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CONTRACTING ORGANIZATION: Molecular Medicine Technologies Inc.
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13. ABSTRACT *(Maximum 200 words)*

The overall goal of this contract was to study the characteristics of the immune response against a malaria parasite antigen using vaccination with DNA. During the tenure of the contract we have analyzed in great detail all the immunologic aspects we had anticipated we would investigate. Collectively, the findings indicate the following: a) A single intraspleen inoculation of DNA of an antigenized antibody H chain coding for a malaria parasite is sufficient to elicit immunity against the malaria. The initial antibody response is primarily IgM. b) A single DNA injection is also sufficient to induce immunologic memory. Following protein booster injection (challenge) a vigorous secondary antibody response develops with isotype switch to IgG1. c) The initial IgM antibodies can be switched to IgG1 by immunizing with a gene chimerized with the DNA coding for the cytokine GM-CSF. d) DNA immunization induces antibodies that react with *P. falciparum* sporozoites, hence suggesting that the immunogenic process mimics natural immunity by the parasite. We deem that these results are new and have brought new understanding on how immunity to the malaria parasite may work and how DNA immunization can be exploited to program the immune system to mount an effective immune response.

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(5) INTRODUCTION

Malaria continues to be a major threat to man kind in may areas of the globe, particularly in underdeveloped countries. *Plasmodium falciparum,* one of the four etiologic agents of malaria in humans, is widely distributed and causes severe, and frequently fatal, malaria in children and nonimmune adults. Its control is hampered by the increased resistance to chemoprofilaxis, chemotherapy as well as eradication of the Anopheles mosquito vectors with insecticides.

The major surface proteins of malaria sporozoites, the circumsporozoites (CS) proteins, have unusual structural and immunological properties. It has now been established that in all *plasmodium* species the central portion of the molecule consists of a repetitive amino acid sequence which contains the protein immunodominant B-cell epitope(s). In *P. falciparum* the central domain contains about 40 repeat of the tetrapeptide Asn-Ala-Asn-Pro (NANP) plus 3 to 4 Ans-Val-Asp-Pro (NVDP) repeats. The presence of internal repeats likely derives from tandem duplication and is considered to have structure-function relevance.

To date there are no efficient methods to immunize against this parasite and much of what is done towards the development of a malaria vaccine is still experimental in nature. In particular, in addition to identify new parasite antigens that may be suitable for immunization and protection, emphasis is also placed on new methods to vaccinate and present antigen to the immunocompetent host.

The goal of our original proposal was to validate a new principle and method of immunization against the malaria parasite, gene immunization, using as a vaccine an immunoglobulin gene re-engineered to encode a discrete epitope of the malaria parasite, "antigenized antibody gene". As a model system we will use the tetrapeptide sequence NANP of the malaria parasite *Plasmodium falciparum* CS protein.

The studies proposed made use plasmid DNA ("naked" DNA) for the heavy chain of an antibody antigenized to express (NANP)3. This gene was used in pilot studies to immunize mice with the intent of inducing anti-NANP antibodies. The studies were designed at verifying feasibility by empirically establishing a series of parameters such as specificity and titer of the antibody response, isotype of NANP-reactive antibodies, duration of the antibody response and priming effect on a subsequent immune response elicited by a protein antigen. The results obtained in the course of these pilot studies (six months) will be reviewed hereunder.

(6) BODY

The work was done using a series of plasmid vectors designed and manufactured in the laboratory. Thes ewere used to inoculate C57Bl/6 mice to study the immune response that was elicited. Therefore, prior to summarizing the data we will briefly describe the material and methods used throught the studies.

Material and Methods:

The plasmid DNA

- (1) γ1NANP. This is a modified version of the γ1WT plasmid *(infra)* where the third hypervariable region was modified to encode three repeats of the tetrapeptide *Asn-Ala-Asn-Pro* (NANP) sequence. This vector also encodes a genomic human γ1 gene human constant region gene and contains a neomycin resistance gene under the control of the SV40 promoter for the selection of stable transformant cells.

