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TITLE: IGG SUBCLASS AND ISOTYPE SPECIFIC IMMUNOGLOBULIN RESPONSES TO LASSA FEVER AND VENEZUELAN EQUINE ENCEPHALOMYELITIS: NATURAL INFECTION AND IMMUNIZATION

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13. ABSTRACT *(Maximum 200 words)*

Venezuelan Equine Encephalitis (VEE) specific immunoglobulin responses to the two vaccines, TC-83 (A live-attenuated vaccine) and C-84 (A formulin inactivated vaccine derived from the TC-83 strain) were evaluated using an antigen and isotype specific enzyme-linked immunoabsorbent assay (ELISA). The VEE-specific ELISA was developed and standardized using selected sera from individuals previously immunized.

Initial experiments demonstrated that non-specific binding in the IgG, G2, A & M analysis was a signficant problem in data interpretation. Preparation of a negative control (tissue culture media derived) antigen were utilized to further standardize the assays. All assays were standardized in methodology and in relation to a uniform reference curve, where available. Initial screening of 28 SERA demonstrated the presence of predominantly VEE-specific G1 and G3 subclasses, IgA & M responses were also demonstrated in the series of subjects who received the TC-83 vaccine as well as in response to a C-84 booster vaccine.

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Subsequent testing included four groups of serum samples (Group A: TC-83 vaccine only; N=20; Group B: TC-83 primary/C84 booster; N=19; Group C(b): C84 primary, C84 booster; N=15; Group C(c): C84 primary only; N=19). Data analysis revealed the following differences:

1. The live attenuated vaccine TC-83 generated significantly higher VEE-specific IgA and IgM responses than either 1 or 2 boosters of the killed vaccine, C84. There was also a significant quantitative difference for IgG1, IgG3 and total IgG.

2. C84 is an effective booster vaccine in subjects with antecedent exposure to TC-83 for VEE specific IgG, G1, G3, G2, A & M.

3. In subjects with C84 exposure only, the booster response to C84 is substantial for IgG, G1, G3, but not IgA or IgM.

4. The highest VEE-specific IgG2, IgA and IgM responses were seen with TC-83, whereas the highest VEE-specific G, G1, and G3 responses were seen with C84 booster in subjects previously exposed to TC-83.

Attempts to establish the Lassa-specific ELISA were thwarted by difficulties in accessing antigen capture antibody or an adequate crude antigen source.

FOREWORD

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ABSTRACT

Venezuelan Equine Encephalitis (VEE) specific immunoglobulin responses to the two vaccines, TC-83 (a live attenuated vaccine) and C-84 (a formalin inactivated vaccine derived from the TC-83 strain of virus) were evaluated using an antigen and isotype specific enzyme-linked immunoadsorbent assay (ELISA). The VEE-specific ELISA for IgG, IgG sublcasses, IgA and IgM were developed and standardized using sera from vaccine-exposed and unexposed human subjects. Paired human sera (PRE and 28 days POST immunization) were tested from laboratory workers vaccinated with either TC-83 (GROUP A: 19 paired sera from subjects receiving a single TC-83 vaccine and with no prior documented history of vaccination) or C-84 in varying schedules (GROUP B: 20 paired sera from subjects who had a distant vaccination history to TC-83 but no evidence of neutralizing antibody; GROUP C(a): 19 paired sera from subjects receiving their first C-84 vaccination and no prior documented history of vaccination; GROUP C(b): 15 paired sera from subjects receiving a C-84 booster vaccination with prior history of C-84 but no TC-83 exposure). Sera were all tested for viral neutralization in vitro using a Vero cell monolayer for culturing virus and establishing 80 percent plaque reduction for each serum tested.

All PRE sera tested demonstrated no plaque reduction neutralization at a level of 80 percent for a dilution of 1:10. ELISA antibody titers for all PRE sera with no prior VEE exposure through vaccination or possible environmental factors were negative at a titer of 1:160 for IgM, 1:80 for IgG, IgA, and G subclasses. All vaccine types and strategies generated a significant IgG response POST vaccination ($P < 0.0001$) and this response correlated with the 80 % plaque reduction neutralization titer (80% PR-VNA) for VEE-specific IgG, G1, G3 and IgA at a P value of < 0.001 for both GROUP A and B. No such correlation was observed for G2 and no G4 responses to immunization were noted in any of the groups tested. There was a significant difference between geometric mean (GM) titers post vaccination for GRP-A or GRP-B versus GRP-C(a) ($P < 0.001$) and for GRP-C(a) versus GRP-C(b) ($P < 0.001$) for IgG. Neither C-84 alone group (1 or 2 doses) demonstrated an IgA response in contrast to the TC-83 exposed groups (GRP A and B). C-84 was an effective booster vaccine in subjects previously exposed to the live attenuated vaccine and generated a significant neutralization antibody response mirrored in the IgG, G1, G3 and IgA titer increases by ELISA.

