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13. ABSTRACT (Maximum 200 words)

During the contract year August 1, 1990 through July 31, 1991, we made substantial progress in several important aspects of our goal to define a strategy to employ synthetic vaccine strategies to immunization against arenavirus infections:

1. Refinements of our model of the arenavirus spike structure to include the GP-1/GP-2 interaction and the predicted conformation of GP-2 and its interaction with the membrane and viral nucleocapsid "core".

2. To define the ionic bonding interaction between GP-1 and GP-2 that stabilizes the tetrameric spike.

3. Refinement and extension of the antibody-mediated protection demonstrated against acute LCMV infection.

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FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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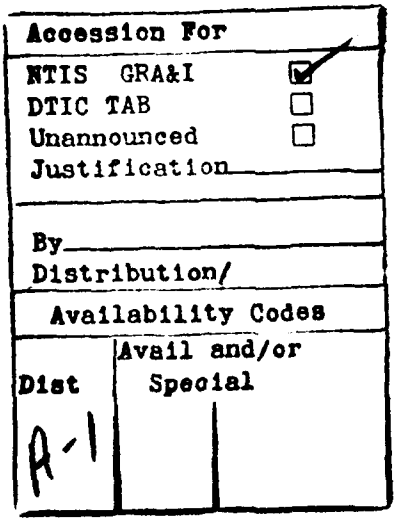

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A. Introduction and Overview

During the contract year 1 Aug 1990 - 31 Jul 1991 we made substantial progress in several important aspects of our goal to define a strategy to employ synthetic vaccine strategies to immunization against arenavirus infections. Highlights of this progress are the following:

1. Refinements of our model of the arenavirus spike structure to include the GP-1/GP-2 interaction and the predicted conformation of GP-2 and its interaction with the membrane and viral nucleocapsid "core." This understanding has allowed us to develop a purification scheme which yields homogenous GP-1 monomers in native form, and to precisely map the internal and external domains of GP-2 and their interaction with the membrane. On this model we can now superimpose conserved GP-2 epitopes defined previously by us (Weber and Buchmeier, 1987) with support from this contract, and cytotoxic T-cell epitopes in GP-2 as defined by the Oldstone group (Whitton et al., 1988a,b).

2. To define the ionic bonding interaction between GP-1 and GP-2 that stabilizes the tetrameric spike. This demonstration of an ionic interaction between GP-1 and GP-2 has allowed purification of native GP-1 monomers from both Old World (LCMV) and New World (Pichinde and Tacaribe) viruses and unifies the model of glycoprotein processing for all of the arenaviruses. Before this demonstration it was widely believed and published that Tacaribe and perhaps also Junin contained only a single glycoprotein molecule "G," while the Old World viruses clearly contained two glycoproteins, "GP-1" and "GP-2," derived by cleavage of the GP-C polyprotein.

3. Refinement and extension of the antibody-mediated protection demonstrated against acute LCMV infection. Our studies during the past year have explored the role of complement in protection, role of antibody subclass, and requirements for antibody Fc region domains. Moreover, we have developed a mother-baby model of protection that clearly demonstrates passive transfer of protective antibody from passively or actively immunized mothers to their offspring.

Details of our progress in each of these areas is presented below.

B. Refinement of a model of the structure of the arenavirus spike structure.

A priori computer predictions of the conformation of proteins such as the arenavirus GP-C polyprotein are of mixed value (Jennings, 1989). On one hand they provide a basis to predict with reasonable certainty gross topographical features such as N-terminal signal sequences and transmembrane domains. These methods are less reliable however when asked to predict more complex secondary and tertiary structures. We have used two such programs, the Chou-Fasman (1978) and Garnier-Osgethorpe-Robson

(1978) algorithms, to attempt to predict the secondary and tertiary structure of GP-C. Figure 1 illustrates the results summarized in one figure by the plot structure (University of Wisconsin Genetics Computer Group software package) utility. Note in the Hopp and Woods (1981) hydrophilicity plot at the top that there are two extensive regions of hydrophobic peaks (peaks with negative overall values). These correspond to the 58 amino acid N-terminal signal sequence (residues 1-58) which we described in the previous Annual Report, and the predicted transmembrane domain near the C-terminus of GP-C (boxed residues 432-458). Note as well a sequence near the amino terminal end of GP-2 (amino acids 266-290; dashed box) which is predicted by both the Chou-Fasman and Garnier algorithms to contain beta sheet-betaturn-beta sheet configuration. This sequence corresponds to the major GP-2 CTL epitope described by Whitton et al. (1988a) for GP-2 of LCMV. Between the CTL site and the transmembrane domain is a predicted extended alpha helix punctuated by a short beta sheet region at 370-390. In the GP-1 sequence there is little evidence of consistency between the two programs, although there are several predicted alpha helical and beta sheet regions predicted by both programs which are consistent with a globular structure.