- (2) γ1WT. This defines plasmid pNγ162 which contains the 2.3 Kb EcoRI DNA fragment carrying the genomic rearrangement of the murine V_H^{62} gene together with a genomic human γ1 gene human constant region gene.

- (3) pSV2neo. This is the original plasmid forming the backbone of the pNγ1 vector without the genomic human γ1 gene human constant region gene.

All plasmid DNAs used for injection were prepared from DH5α *Escherichia coli* according to standard procedures. The DNA is stored at -20°C until use.

Serological assays

- Detection of serum antibodies. This was done on sera collected at regular day intervals. The detection of antibodies to γ1NANP was done on a 96-well polyvinyl microtiter plates (Dynatech, Gentilly, VA) coated (2.5 µg/ml) by drying at 37°C. After coating, wells were blocked with 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) 0.15 M, pH 7.3. Wells were incubated overnight at 4°C with mice sera diluted in 1% BSA-PBS, containing 1% Tween-20 (PBSA). The bound antibodies were revealed using a goat antibody to mouse γ-globulin absorbed with human γ-globulins and conjugated with horseradish peroxidase (HP) (Sigma, St. Louis, MO) The bound peroxidase was revealed by adding o-phenylenediamine dihydrochloride and H_2O_2. Plates were read after 30 minutes in a micro-plate reader (V*max,* Molecular Devices) at 492 nm. Tests were done in duplicate. The detection of antibodies against the NANP peptide was done similarly using microtiter wells coated with the synthetic peptide $KK(NANP)_3$ (2.5 µg/ml). As a control, we used a peptide of human vitronectin. In the experiments reported herein, the peptide was diluted in 0.9% NaCl and adsorbed on the plate by drying at 37°C. (Experiments that will be reported in the future will be done using a more gentle approach of binding the peptide to the plate, that is a solution in carbonate buffer 0.01 M at pH 9.6).

- Detection of the Transgene Product. This was done on sera done on sera collected at regular day intervals. The presence of γ1NANP H chain transgene polypeptide in the serum of mice as detected using a capture assay. Briefly, 1:10 dilution of individual mouse sera in PBSA were incubated on 96-well plate coated with a goat antibody to human γ-globulin (10 µg/ml). To determine the concentration of the transgene product in the serum, O.D. values were plotted against a standard curve constructed with known amounts of human γ-globulins diluted in PBSA containing 10% normal mouse serum.The bound antibodies were revealed using a HP-conjugated goat antibody to human γ-globulin (H chain specific) absorbed with murine immunoglobulins (Sigma, St. Louis, MO). The bound peroxidase activity was revealed by adding 0-phenylenediamine dihydrochloride and H_2O_2. Because antibody titers were found only in the groups immunized intraspleen, the transgene was searched for only in these groups. Tests were done in duplicate.

- Isotype analysis of the immune response. Ig class determination was done by ELISA using isotype-specific rabbit antibodies (Mouse Typer Subisotyping Kit, Bio-Rad, Hercules, CA) used at the dilution recommended by the manufacturer.

Molecular biology techniques.

The expression of the transgenic mRNA was studied in the spleen of an immunized mouse inoculated via the intrasplenic route by reverse PCR. Briefly, the spleen was harvested on day 20 after the initial inoculation, frozen at -170 °C. The cells were prepared by gentle teasing. Typically, $PolyA^+$ mRNA was isolated from 11 mg of spleen