INTRODUCTION

Venezuelan equine encephalitis (VEE), an arthropod-borne RNA virus representative of the alpha-viruses in the Togaviridae group, produces epidemic and endemic disease in Central and South America as well as the southern United States.(1,2) The equine population serves as the principal viremic host for epidemic strains of VEE and rodents are the major vertebrate amplifiers of endemic strains. Morbidity associated with this disease is considerable but mortality in adults is low (perhaps less than 1%). In children, however, the case fatality rate with encephalitis is as high as 35 %. The infection/viremia generates a brisk and promptly effective (neutralizing) antibody response in the host and hyperimmune serum provides effective passive immunity.(2) The IgG subclass responses to natural infection and vaccination have not been well described for the VEE virus. The role of serum neutralizing antibody in preventing viremia may not be sufficient in protecting the host against mucosal infection when droplet or small particle aerosols are involved. Understanding the role of different immunoglobulin isotypes and subclasses in providing protective, viral neutralizing humoral immune responses may improve understanding of future observations of variations in clinical responses to different vaccine constructs.

Two types of vaccines are currently available for the prevention of VEE in man and horses.(1) TC-83, a live attenuated vaccine produced by serial passage of the wild virus utilizing guinea pig fetal heart cell culture, has proved to be efficacious (providing long term immunity) and

relatively safe for immunizing horses and man. However, up to 25 percent of individuals vaccinated develop clinical illness with a low grade viremia.(3) In addition, this vaccine may have abortogenic and teratogenic potential and is relatively ineffective in boosting marginal antibody responses.(1,3)

C-84 is a formalin-inactivated vaccine derived from the TC-83 strain of virus which has been shown to be safe and effective in inducing serum antibody.(1,4) This vaccine produces only mild local and systemic reactions and induces a high titer of neutralizing antibody in both non-immune subjects after 3 immunizations and in sero-positive TC-83 recipients (positive booster effect).(4,5) This vaccine provides effective protection for experimental animals infected by virulent VEE strains by injection but not by aerosol.(6,7) The mechanism for this is not understood. Vaccinated humans have not been exposed to virulent virus to permit any conclusions about protective efficacy in man. The limited experience with protection of laboratory workers using primary C-84 does not provide adequate information regarding protection at the mucosal level. The excellent record of TC-83 in preventing laboratory infections is nevertheless mitigated by the clearly documented infections that have occured in a setting of waning serum neutralization titers against the offending serotype of VEE virus. (Franck and Peters, unpublished observations)

The purpose of the study was to develop specific and sensitive assays

for the measurement of IgG, IgG subclasses, IgA and IgM directed against VEE antigens. These assays were then applied to the evaluation of specific antibody responses to the live attenuated (TC-83) and the formalin-inactivated vaccine (C-84).

MATERIALS AND METHODS

Human sera positive and negative for VEE specific antibody by viral neutralization assay were obtained through the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Frederick, Maryland. These sera had been previously collected under Human Use Committee approved informed consent including permission for utilization in other studies as long as the confidentiality of records was maintained. Twenty sera were drawn from a serum bank of human subjects between the ages of 18 and 65 with no VEE exposure and no laboratory work history. These sera were utilized to define background acivity in each of the assays. Four groups of paired sera were drawn from prior vaccination programs for laboratory workers and defined as as follows and as outlined in Table 1:

GROUP A (GRP-A): subjects who received a single dose of TC-83 vaccine, N = 20;

GROUP B (GRP-B): subjects with a history of exposure to a TC-83 vaccine dose, waning immunity by viral neutralization assay, who received a single booster dose of C-84 vaccine, N = 19;

GROUP C (GRP-C): subjects with a history of C-84 vaccine exposure only,
C(a): PRE and POST a single C-84 vaccine dose, N = 19;
C(b): PRE and POST a second booster C-84 dose, N = 15.

Sera had been stored at minus 70 ^{o} centigrade for as long as 15

years without intervening freeze-thaw cycles.

VEE antigen was prepared at the USAMRIID facility as follows: TC-83 strain VEE concentrate grown in BHK-21 cell line (Clone 13); gradient purified preparation inactivated by 6 times 10^6 rads of cobalt irradiation. A positive pooled reference serum (with significant high titer 80% plaque reduction on viral neutralization assay) was prepared and utilized for initial testing. ELISA assays for VEE-specific IgG, IgA, IgM and IgG subclasses were developed utilizing previously established methodologies (8,9) and customized as necessary. Optimum coating concentrations and conditions were tested and fixed as follows:

1. Dynatech Immulon-2-flat microelisa plates (011-010-3650) were coated with VEE antigen using a carbonate buffer at pH 9.6 overnight at 4° C. Plates can be stored coated without loss of activity for at least a one week period. Each new batch of VEE antigen must be tested with the reference serum in order to decrease inter-assay variability with changes in antigen lots. In addition, storage of aliquoted antigen frozen at minus 70° C. longer than 6 months results in some decay of specific binding at a fixed coating concentration. Coating concentrations varied from a 1:150 dilution to a 1:400 dilution. Assays over time were noted to remain uniform if conditions were always corrected to the same reference serum curve.
2. As described previously, a VEE negative antigen derived from the same culture media as the positive antigen has been utilized to

correct for the background binding which was highly variable between individual sera. Alternate columns were subsequently coated with positive and negative antigen, and each serum was run in parallel with at least 4 dilutions on the same plate. Later experiments showed that different batches of VEE negative antigen gave quite similar results in terms of background binding and could therefore be used with any VEE positive antigen batch. We attempted to maintain as much uniformity as possible with batches of negative and positive antigen.