Looking more closely at these structures we sought evidence of conservation among known GP-C sequences for various arenaviruses. Examining the hairpin loop, membrane spanning and carboxy terminal cytoplasmic domains (Figure 2), it was evident that there was a high degree of sequence similarity between LCMV and Lassa and less between the Old World (LCM, ARM and WE; Lassa) viruses and the New World viruses (Tacaribe and Pichinde) in these regions. A similarity of considerable potential interest was observed in the region of predicted extended alpha helix in GP-2 (between GP-C residues 318 and 370). In this region which contains an extended alpha helical "heptad repeat" reported by Auperin et al. for Lassa virus (1986), we found that this predicted heptad repeat structure was conserved for all of the viruses for which sequence information is available. Although the amino acid sequences diverged (Figure 3), the predicted heptad repeat periodicity indicative of alpha helix was conserved. This type of structure is found in the stalk region of Influenza HA (Wilson et al., 1981) and of the coronavirus spike glycoprotein S (deGroot et al., 1987), suggesting that this region of GP-2 serves as the stalk for the arenavirus spike. Also of interest is the fact that the highly conserved B cell antigenic site described by this laboratory (Weber and Buchmeier, 1987) lies immediately adjacent to the heptad repeat in amino acids 370-380.

To get a better look at the spike we performed cryoelectron microscopic (Milligan et al., 1984) examination on highly purified LCMV. This technique, which involves no fixation, images the virus directly in vitreous ice and offers minimal distortion of surface structures such as the spike. Purified LCMV-ARM (1 mg/ml; > 10^{10} pfu/ml) in TNE buffer was applied to carbon coated holey support films on grids. The grids were