tissue using the the Micro-FastTrack mRNA isolation kit (Invitrogen, San Diego, CA). cDNA was synthesized by reverse transcription using the cDNA Cycle Kit (Invitrogen, San Diego). Specific PCR primers for the murine V_H gene and the human γ1 C region gene (the transgenes) were designed using Oligo 4.0 Primer Analysis Software (NBI, Inc., Plymouth, MN) from the sequences of the murine V_H^{62} and human γ1 genes obtained from the National Center for Biotechnology Information (NCBI) GenBank (Bethesda, MA). The PCR reaction conditions were as follows: 1 min. at 92 °C followed by 2 min. at 40 °C and 3 min. at 72 °C. This cycle was repeated 30 times. cDNA amplification and size determination (VH = 240 bp; human γ1 C region gene = 466 bp) were checked by electrophoresis using 4% NuSieve 3:1 agarose gel (FMC, Rockland, ME). To generate sticky ends on the PCR products we used the restriction half-site strategy where the oligonucleotide primers used contained three nucleotides (TTC) of the 6-bases recognition site of EcoRI site at their 5' end. The fragments were then cloned in pBluescript KS vector (Stratagene, San Diego, CA) for sequencing by the dideoxy method Sequenase 2.0 DNA sequencing Kit (USB, Cleveland OH). For Southern blot studies the cDNA was analyzed after electrophoresis on a 1% agarose gel and transfer to Hybond-N^+ nylon membrane (Amersham). The Blot was hybridized with oligonucleotide probes labeled using T4 polynucleotide kinase forward reaction in presence of γATP^{32}.

Histology of spleens after a single DNA inoculation.

Spleens were fixed in 4% paraformaldheide for 24 hours, rinsed with PBS and included in Tissue-Tek (Miles, Elkahrt, IN) and frozen at -70 °C. Blocks of frozen tissue were cut (5 μm) on a cryostat and stained with hematoxilin and eosin. Sections were inspected by two investigators.

Results

The results collected over the past six months can be summarized as follows:

1) Effect of the route of DNA inoculation

Only the intra-spleen route of immunization yields antibodies to both the γ1NANP protein and the synthetic peptide NANP. The response was specific in that none of the mice immunized with control DNA had a measurable antibody response. Notably, control mice primed by inoculation intra-spleen of antigenized antibody γ1NANP mounted a weak primary antibody response. These results are summarized in Table 1.

Table 1. The Route of Inoculation Dete rmines the fate of DNA Immunization

Route of Inoculation	No. of Mice	No. of Injections	Antibody Titers (Log)		
			Day 0	Day 14	Day 35
Intramuscular	5	4	≤2.3	2.4±0.2	2.4±0.2
Subcutaneous	5	4	≤2.3	≤2.3	≤2.3
Intravenous	5	4	≤2.3	≤2.3	≤2.3
Intradermal	4	3	≤2.3	≤2.3	2.4±0.2
Intra-spleen	5	1	≤2.3	**3.1±0.4**	**3.1±0.2**
Intra-spleen + Intravenous	5	1 + 3	≤2.3	2.8±0.2	2.8±0.2

2) Production of transgene immunoglobulins

Transgene immunoglobulins were detected in the serum of all mice immunized intraspleen. These results are summarized below in Table 2.

Table 2. Detection of transgene immunoglobulins in the serum of immunized mice

Route	Antigen	No. of Mice	No. of Injections	Transgene Product (ng/ml) Day 0	Day 7	Day 14
i.s.	γ1NANP (DNA)	10	1	Undetectable	3.5±3.2	11.8±5.6
i.s.	pSV2Neo (DNA)	4	1	Undetectable	Undetectable	Undetectable
i.s.	γ1NANP (protein)	4	1	Undetectable	6±5	Undetectable
i.s.	OVA	4	1	Undetectable	Undetectable	Undetectable

3) The antibody response

The response anti-transgene immunogloblins and anti-NANP peptide is specific for DNA immunization into the spleen. These data are summarized in Tables 3 and 4. Notably, mice inoculated intrtaspleen with 50 μg of equivalent transgene immunoglobulin did not make any measurable response.