Specific reagents tested and utilized during the assays and special procedural considerations are outlined as follows:

1. Sera were tested at a dilution of 1:80 with a serial 1:2 dilution on the plate to include at least 4 dilutions. Post vaccine exposure sera were started at 1:160. IgG subclasses were tested at 1:40 and POST IgG tests were stared at titers of 1:320. Individual subject sera PRE and POST vaccination were always run in parallel on the same plate in order to decrease intra-assay variability and optimize titer-fold reproducibility (POST divided by PRE vaccine titers). Sera were diluted in phophate buffered saline (PBS) with 0.05% Tween-20 and 0.1% bovine serum albumin (0.02% azide as preservative) (PBS-Tween-BSA) and incubated overnight at 4^{O} C.
2. Detection of specific isotypes and subclasses utilized reagents tested in multiple experiments for specificity (using purified

myeloma proteins) and sensitivity. Final selection of the following reagents for the detection of antibody included the following: (NOTE: all reagents were diluted in PBS-Tween-BSA and incubated on the plate at least 90 minutes at 37 degrees C.)

a. Affinity-purified goat anti-human IgG, alkaline phosphatase conjugated (TAGO, Inc., Palo Alto, CA, catalogue no. 4300);

b. Affinity-purified goat anti-human IgM, alkaline phosphatase (AP) conjugated (TAGO catalogue number 4302);

c. Affinity-purified goat anti-human IgA, flourescein isothiocyanate (FITC) conjugated (TAGO catalogue number 4201) followed by an affinity-purified goat anti-FITC, AP conjugated (special order RD009 from TAGO, Inc.);

d. Mouse monoclonal antibodies specific for human IgG subclasses were selected for specificity following testing with a panel of purified G subclass myeloma proteins. The selected antisera are listed below and are currently recognized by the World Health Organization as specific for human IgG subclasses: G-1 (HP 6001); G-2 (HP 6014); G-3 (HP 6050); G-4 (HP 6025). These reagents were graciously provided by Dr. Reimer of the Center for Disease Control, Atlanta, GA.

3. Subsequent reagents utilized in the G subclass assays included affinity-purified goat anti-mouse-FITC conjugated (Coulter catalogue number 6602159) followed by the previously listed anti-FITC.

The intermediate steps between reagents and the final development step

with the substrate, p-nitrophenylphosphate (Sigma Chemicals), are well described previously.(8) Each assay was stopped with 3N NaOH solution.

Plates were read utilizing an MR-600 Dynatech Microflour reader utlized in conjunction with an Apple IIe computer and the software Immunosoft version 2.4. VEE antigen negative (VEE -) optical densities were subtracted from VEE positive (VEE +) antigen binding in parallel dilutions. The negative binding was significantly above plate/reagent background for the IgG, IgA, IgM, and IgG-2 assays but not for G-1 or G-3 (or G-4). The assays for VEE-specific IgG, G1 and G3 were standardized to the uniform reference curve and end-point titers were calculated using log-logit transformations and curve fitting.(9) Results are expressed in the inverse of the dilution titer. Since the pooled reference serum did not generate a greater than 4 point standard curve for IgA, IgM, G2 and G4, these assays were read as the positive dilution where the optical density was 1.5 times the reference curve lower plateau O.D. Reagent background for each of the assays was less than 0.200 O.D. Intra-assay and inter-assay variability was calculated by running 2 pairs of sera four times (2 determinations per plate, 4 plates per day) on 3 different days. There was no more than a twofold difference in titer of any given serum on any of the 4 plates and 8 determinations. The inter-assay coefficient of variation was less than 15% for low titer pair sera for all specific antibody types and less than 30% for high titer pairs.

Plaque-reduction neutralization tests were performed with heat-

inactivated sera in Vero cell monolayer cultures as previously described. (10) The titer of neutralization antibody was expressed as the highest dilution of serum that causes an 80% reduction in the number of plaque forming units. Viral neutralization assays specific for VEE are performed routinely at the USAMRIID laboratories and 80% plaque reduction titers (80% PR-VNA) for each of the sera studied were provided courtesy of J. Mangiofico.

STATISTICAL ANALYSIS

The geometric mean titers (with standard errors of the mean and the 95% confidence intervals) for VEE specific IgG, IgG subclasses 1 through 4, IgA, and IgM along with the 80% PR-VNA mean titer were calculated for each group using the NWA STATPACK Multi-function statistics library, Northwest Analytical, Inc., Version 3.1, Portland, Oregon. Additional statistical analysis, using the same software described above, included a comparison of geometric mean titers for significant differences using the unpaired t-test on the logarithms of the inverse titers (significance was confirmed by the non-parametric comparison using the Kruskall-Wallis analysis and Duncan's analysis of variance). Dynamic titer-fold increases were calculated by dividing POST vaccination antibody levels by the PRE vaccine levels for each isotype; geomteric means were calculated for each group. Again, comparisons between groups were made using the methods described above. Correlation between the 80% PR-VNA value and the different isotype and G subclass responses were calculated for each group using the Pearsons Product moment correlation with the Logarithm

(Log) transformed value of the respective titers.

