein (PyCSP) induces high levels of antibodies in mice.20 This DNA plasmid is highly immunogenic in mice, inducing antibodies as high as those induced by the best synthetic peptide/adjunct combination.21 Therefore, we initially assessed the immunogenicity of this vaccine in *Aotus* monkeys to determine optimal dose and route of administration (Gramzinski, manuscript submitted). The results of a series of experiments in which we immunized monkeys with as little as 5 µg and as much as 2000 µg of DNA by the i.m. route demonstrated that immunization with as much as 2000 µg of this plasmid i.m. elicited no detectable antibodies by an immunofluorescence antibody titer (IFAT) test or by an enzyme linked immunosorbent assay (ELISA) to recombinant circumsporozoite protein. However, if the same vaccine is administered by intradermal (i.d.) immunization the vaccine elicited an excellent antibody response in all animals with the lowest dose tested (125 µg) [as determined by ELISA to a PyCSP purified recombinant protein and to air-dried sporozoites in an indirect fluorescent antibody test (IFAT)]. There was a definite dose response (i.e. 2000 µg was better than 500 µg which was better than 125 µg), and antibody levels increased with each of three doses in the primary series, peaking 2 weeks after the third dose.

Having established that mice made antibodies after injection with the PyCSP plasmid by the i.m. route, but
that the monkeys made no antibodies after i.m. injection, but excellent antibody responses after i.d. immunization, we studied antibodies after i.m. or i.d. immunization in mice. In mice, i.d. immunization led to similar levels of antibodies as did i.m. immunization. These data clearly demonstrate that the antibody responses after DNA immunization may vary between species of animals, and that the results of immunization of mice may not necessarily predict results in nonhuman primates. The disparate relationship between results seen in mice and nonhuman primates to the immunogenicity of these vaccines in humans is not known. However, the difference between mice and monkey plasmid DNA immunization may be important because of the implications these data have to human application of these vaccines. Since monkeys are phylogenetically closer to humans it is reasonable to speculate that the optimal dose and route of immunization regimens in monkeys will more closely predict optimal regimes for humans than will mice. If this is true then based on these data, one must consider i.d. immunization as a possibility in the design of any human plasmid vaccine trial designed to induce protective antibodies.

We have previously shown that sera with high levels of antibodies against P. yoelii sporozoites from mice immunized with the PyCSP DNA vaccine have much less capacity to inhibit invasion and development of sporozoites into hepatocytes than expected. We attribute this lack of inhibitory activity to the fact that the antibodies are not primarily against the immunodominant central repeat region of the PyCSP, but to the flanking regions. Since all protective monoclonal antibodies against PyCSP and other CSPs are against the central repeat region, this difference in fine specificity probably accounts for the lack of biologic activity of the PyCSP DNA vaccine-induced antibodies. Consistent with the murine findings, sera from monkeys which had been i.d. injected with the P. yoelii DNA vaccine and had high antibody titers by IFAT and ELISA had poor inhibitory activity in vitro as assessed by inhibition of liver stage development assay (ILSDA) (Graminski submitted).

**Plasmodium falciparum CSP (PICSP) DNA vaccine**

Additionally, we have compared i.m. and i.d. immunization of Aotus monkeys with a PICSP DNA vaccine. Identically as determined with the PyCSP DNA vaccine i.m. immunization with PICSP DNA did not induce any detectable antibodies to the PICSP. In striking contrast, i.d. immunization induced excellent antibody responses in seven of eight monkeys after two doses of the DNA vaccine (three doses at 4 week intervals and a fourth dose at week 21) and 2 weeks after the fourth dose (week 21) eight of eight monkeys had seroconverted with antibodies to the PICSP by ELISA and to air-dried sporozoites by IFAT. Like the PyCSP vaccine, immunization of mice by the i.d. or i.m. routes led to antibody responses.

**Aotus monkeys as models for protective efficacy**

Erythrocytic stage vaccines. Human malaria strains have been adapted to Aotus monkeys and have been used extensively in anti-malarial drug development and relatively recently malaria vaccine development. Many P. falciparum strains have been successfully adapted to Aotus and are maintained by serial in vivo passage of parasitized erythrocytes in these animals. Infection of Aotus with parasitized erythrocytes and its parameters has been extensively studied by Schmidt and Collins and as a model for testing the protective efficacy of erythrocytic stage malaria vaccines Aotus is an excellent model. However, since little is known about the Aotus immune response and its similarities or differences between human immunity, one must use caution when making direct comparisons between the success or failure of a vaccine candidate in Aotus to its potential success or failure in humans. The predictive value of Aotus for human vaccine success or failure will have to await studies in which vaccines have been tested both in Aotus and in humans and the outcomes known.

**Pre-erythrocytic stage vaccines**

Sporozoite infection of Aotus monkeys with the P. falciparum (St. Lucia strain) was originally described by Collins in Columbian Aotus (Aotus lemurinus geoffroyi) and recently demonstrated in Panamanian Aotus (Aotus lemurinus lemurinus) (Graminski, manuscript submitted). Therefore it is possible to test the efficacy of pre-erythrocytic vaccines using this model. To this end we have immunized A. lemurinus lemurinus monkeys with a DNA vaccine consisting of either a mixture of three pre-erythrocytic stage antigens from P. falciparum (PICSP, PfExp-1, and PfSSP2) or with PfSSP2 alone by either the i.m. or i.d. route. Of the monkeys that received the combination vaccine (PICSP, PfExp-1, PfSSP2) by the i.d. route after three doses at 4 week intervals and a fourth dose at week 21 eight of eight animals had antibodies to PICSP, one of eight animals had antibodies to PfExp-1, and zero of eight animals had antibodies to SSP2. All animals that received the combination vaccine i.m. or the single SSP2 DNA vaccine by either the i.d. or i.m. route developed no detectable antibodies after the fourth dose at week 21. Currently these animals are scheduled to be challenged with P. falciparum (St. Lucia) and protection data are unavailable.

Thus we are currently developing malaria DNA vaccines for human use and testing the immunogenicity and efficacy of these vaccines in Aotus monkeys. Consistently, monkeys i.d. immunized Aotus monkeys have had superior antibody responses compared to monkeys immunized i.m. We are currently evaluating the immunogenicity and protective efficacy of both pre-erythrocytic and erythrocytic DNA vaccines in Aotus monkeys.

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