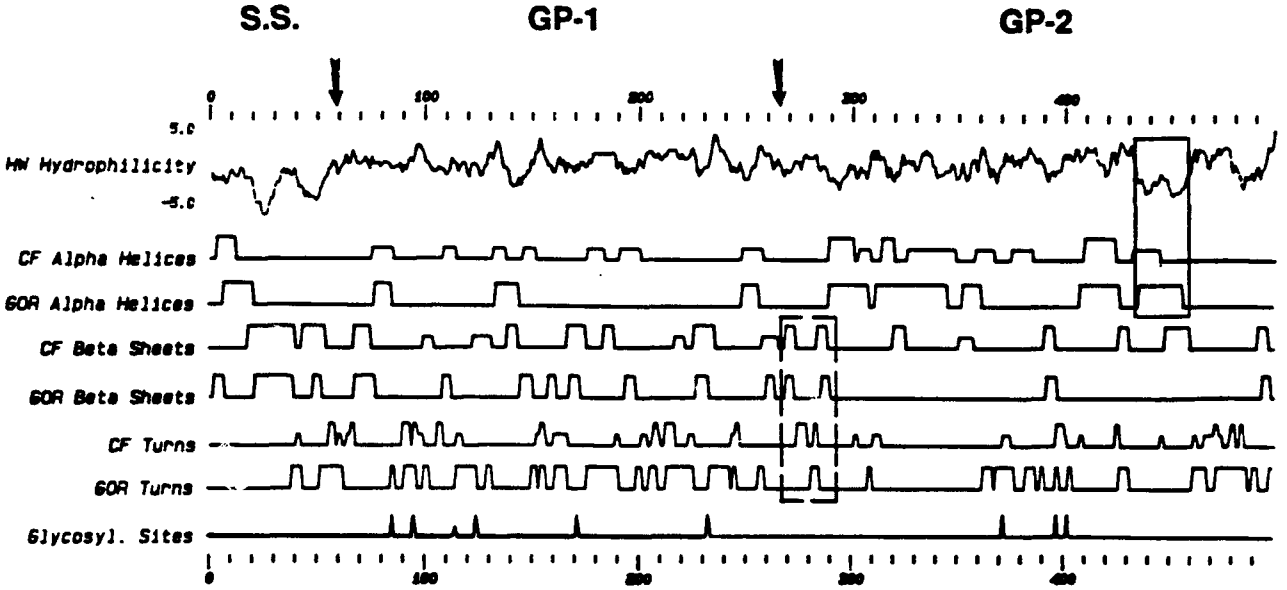


Figure 1. Predicted secondary structure of the LCMV glycoprotein precursor, GP-C. Predicted secondary structure plots of LCMV GP-C were generated on a VAX computer using the reported amino acid sequence of GP-C and the predictive algorithms of Hopp and Woods (1988), Chou and Fasman (1978) and Garnier, Osguthorpe and Robson (1978). The location of potential N-linked glycosylation sites are identified based on the presence of the amino acid consensus Asn-X-Ser/Thr. The proposed signal sequence (S.S.) and GP-1:GP-2 cleavage sites are indicated on the top line. The predicted beta sheet-reverse turn-beta sheet region of the proposed hairpin loop (dashed box) and the hydrophobic alpha-helical membrane spanning domain (solid box) of GP-2 are marked.

1. Hairpin Loop Structure:

Arm:	G T F T W T L S D S S G V E N P G G Y C L T K W M I L
WE:	- -
Lassa:	- - - - - - - - - - E - K D T - - - - - - - R - - L I
Pichinde:	- F - - - D - - - - - - Q H V - - - - - - E Q - A - I
Tacaribe:	A F - S - S - T - P L - M - A - - - - - - S - - - L V

2. Membrane Spanning Domain:

Arm:	P L A L M D L L M F S T S A Y L V S I F L H L V K I P
WE:	- - - - - - - - - - - - - - - - - I - - - - - F - R - -
Lassa:	- - G - V - - F V - - - - F - - I - - - - - - - - - -
Pichinde:	- - - - T - I C F W - L V F - T I T V - - - I - G - -
Tacaribe:	- I T - V - I C F W - - V F F T S T L - - - - I G F -

3. Carboxy-Terminal Cytoplasmic Domain:

Arm:	C S C G A F K V P G V K T V W K R R
WE:	- - - - - - - - - - - - - I - - - -
Lassa:	- - - - L Y - Q - - - P V K - - -
Pichinde:	- - - - Y Y - Y G R N L - N G
Tacaribe:	- R - - K Y L P L K K P - I - H - - H

Figure 2. Comparison of the sequences of topographic landmarks of several arenavirus glycoproteins.

CONSERVATION OF HEPTAD REPEAT REGIONS AMONG ARENAVIRUS GLYCOPROTEINS SUGGESTS A COMMON ALPHA-HELICAL AND COILED-COIL STRUCTURE