Because a single subject of Asian background was noted to have a very high titer of VEE-specific IgG2 (1:40,960) in response to the TC-83 vaccine, 7 additional subjects of Asian descent were tested (post vaccine sera only) in order to determine if any racial variation in subclass responses could be determined. These results were not included in the group analysis because pre-vaccine sera were not available.

RESULTS

Table 2 summarizes the geometric means of the inverse antibody titers for VEE-specific 80% PR-VNA, IgG, IgG1, IgG3, IgG2, IgA and IgM prior to vaccination (PRE). In Groups Cb and Ca, 80% PR-VNA data was only available for 9 and 13 subjects respectively because of a shortage of serum and no recoverable historical data. Standard errors of the means are in parentheses. There was no significant difference between groups by parametric or non-parametric analysis. It is noteworthy that all 20 negative control serum samples from non-laboratory workers without an extensive immunization history demontrated optical density readings at 1:80 serum dilution less than 0.2 for all isotypes and G subclasses except for IgM where 2 of the 20 subjects fulfilled this criteria for a titer of 1:160. There was a greater variation in baseline titers noted in the study groups than the negative controls. Higher PRE titers for GRP-B and GRP-C(b) could be explained by antecedent vaccine exposure history and variations in persistence of antibody.

Table 3 summarizes the geometric means of the inverse antibody titers for VEE-specific 80% PR-VNA, IgG, IgG1, IgG3, IgG2, IgA and IgM POST vaccination. Standard errors of the means are in parentheses. There is a significant difference ($P < 0.01$) by both parametric and non-paramteric analysis for GRP-A versus GRP-B for the G1 subclass (with the greater response in the TC-83/C-84 booster GRP-B) and a marginally significant difference for IgA ($P = 0.02$) and G2 ($P = 0.04$). The 80% PR-VNA for GRP-A versus GRP-B was only significantly different by Duncan's ANOVA ($P < 0.01$). Similar differences ($P < 0.01$) were noted in a comparison of GRP-A and GRP-B with GRP-C(b) with G1, IgA and IgM being most significant, G2 marginally, and IgG for GRP-B versus GRP-C(a) being very significant as well. For varying isotypes and IgG subclasses, there was a prominent difference in the comparison of GRP-C(a) versus GRP-A ($P < 0.001$), versus GRP-B ($P < 0.001$ for G, G1, G3; $P < 0.05$ for IgA; $P < 0.02$ for IgM), versus GRP-C(b) ($P < 0.01$ for G, G1, G3). There was no G4 subclass response in any groups and the strongest IgA response was noted in the groups exposed at some time to TC-83. Of interest, the strongest 80% PR-VNA, IgG, G1, G3 and IgM responses were noted in the C-84 booster group that had been exposed to TC-83 in the past.

Table 4 summarizes the geometric means (with standard errors of the mean) for titer-fold increases for each group by isotype, G subclass or 80% PR-VNA. Although some of the stastical differences are comparable to those noted for Table 3, they are different because the higher PRE vaccine titers seen in the booster GRP-B and GRP-C(b) decreases the

relative increases seen; this is particularly noted for IgG, IgA and IgM when comparing GRP-A versus GRP-B (no longer significantly different).

Table 5 summarizes, for each group, the significant correlations (R values with $P < 0.01$) between the 80% PR-VNA titer and the individual VEE specific antibody responses. It is noteworthy that a significant IgA response is observed in the groups who received the live attenuated vaccine TC-83 but not in the groups having received the killed vaccine C84 alone (either 1 or 2 doses). All groups demonstrated a significant correlation for IgG, G1 and G3 but the G2 response is only noted in the TC-83 groups.

Figure 1 graphically displays the comparison of geometric mean antibody levels for each of the 4 groups (PRE to POST vaccine exposure) for IgG, IgA and IgM. Figure 2 provides a similar graphic diplay but for the IgG subclasses 1, 3, and 2. These illustrations again emphasize the dramatic differences in response for VEE specific IgA and IgG2 between the TC-83 and C-84 only groups.

CONCLUSIONS

A single exposure to the live-attenuated Venezuelan equine encephalitis vaccine TC-83 generates a brisk antibody response with protective titers as measured by the plaque reduction viral neutralization assay. This response is paralleled in the IgG, IgA and IgM response with the IgG represented dominantly by the IgG1 and IgG3 subclasses. No IgG4 was observed and IgG2 booster responses were generally low with less than a

four fold increase in a majority of patients. However, the most consistent IgG2 responses were noted with primary TC-83 exposure. In subjects with a prior exposure to TC-83 but loss of protective titers by 80% PR-VNA, the killed vaccine C-84 provides an effective booster response in essentially the same antibody distribution. There is a significantly increased IgG and IgG1 response with the C-84 booster compared to a single dose of TC-83. Once again, the IgA response is dominant with TC-83 challenge. The IgG3 booster response is most prominent with the C-84 booster both for the subjects who had seen TC-83 and those only exposed to the killed vaccine C-84.