Table 3. Detection of Anti-γ1NANP Antibodies in Mice Immunized Intraspleen

Immunogen	No. of Mice	No. of Injections	No. of Responders	Antibody Titers (Log) day 0	14	28	53
γ1NANP (DNA)	8	1	8/8	2.3	2.8	2.9	2.9
pSv2Neo (DNA)	4	1	0/4	2.3	2.3	2.3	2.4
γ1NANP (protein)	4	1	3/4	2.3	2.4	2.4	2.5
OVA (protein)	4	1	0/4	2.3	2.3	2.3	2.3

Table 4. Detection of Anti-Peptide Antibodies in Mice Immunized Intraspleen

Immunogen	No. of Mice	No. of Injections	No. of Responders	Antibody Titers (Log) day 0	14	28	53
γ1NANP (DNA)	8	1	8/8	2.3	2.5	2.8	2.8
pSv2Neo (DNA)	4	1	0/4	2.3	2.3	2.3	2.3
γ1NANP (protein)	4	1	0/4	2.3	2.3	2.3	2.3
OVA (protein)	4	1	0/4	2.3	2.3	2.3	2.3

4) Induction of immunologic memory

Intraspleen DNA immunization induces immunologic memory. We verified that mice primed by virtue of a single intraspleen DNA inoculation had acquired immunologic memory as defined by the ability to mount a vigorous response upon challenge with a protein antigen. These data are summarized in Table 5 and 6.

Table 5. Detection of antibodies to γ1NANP in mice primed intra-spleen and boosted subcutaneously

Priming	Booster	No. of Mice	Day 0	Day 14	Day 28
γ1NANP DNA	γ1NANP DNA	4	2.8±0.4	2.9±0.1	2.9±0.1
γ1NANP DNA	γ1NANP protein	4	2.5±0.2	3.4±0.2	3.5±0.3
pSVneo DNA	γ1NANP protein	4	2.3±0	2.4±0.2	2.5±0.2
γ1NANP protein	γ1NANP protein	4	2.5±0.2	2.9±0.2	2.9±0.2
OVA protein	OVA protein	4	2.3±0	2.3±0	2.3±0

Titers are expressed as means ± SD of Log values of individual mice. Day 0 refers to the day of the booster immunization, that is 200 days after the initial immunization. DNA booster (100 μg) was given in saline solution intravenously. Protein booster (50 μg) was given in IFA subcutaneously.

Table 6. Detection of antibodies to synthetic peptide NANP in mice primed intra-spleen and boosted subcutaneously

Priming	Booster	No. of Mice	Day 0	Day 14	Day 28
γ1NANP DNA	γ1NANP DNA	4	2.9±0	2.9±0	2.9±0
γ1NANP DNA	γ1NANP protein	4	3±0.2	3.6±0.3	3.7±0.4
pSVneo DNA	γ1NANP protein	4	2.3±0	2.3±0	2.3±0
γ1NANP protein	γ1NANP protein	4	2.4±0.3	2.5±0.4	2.6±0.6
OVA protein	OVA protein	4	2.3±0	2.3±0	2.3±0

Titers are expressed as means ± SD of Log values of individual mice. Day 0 refers to the day of the booster immunization, that is 200 days after the initial immunization. DNA booster (100 μg) was given in saline solution intravenously. Protein booster (50 μg) was given in IFA subcutaneously. A synthetic peptide from the sequence of human vitronectin was used as a control. The average reactivity on control peptide was reproducibly 2.3.

As indicated a single booster injection with the γ1NANP protein in mice primed with DNA intra-spleen augmented markedly the antibody response to both the transgene product as well as against the NANP peptide. The effect was specific in that none of the other groups (DNA -> DNA; or protein intra-spleen -> protein; or control DNA -> protein) had such a marked antibody response. it is worth noting that a booster injection with DNA γ1NANP failed to induce an increase in the antibody titer over the primary immune response.

The antibody response (time course and relative binding) is also shown in Figure 1.