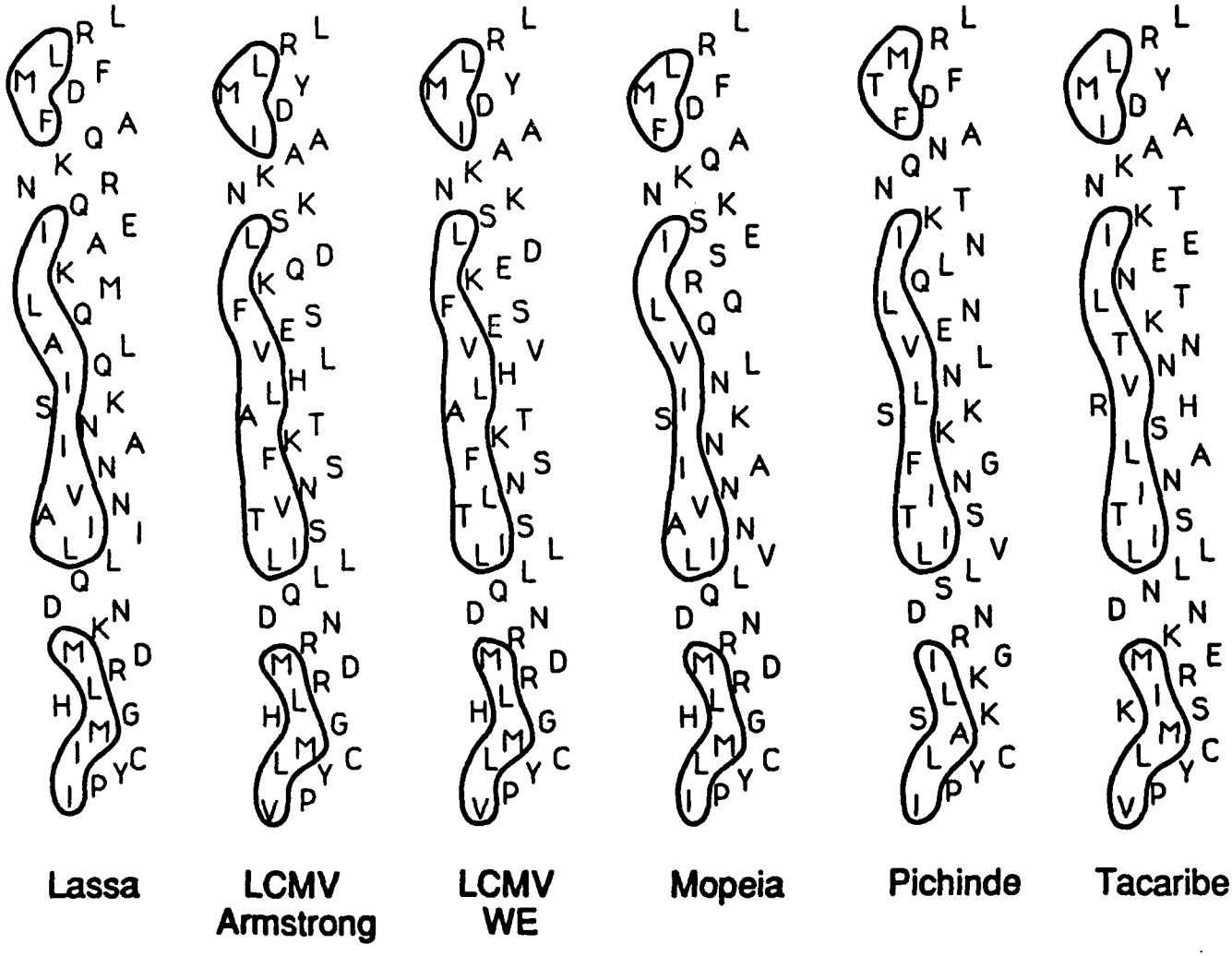


Figure 3. Helix-net array of the heptad repeat regions of the arenavirus GP-2 (equivalent) molecules. The published amino acid sequences of the arenavirus GP-2 heptad repeat regions are depicted in side-by-side helix net arrays aligned with the reported coiled coil domain of Lassa virus. Amino acids 309 (F) and 360 (I) of Lassa virus aare marked for reference. The hydrophobic face of each GP-2 molecule is outlined to illustrate the high degree of conservation among the viruses.