The observation that subjects receiving C-84 vaccine only (1 or 2 boosters) did not mount a significant IgA response is of interest because of earlier observations that protection against aerosal infection with VEE was not consistent in hamsters that had only received C-84 inactivated vaccine.(6) IgA is the dominant secretory antibody providing protection at mucosal surfaces. Virulent VEE is considered a neurotropic virus, and it exhibits significant infectivity via the respiartory tract. The potential of VEE virus to invade the central nervous system via the cribriform plate has been documented for nonhuman primates (7,11), and in hamsters VEE virus has been shown to invade the olfactory bulb.(12) Mucosal immunity may prevent invasion of the cribriform plate. IgA may be a critical isotype for this type of protection yet some patients with IgA deficiency do not appear to be more susceptible to mucosal viral

infections than normal individuals.(13) Live replicating antigen is superior to inactived antigen in local immunity in other infectious disease systems such polio(14); and this might be attributed to the differences in stimulation of IgA secretion. Subsequent work will need to focus on understanding in vivo differences in risk of infection in association with the types of vaccine responses noted serologically. It is interesting to note that the viral neutralization assay provides the best marker of clinical "protection" and this assay is completely independent of complement activity which also does not play a role in IgA mediated defense.

Immunoglobulin responses to a wide range of infectious agents have been associated with protective immunity, either short or long term. In the case of viral infections, total virus-specific antibody measurements may not quantitatively correlate with in vitro neutralization potency or with the degree of in vivo effective natural immunity. These observed variations may in some cases be due to differences in viral antigens recognized, but they may also reflect differences in isotype or IgG subclass responses that have different efficiency in mediating effective immunity.

Antigen-specific IgG production is generally associated with long-term immunity to a wide range of bacterial and viral infectious agents.(15,16) Human IgG is subdivided into 4 subclasses, each having distinct biologic properties and functions.(15)

Virus specific IgG subclass responses to vaccines may vary with vaccine

type and may play a role in the difference in protective potential between the inactivated versus live attenuated vaccines. Selective stimulation of certain subclasses of antibody may be more important in the future when adjuvants and smaller antigens may be used for immunizing agents. In addition, the understanding of the IgG subclass predominantly associated with neutralizing antibody could be useful if serum or monoclonal antibodies were to be used for passive immunization against VEE. It is interesting to note that IgG1 and IgG3 were the dominant antibodies associated with a vaccine response and these are the most efficient complement activating subclasses, further enhancing their potential role in protective immunity. In contrast to such inactivated viral vaccines vaccines such as hepatitis B surface antigen, where a significant IgG4 response has been demonstrated, neither VEE vaccine elicited any response in this subclass. This may be related to the fact that one is a protein antigen rather than whole virus vaccine such as the VEE vaccines.

The search for an improved understanding of the mechanisms underlying protection from viral infections after vaccination is an essential part of future vaccine development which will include recombinant DNA constructs and represent only limited antigenic epitopes.(17) Aside from identifying epitopes whose binding results in viral neutralization, efficacy may also be determined by the type of humoral immune response generated with a particular vaccine construct.

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TABLE 1: GEOM MEANS (SEM)
VEE-SPECIFIC ANTIBODY TITERS PRE VACCINES

VEE AB	GRP-A n=20	GRP-B n=19	GRP-C(b) n=15	GRP-C(a) n=19
G-TOTAL	143(1.6)	217(1.8)	150(2.4)	164(2.5)
G-1	55(1.4)	77(2.6)	47(1.5)	45(1.4)
G-3	53(1.7)	52(1.6)	50(1.7)	51(1.6)
G-2	96(2.1)	83(2.1)	66(1.8)	52(1.5)
A	80(2.1)	70(2.8)	52(1.7)	50(1.7)
M	178(2.5)	177(1.7)	153(2.0)	124(2.0)

NOTE: sera (at 1:10) showed no viral neutralization by *in-vitro* 80% plaque reduction assay

GRP-C: a. C-84 VACCINE, 1st Dose; b. C-84, 2nd Dose
GRP-A: TC83 ONLY
GRP-B: TC83/C84 BOOSTER

TABLE 2: GEOM MEANS (SEM)
VEE-SPECIFIC ANTIBODY TITERS POST VACCINES

VEE AB	GRP-A n=20	GRP-B n=19	GRP-C(b) n=15	GRP-C(a) n=19
80% PR-VNA	171(4.0)	499(7.5)	127(5.5) n=9	19(5.9) n=13
G-TOTAL	6386(2.3)	10054(3.9)	2777(3.0)	738(1.9)
G-1	522(2.3)	1793(4.3)	294(2.5)	67(1.9)
G-3	535(3.0)	987(6.6)	367(5.2)	111(1.9)
G-2	286(3.4)	131(2.9)	61(1.6)	46(1.4)
A	1707(2.3)	811(3.0)	110(2.7)	75(2.0)
M	3721(2.7)	1317(4.5)	378(2.9)	320(3.0)