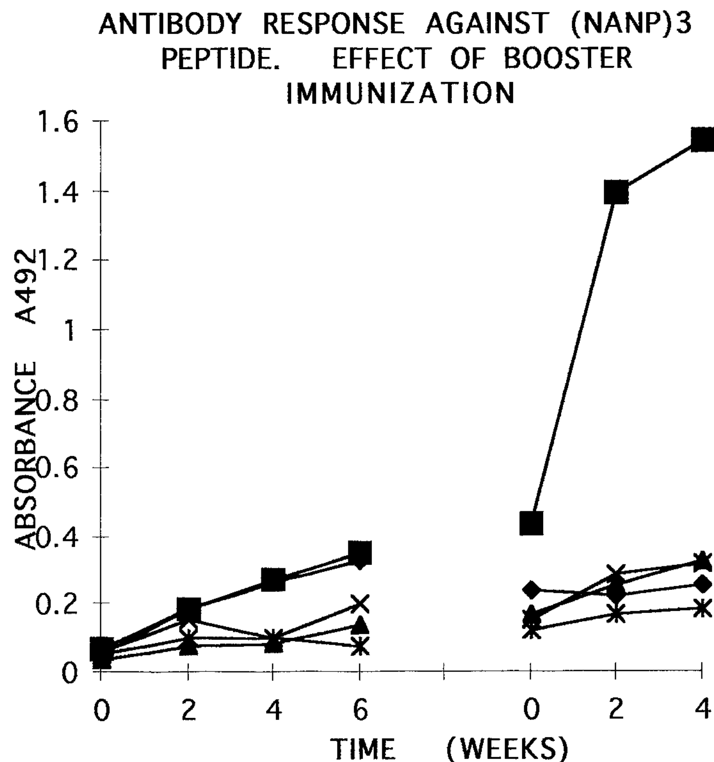

Figure 1. Time course and effect of booster immunization on the immune response to γ1NANP (top) and NANP peptide (bottom). Sera were tested individually at 1:1600 dilution. Experimental groups are as described in the text. Time points (week) on left refer to bleeds after the primary injection. Time points on the right correspond to bleeds after the booster immunization. Time 0 refers to the day of the booster injection.

5) Isotype analysis of the immune response during the primary and secondary response

Much to our surprise we found that the antibodies produced in response to a single intra-spleen immunization are prevalently of the IgM isotype. This suggested that priming of the immune response by intra-spleen DNA immunization may occur in conditions of limited production of cytokines, including those necessary for isotype switching. We also found that after booster, mice immunized once intraspleen with DNA undergo rapid IgM->IgG1 switch as shown below in Figure 2.

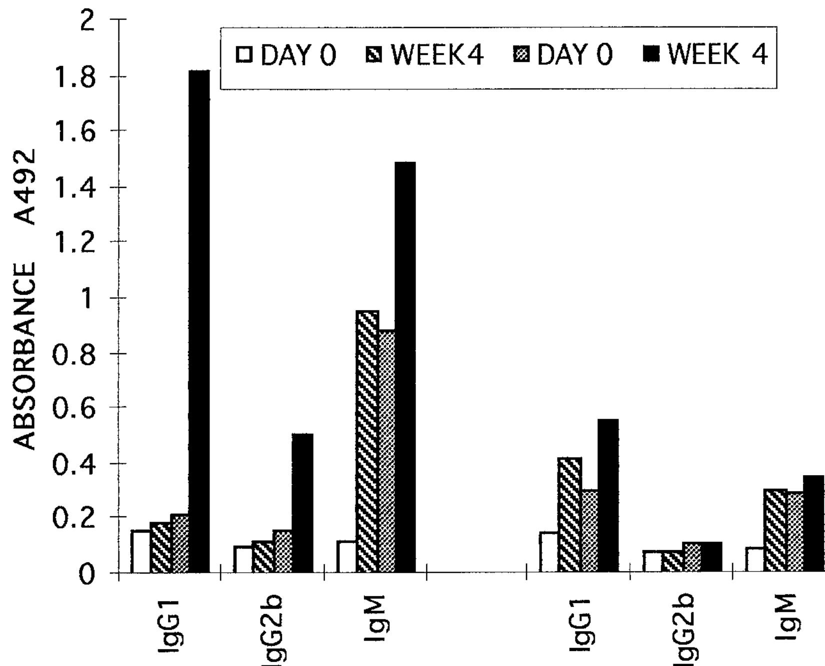

Figure 2. Isotype analysis of the immune response in mice primed intra-spleen with γ1NANP DNA (Left) or γ1NANP protein (Right), and boosted subcutaneously with the transgene protein in IFA. Symbols are indicated in the Figure. Sera were tested as a pool at a 1:1600 dilution. The first two columns in each group refer to day 0 and week 4 after priming; the last two columns refer to the day of the booster injection (Day 0) and week 4 after the booster, respectively.