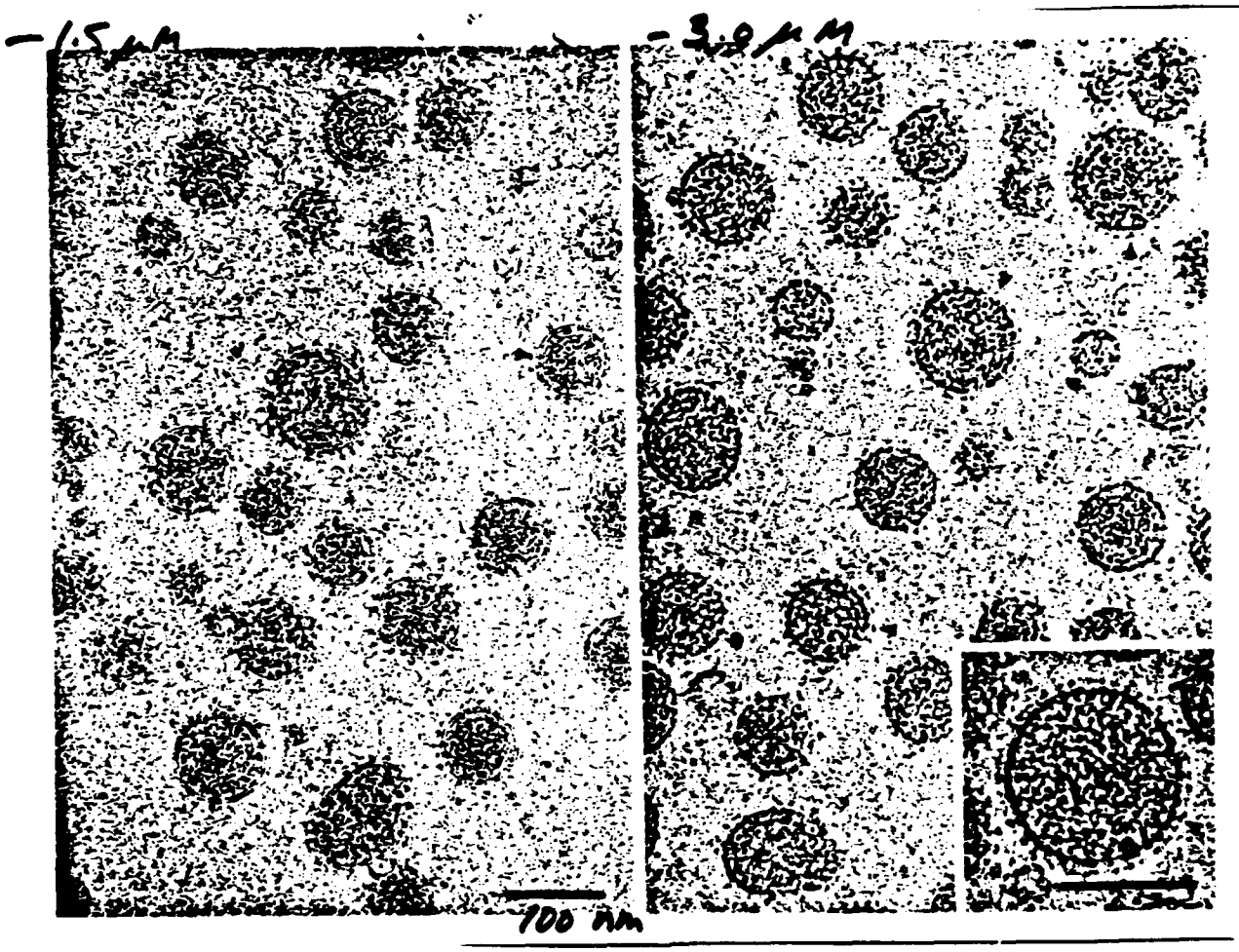
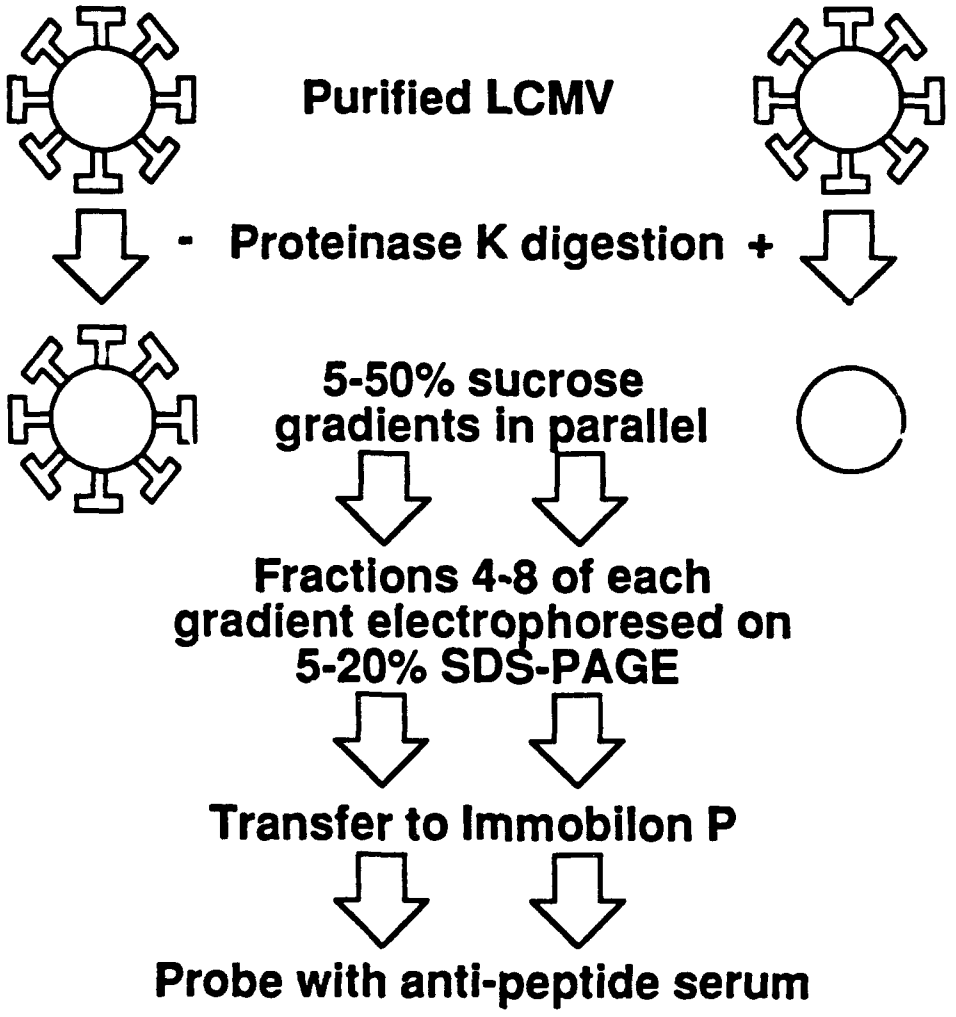