80%PR-VNA: 80% PLAQUE REDUCTION (NEUTRALIZATION ASSAY)
GRP-C: a. C-84 VACCINE, 1st Dose; b. C-84, 2nd Dose
GRP-A: TC83 ONLY
GRP-B: TC83/C84 BOOSTER

TABLE 3: POST VEE VACCINE

GEOM MEAN (SEM) TITER-FOLD INCREASES

VEE AB	GRP-A n=19	GRP-B n=20	GRP-C(b) n=15	GRP-C(a) n=19
80% PR-VNA	172(4.0)	499(7.5)	127(5.5) n=9	19(5.9) n=13
G-TOTAL	46(2.5)	46(3.9)	19(5.1)	4.5(2.6)
G-1	9.5(2.7)	23(5.2)	6.2(2.8)	1.5(1.9)
G-3	9.2(2.9)	21(8.5)	7.3(6.4)	2.2(2.0)
G-2	2.0(2.9)	1.6(2.3)	1.0(1.4)	0.9(1.3)
A	21(2.9)	11.6(4.2)	2.1(2.7)	1.5(1.9)
M	20(3.2)	7.4(4.4)	2.5(3.5)	2.6(3.8)

80%PR-VNA: % PLAQUE REDUCTION (NEUTRALIZATION ASSAY)
GRP-C: a. C-84 VACCINE, 1st Dose; b. C-84, 2nd Dose
GRP-A: TC83 ONLY
GRP-B: TC83/C84 BOOSTER

TABLE 4: CORRELATION R VALUES ($P < 0.01$)
80% PR-VNA VERSUS VEE-SPECIFIC ANTIBODY

80% PR-VNA VERSUS:	GRP-A n=38	GRP-B n=40	GRP-C(b) n=18	GRP-C(a) n=26
G-TOTAL	0.89	0.94	0.85	0.61
G-1	0.83	0.86	0.87	0.48
G-3	0.75	0.83	0.73	0.46
G-2	0.40	0.30	----	----
A	0.87	0.82	----	----
M	0.85	0.66	0.53	----

PR-VNA: % PLAQUE REDUCTION (NEUTRALIZATION ASSAY)
GRP-C: a. C-84 VACCINE, 1st Dose; b. C-84, 2nd Dose
GRP-A: TC83 ONLY
GRP-B: TC83/C84 BOOSTER

VEE-Specific Antibody Responses

to TC83 (Grp A), TC83/C84 Booster (Grp B), and C84 (Grp C: 2 doses (b), 1 dose (a))