The results indicate that after booster with the transgene protein mice make a strong antibody response of the IgG1 and to a lesser extent IgG2b isotype, but no significant increase of the IgM response over the primary immunization is observed. In contrast, mice primed intra-spleen with the transgene protein (γ1NANP) make a primary IgG1 response but not IgG2b antibodies.

Therefore, a booster immunization with protein in animals primed with DNA via the intra-spleen route readily induced isotype switch and production of IgG1 antibodies much greater than in the group primed with the transgene protein directly into the spleen. Intra-spleen immunization with DNA induces IgG2b antibodies in the secondary response. Because these effects were of more limited magnitude in mice intra-spleen with the antigenized antibody, we conclude that these effect are not solely the result of the booster immunization but also of a state of immunologic memory established by priming with DNA intra-spleen.

6. Strategies of DNA immunization intraspleen to modulate the quality of the immune response

Isotype analysis of the primary and secondary antibody responses indicates that IgM is the prevalent isotype during the primary immune response following a single intra-spleen inoculation of DNA. This has suggested to us that the T cell help provided is insufficient to promote switch. Studies of the secondary immune response, however, indicate that the initial IgM response switches to IgG1 upon new contact with antigen.

For the purpose of vaccination it may be desirable to induce IgG1 antibodies at the outset of immunization. In immunization with protein antigens this is achieved by incorporating the antigen in an immunologic adjuvant (e.g., CFA, alum etc.). Induction of an IgG1 response by immunization with naked DNA may require multiple DNA injections.

There are, however, possibilities to direct isotype switch at the time of immunization without using conventional immunologic adjuvants. For instance, the use of cytokines at the time of immunization or the use of antigen/cytokine chimeras may be of advantage to immunization. Relevant to our experiment is the observation by R. Levy and colleagues (Stanford University) that an antibody/GM-CSF chimera induces a marked primary IgG1 anti-idiotype response.

Although these new studies were not contemplated in the original SBIR proposal, we decided to use as the immunogen DNA (e.g., γ1NANP) chimerized with GM-CSF or IL-2. We studied the effect(s), if any, of DNA chimeras on: *i*) the isotype response, and *ii*) the quantitative aspects of the overall response. The results obtained are shown in Table 7, 8 and 9.

Table 7. Induction of IgG1 antibodies during the primary response after intraspleen inoculation with γ1NANP/GM-CSF DNA chimera

Exp. #	Priming (DNA)	Booster	No. of Mice	Primary Response (Days)		
				0	14	28
I	γ1NANP	γ1NANP	6	0.239	0.308	0.315
I	γ1NANP/GM-CSF	γ1NANP	6	0.217	0.967	0.819
I	γ1NANP/IL-2	γ1NANP	6	0.237	0.241	0.233
II	γ1NANP	γ1NANP	3	0.240	0.278	0.320
II	γ1NANP/GM-CSF	γ1NANP	3	0.273	0.847	0.638
II	γ1NANP/IL-2	γ1NANP	4	0.218	0.283	0.222
III	γ1NANP	γ1NANP	4	0.250	0.283	0.305
III	γ1NANP/GM-CSF	γ1NANP	4	0.240	0.791	0.617
III	γ1NANP/IL-2	γ1NANP	4	0.282	0.283	0.279

Values correspopnd to ELISA readings (A492) of pool of sera collected on the day indicated in the Table and tested at 1:1600 dilution.