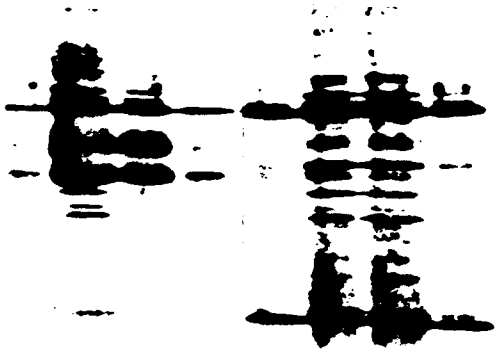
Figure 4. Cryo-electron micrographs of purified LCM virions. Purified LCMV preparations were analyzed by cryo-electron microscopy using defocus values of 1.5 u (left panel) or 3.0 u (right panel and inset). In the left panel the lipid bilayer of the virion envelope is emphasized (see arrow head). The right panel and inset emphasize the surface topography and t-shaped glycoprotein spikes (see arrow heads). Magnification of the left and right panels is 167,100x and the inset magnification is 232,750x. Bar scale equals 1000 A.

blotted and quick-frozen in liquid ethane slush in LN_2 and stored in CN_2. For examination the grids were mounted on a cold stage and visualized using a Phillips CM12T electron microscope at 100 kV. A number of images were taken at various levels of defocus to emphasize various aspects of the virion structure. As evident in Figure 4, the virions were spherical particles of variable diameter consisting of a dense (nucleoprotein) core enclosed by a lipid bilayer. The outer surface of the bilayer is studded with projections, the LCMV glycoprotein spikes. At a focus of -1.5 microns, where 50 A spacings are emphasized in the images, the bilayer is clearly visible (Figure 4, left panel). The high electron-scattering density of the phosphate head groups of the lipids give rise to the characteristic trilamellar appearance of the bilayer. The spikes, while apparent in these images, are more clearly visualized in more strongly defocussed images (-3.0 microns, Figure 4, right panel). Details in the images suggest that the spikes are T-shaped, with the rodlike stalk anchored in the lipid bilayer and the crosspieces lying parallel to the bilayer at a distance of approximately 80-100 A (8-10 nm) from the surface.

Having established these features of the spike structure, we sought to establish precisely the interaction of GP-2 with the envelope. Previous studies from this laboratory (last Annual Report; Burns and Buchmeier, 1991) established that GP-2 was an integral membrane protein which could be crosslinked to the nucleocapsid protein, NP, using the membrane-permeable crosslinker dimethyl suberimidate (DMS). Briefly, we performed an exhaustive proteinase K digestion of highly purified LCMV followed by rebanding of the virus and separation and identification of the proteins remaining in the digested virions by SDS-PAGE and Western blotting. A schematic diagram of this protocol is represented in Figure 5. Peptide antisera used for this experiment corresponded to amino acids 130-144 of NP (antibody A), amino acids 59-79 of GP-C (1-20 of GP-1; antiserum B) and residues 483-498 of GP-C (exact C-terminus of GP-2; antiserum C). Figure 6 illustrates the results of this experiment. NP remained unaltered after proteinase K digestion due to its internal localization in the virion. GP-1 and GP-2 in contrast were largely digested by PK and a new polypeptide band of approximately 7800 da was evident. This band was detected only with antiserum C and not with A or B or with two additional peptide antisera representing amino acids 272-285 or 378-391 of GP-C. We sequenced this low molecular weight band by Edman microsequencing and identified an unambiguous N terminal sequence of gly-ser-thr-pro-leu, which corresponds to residues 430-434 of GP-C. Based on this data we conclude that the spike is anchored by a 68 amino acid (430-498) transmembrane and cytoplasmic domain at the C-terminus of GP-2. This domain includes the predicted transmembrane hydrophobic domain (Figure 1) and interestingly, in LCMV also contains five basic amino acids (Lys or Arg) in the last 12 residues at the C terminus of the cytoplasmic tail. These residues are likely to interact with the viral RNA and/or ribonucleoprotein complex within the virion. Considering all of the accumulated data we feel confident in proposing a working