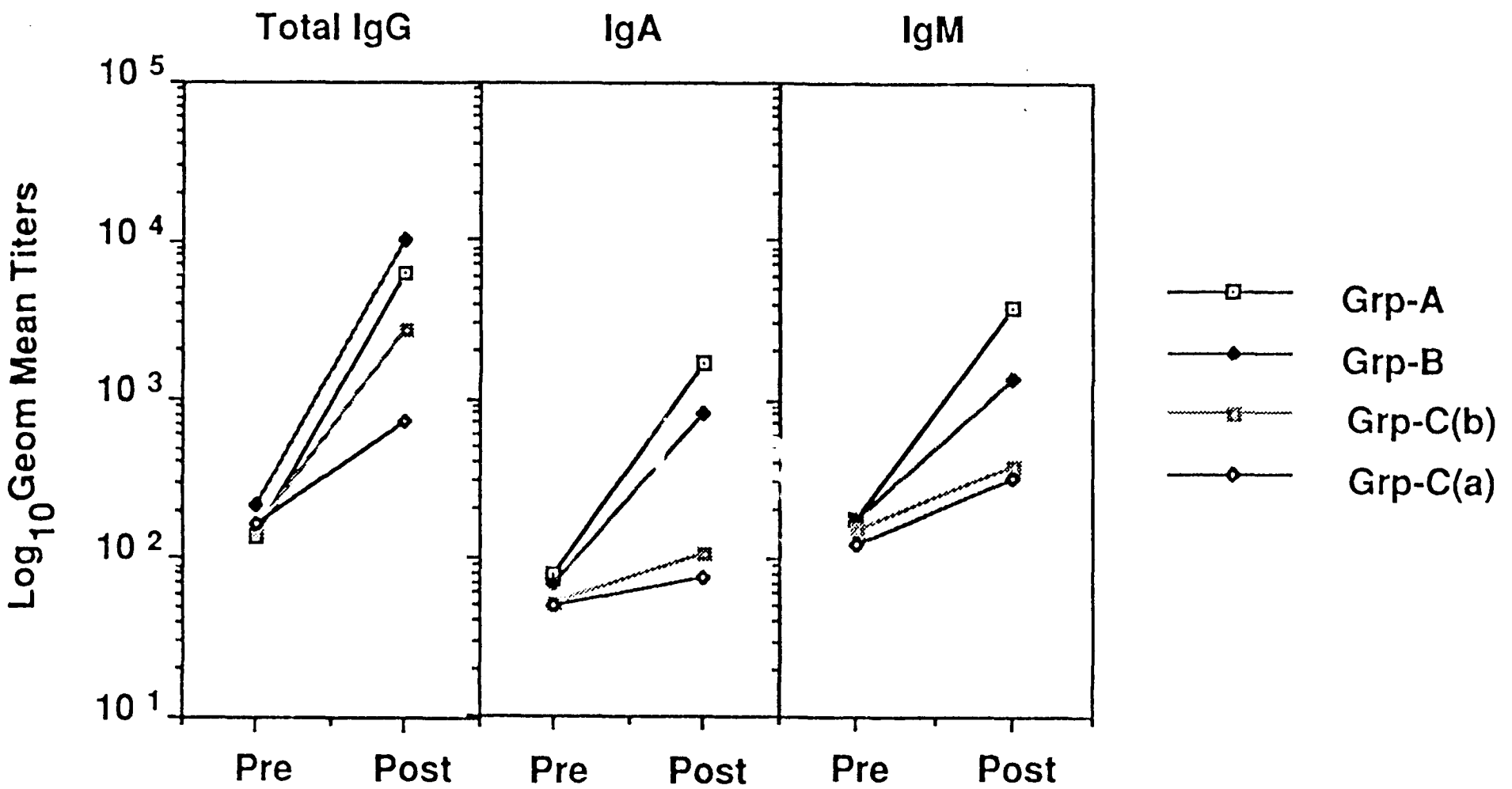
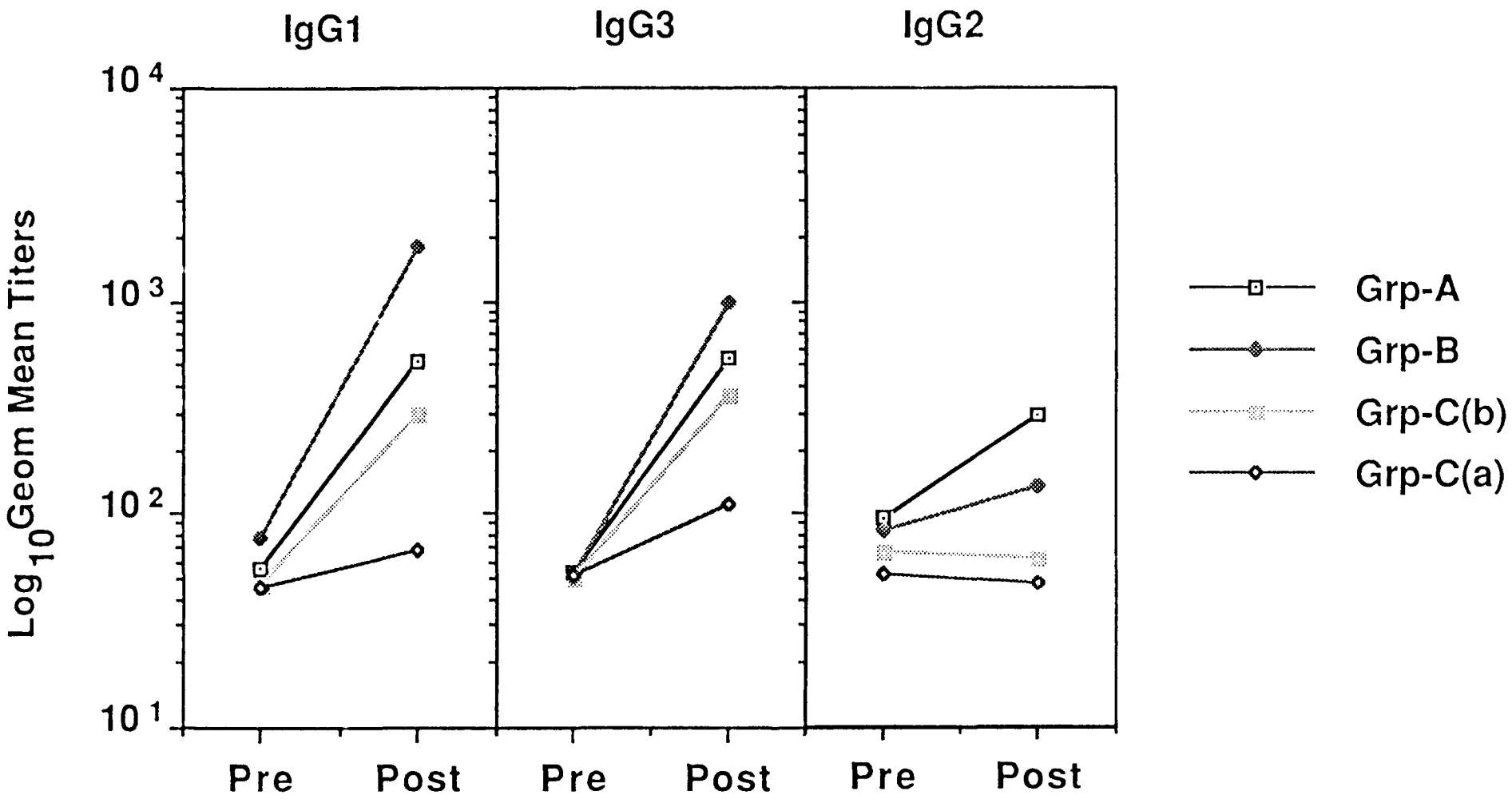