Table 8. The antibody response against γ1NANP protein in mice inoculated intra-spleen with γ1NANP/cytokine chimera is similar to that elicited by γ1NANP alone

DNA Immunogen	Day 0	Day 14	Day 35	**Day 14 After boost**	**Day 28 After boost**
γ1NANP/GM-CSF	2.6	3.2±0.3	3.2±0.2	3.7±0.3	4±0.6
γ1NANP/IL-2	2.6	2.8±0.2	2.8±0.4	3.6±0.2	3.6±0.2
γ1NANP	2.6	3.2±0.1	3.2±0.3	3.8±0.2	4.1±0.6

Mice were immunized by a single inoculation of DNA intra-spleen (100 μg). A booster injection was given on day 40 by injection of γ1NANP protein (50 μg) in IFA subcutaneously. Values represent the means ± SD of 4 mice/group tested individually.

Table 9. Antibody NANP peptide in mice inoculated intra-spleen with γ1NANP/cytokine chimera

DNA Immunogen	Day 0	Day 14	Day 35	**Day 14 After boost**	**Day 28 After boost**
γ1NANP/GM-CSF	2.3	2.6±0.2	2.8±0.2	3±0.2	3±0.1
γ1NANP/IL-2	2.3	2.5±0.2	2.6±0.2	2.6±0.1	2.6±0.1
γ1NANP	2.3	2.5±0.2	2.6±0.2	2.7±0.2	2.7±0.2
γ1NANP/GM-CSF	2.3	2.7±0.2	2.8±0.2	3.1±0.2	3.1±021
γ1NANP/IL-2	2.3	2.7±0.3	2.7±0.3	3.1±0.3	3.1±0.3
γ1NANP	2.3	2.6	2.6	2.9	2.9
γ1NANP/GM-CSF	2.3	2.8±0.2	2.8±0.2	3±0.3	3.1±0.3
γ1NANP/IL-2	2.3	2.8±0.3	2.8±0.3	3±0.2	3±0.2
γ1NANP	2.3	2.7±0.3	2.8±0.2	3.1±0.2	3±0.2

As it can be seen GM-CSF does not appear to have an effect on the quantitat ive aspect of the anti-peptide response eiother before or after booster. We conclude that while the addition of GM-CSF produced a marked and reproducible effect on the isotype, it was unable to modify the amounts of antibodies produced.

7) Detection of transgene product in mice immunized with γ1NANP/cytokine chimera

Mice immunized with the γ1NANP/GM-CSF chimera produce IgG1 antibodies from the onset during the primary response. We compared, therefore, the levels of transgene immunoglobulins in the serum of these mice in order to reveal any difference that may help understanding the qualitative effect of GM-CSF. Table 10 summarizes the results.

Table 10. Transgene immunoglobulins in mice immunized with DNA/cytokine chimera

IMMUNOGEN	MICE (No.)	Producers	Transgene Product (ng/ml)
γ1NANP	11	10/11	11.5±8.4
γ1NANP/GM-CSF	13	1/13	2.2
γ1NANP/IL-2	14	3/14	1.2±0.1

As indicated mice inoculated with the DNA/cytokine chimera did not produce detectable amounts of the transgene. Therefore, the effect on isotype IgM->IgG1 switch can not be attributed to higher amounts of transgene produced.

8) Histology of spleens after a single DNA inoculation

To check for histological abnormalities subsequent to intraspleen immunization, spleens from DNA-inoculated, saline-inoculated and naive mice were collected 3, 7, 14 and 21 days from the time of injection. We did not observe any gross abnormality in the architecture of the organ or infiltrates of inflammatory cells as compared with spleens from naive mice of the same age or from mice given a sham inoculation of sterile saline solution. Immunohistochemical analysis of frozen sections using monoclonal antibodies to B and activated T lymphocytes showed no changes from controls. Collectively, the intraspleen inoculation of DNA did not cause apparent tissue damage nor appreciable changes in the number and tissue distribution of B and T lymphocytes.