Sample Preparation and Immunoblotting of Control and Proteinase K-Digested Virions to Establish the Membrane Orientation of GP-2

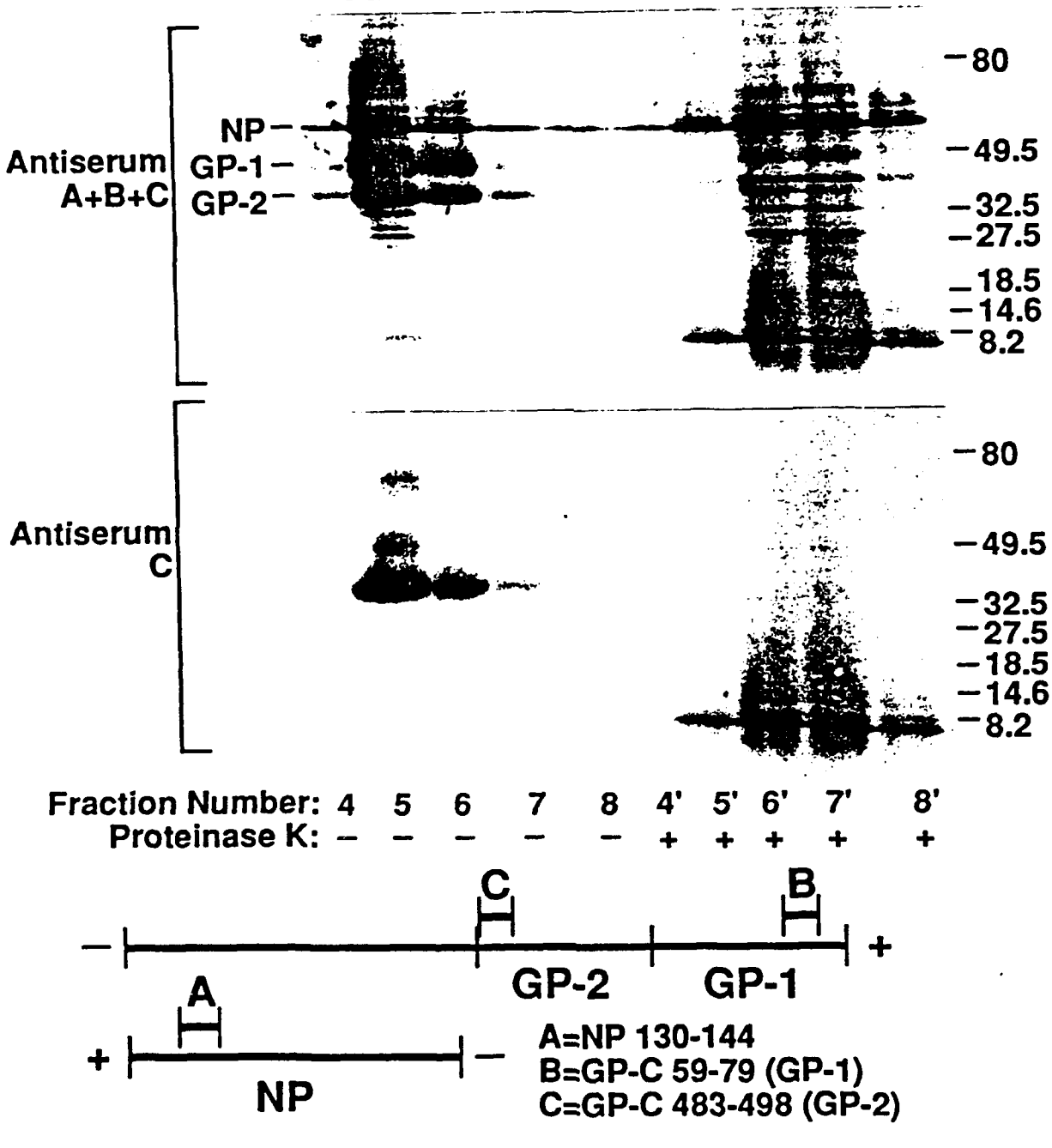




Control Digested

Figure 6. Isolation of a transmembrane fragment of GP-2. Purified LCM virions were digested with Proteinase K (500 ug/ml) for 30 min. at 37C. Digestion was terminated by the addition of PMSF to a final concentration of 25 mM. Control (non-digested) virions were incubated and PMSF treated in parallel. The protease-digested and control virus preparations were re-purified on 5-50% sucrose (w/w) gradients and fractionated by bottom puncture. Aliquots of fractions containing control (lanes 4, 5, 6, 7 and 8) or protease digested (lanes 4', 5', 6', 7' and 8') virions were analyzed on parallel immunoblots after disruption and electrophoresis on 5-20% SDS-polyacrylamide gradient gels. The blot shown in the upper panel was probed with a mixture of rabbit anti-peptide sera A, B and C (specific for NP, GP-1 and the carboxy-terminus of GP-2, respectively). The blot shown in the middle panel was probed only with anti-peptide sera C, specific for the carboxy-terminus of GP-2. Anti-peptide sera D and E reacted with GP-2 in the control preparation but were unable to detect any proteolytic cleavage fragments in the digested virus preparation (data not shown). The bottom panel shows the location and identity of the peptide sequences used to generate rabbit antisera.

IMMUNOBLOT OF PROTEINASE K DIGESTED Arm 4 IDENTIFIES A CARBOXY-TERMINAL MEMBRANE ANCHOR ON GP-2



model (Figure 7) for the structure of GP-2.

Forces that stabilize GP-1/GP-2 interaction have also been studied in detail. From Triton-X 114 extraction, as well as studies with nonionic detergents and urea, we can conclude that GP-1/GP-2 macromolecular spikes are not disulfide linked (Burns and Buchmeier, 1991). These molecules are however separated by incubation with high concentrations of salts such as 1M NaCl or LiCl. Figure 8 illustrates this fact. Following incubation for 30-60 min in 1M LiCl, virions were banded on 10-50% sucrose gradients. In the