FIGURE 2

VEE-Specific Antibody Responses

to TC83 (Grp A), TC83/C84 Booster (Grp B); and C84 (Grp C: 2 doses (b), 1 dose (a))



KEY WORDS: LASSA FEVER; IgG SUBCLASSES; ARENAVIRUS/HEMORRHAGIC FEVER

LASSA FEVER SPECIFIC ANTIBODY

Lassa Fever (LF), one of the rodent borne Arenaviruses producing acute hemorrhagic fever in man, can cause a mild to severe and fatal (in up to 20% of cases) systemic disease as a result of microvascular damage and changes in vascular permeability.(1,2) The virus is found predominantly in West Africa but is related to the Argentine and Bolivian hemorrhagic fevers (Junin and Machupo) found in South America.

Immunologically, this disease is associated with late appearance of neutralizing antibody, and serum from the early convalescent phase of the disease does not provide passive immunity.(3) In contrast, sensitized spleen cells have been shown to provide passive protection. No effective vaccine is currently available and this virus is representative of a group of RNA viruses that generate an initial antibody response with natural infection that does not provide effective immunity. At the same time, in the primate model, passive administration of neutralizing antibody can significantly decrease mortality particularly when administered early in the disease course or in combination with antiviral agents such as ribavirin.(4,5) Also of interest with this disease, the quantitative levels of viral specific antibody are not significantly different between the early and late convalescent phase of the disease, yet neutralizing capability of this antibody is significantly different.(5) It is possible that this

difference is due to the virus peptide specificity of the antibody or to its avidity. However, Western blot studies have shown that non-protective early convalescent antibody does react with all three virion peptides.(Jahrling, unpublished observations) Thus, this difference may be related to IgG subclass differences; if so, plasma screening of G-subclass specific anti-viral antibody may be useful in the selection of optimum donors for the preparation of hyperimmune globulin. In addition, the efficacy of an anti-viral monoclonal antibody may be dependent on the subclass created.

Passive immunoglobulin may play a role in both treatment and prevention, particularly if effective monoclonal antibodies could be developed. Furthermore, immunogens under development for a variety of human pathogens may well elicit a more restricted isotype response than natural infection or currently utilized vaccines. Thus, knowledge of specific isotypes or IgG subclasses associated with immunity is important.

Immunoglobulin responses to a wide range of infectious agents have been associated with protective immunity, either short or long term. However, in the case of viral infections, virus-specific anti-body measurements are not consistently correlated with <u>in vitro</u> neutralization potency or with effective natural immunity. These observed variations may in some cases be due to differences in viral antigens recognized, but they may also reflect differences in isotype or IgG subclass responses that have different efficiency in mediating effective immunity.

Antigen-specific IgG production is generally associated with

long-term immunity to a wide range of bacterial and viral infectious agents.(7,8) Human IgG is subdivided into 4 subclasses, each having distinct biologic properties and functions. IgG1 and IgG3 are the most efficient in their complement binding and attach to monocytes, neutrophils and platelets with the greatest affinity. IgG2 and IgG4 are the principal surface immunoglobulins on B cells and are associated with polysaccharide antigen responses.(7)

In the treatment of LF, it may be important to assure that hyperimmune globulin or monoclonal antibody preparations reflect the subclasses of IgG that are associated with optimum viral neutralization and/or the convalescence of a natural infection.(1-3) These same considerations apply to the antibody responses elicited by vaccines.

EXPERIMENTAL METHODS

A panel of sera from Sierra Leon have been collected from the serum bank of USAMRIID; these sera are representative of subjects who had been infected with the Lassa virus or had no history of exposure and no neutralizing antibody. These sera were used to prepare a positive pooled sera as a reference and provided a panel of negative control sera. Multiple batches of inactivated Lassa antigen were tested using passive coating techniques, special blocking techniques and inhibition strategies in order to determine isotype and IgG subclass responses. Unfortunately after a series of experiments, it has become clear that as described in reference 6, this may not be a feasable approach and a supply of antigen capture

antibody will be required in order to proceed with this phase of the study in the future.

Additional experiments with capture antibody stored at Fort Detrick were performed. Unfortunately, the antibody prurification plus strorage conditions appeared to have damaged the quality of the capture antibody again preventing any meaningful studies to be performed with the available serum samples. It will be necessary to produce a new batch of preferrably monoclonal antigen capture antibody in the future but will require more time than the current grant period allows.

Continued attempts to evaluate alternative antigen sources and antibody capture techniques have failed to provide a sufficiently specific and sensitive assay for the measurement isotype and IgG subclass specific responses.

CONCLUSIONS

Although the original purpose of this part of the grant proposal remains worthy of study, technical and administrative difficulties as outlined have made it impossible to achieve the desired goals. With the expansion of interest in recombinant vaccine constructs it appears even more essential to fully understand the nature of the immune response with natural infection and what defines protective immunity. An organization of test sera amenable to further study was achieved and this can be utilized in future experiments pursuing the questions outlined.

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