9) Detection of anti-sporozoite antibodies in the serum of mice immunized with DNA

In collaboration with the laboratory opf Dr. S. Hoffman (Navy Research Laboratory) we have ascertained that antibodies reactive with the NANP peptide by ELISA also react with air dried sporozoites in indirect immunofluorescence.

The results of such studies have been received 24 hours prior to preparing this final report and they will be reviewed only:

- The pool of sera from mice given a single DNA immunization and subsequently boosted with the NANP-containing protein, was positive (Titer 1:40-1:80). The serum of a mouse immunized only once with γ1NANP DNA was positive (titer 1:20). This suggests that one DNA immunization alone is sufficient to elicit anti-sporozoite antibodies.

- The serum of mice primed with γ1NANP/GM-CSF or /IL-2 DNA chimeras, and boosted with the NANP-containing protein failed to react by IFA. The discrepancy could be explained on the basis of the isotype produced in response to the different immunization regimens. In fact, priming with the cytokine chimera predisposes to a great diminution of the IgG2b secondary response. Therefore, it is possible that the difference observed may reflect a more profound qualitative change in the antibody response. it should be mentioned that many reports in the mouse have concluded that antibody-mediated protection is mediated by IgG2a antibodies.

- The positive (+) control and the various negative (-) controls they all performed as expected. Therefore, the test was internally consistent.

10) Detection of the transgene mRNA in the spleen of inoculated mice

The cDNA was analyzed by Southern blot after electrophoresis on a 1% agarose gel and transfer to Hybond-N+ nylon membrane (Amersham). The Blot was hybridized with oligonucleotide probes labeled using T4 polynucleotide kinase forward reaction in presence of γATP^{32}. A strong hybridization band was seen with the amplification product of this reaction (240 bp) but not with the amplification product of a spleen from an unmanipulated mouse germline gene. This result was confirmed by sequencing the amplified and reverse-transcribed V_H. This suggests that after intraspleen inoculation the transgene persist in the spleen (B cells) for a prolonged period of time.

(7) CONCLUSIONS

In conclusion, we have answered all the questions posed in the contract under whose tenure the present work was performed.

1. **The main point of our studies is that a single intraspleen inoculation of DNA of an antigenized antibody H chain coding for a malaria parasite is sufficient to elicit immunity against the malaria. The initial antibody response is primarily IgM.**

2. **A single DNA injection is also sufficient to induce immunologic memory. As shown following a protein booster injection (challenge), a vigorous secondary antibody response develops with isotype switch to IgG1.**

3. **The initial IgM can be switched to IgG1 by immunizing with a gene chimerized with the DNA coding for GM-CSF.**

4. **DNA immunization induces antibodies that react with *P. falciparum* sporozoites, hence suggesting that the immunogenic process mimics natural immunity by the parasite.**

Future work will have to better define the immunological parameters to maximize the immunogenicity with one injection only as well as to identify the methods to produce high levels protective antibodies, e.g., correct isotype.

(8) REFERENCES

There is very little published so far on the induction of immunity against malaria using DNA vaccination. The one relevant publication is:

Sedegah, M., Hedstrom, R., Hobart, P. and Hoffman, S. L. 1994. Protection against malaria by immunization with plasmid DNA encoding circumsporozoite protein. *Proc. Natl. Acad. Sci. USA* 91:9866.

Additional references related to current approaches to DNA vaccination dealing with the induction of an antibody response are listed below. It should be noted that in most if not all instances induction of antibodies required multiple injections of DNA. Our study show that one DNA injection is sufficient to elicit an antibody response directed against the malaria peptide and the malaria parasite (native antigen).

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REPLY TO
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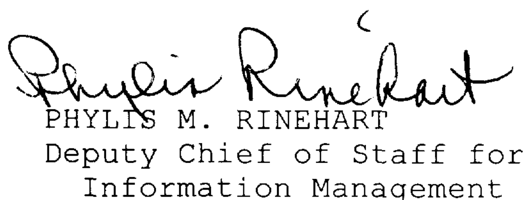

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