salt-stripped virions a band of virions stripped of GP-1 but still containing GP-2 was found near the bottom of the gradient in fractions 4-6; the same fractions contained intact virions in the controls. Near the top of the gradient we found isolated GP-1 in fractions 11-13. These results have been extended to include LCMV, Pichinde and Tacaribe viruses, all of which contain GP-1 recoverable by this method (Figure 9). We have established using conformation-dependent MAbs that the GP-1 recovered in this way is immunochemically native (Figure 10). The observation of a GP-1 (G1) in Tacaribe virus was very satisfying since a number of groups have concluded that TAC had only one glycoprotein molecule, G (Gard et al., 1987). This erroneous conclusion was reached because of the comigration of TAC G1 and G2 glycoproteins in SDS-PAGE. Tacaribe clearly has a full length GP-C precursor as indicated by recently published sequence data (Franze-Fernandez et al., 1987). The MAb used to demonstrate TAC G1, 2.2.1, is a neutralizing MAb raised in this laboratory with collaboration of Dr. C. R. Howard (Howard et al., 1985).

C. Forces which stabilize GP-1 tetramers and GP-1/GP-2 spikes.

The influence of disulfide bonds on GP-1 tetramer stability has been explored in detail (Burns and Buchmeier, 1991). Briefly, GP-1 homotetramers can be stabilized and made resistant to sulfhydryl reagents by crosslinking with sulfo-DST. At a SDST concentration of 10 mg/ml, monomeric through tetrameric GP-1 species were seen. Conversely, in the absence of crosslinkers GP-1 homotetramers were exquisitely sensitive to reducing agents. The following sequential changes were noted. At 0 mM DDT (unreduced), monomeric through tetrameric GP-1 species were seen. At 1-3 mM DDT, only monomers and dimers remained. At higher concentrations of DDT ($\geq$ 200 mM) monomeric GP-1 predominated; moreover, between 3 and 30 mM the apparent mobility of GP-1 changed from ca. 38,000 to 44,000 kDa. Coincident with this mobility shift we observed a loss of GP-1 immunoreactivity with the disulfide-dependent conformation-sensitive MAb 2-11.10 (Wright, Salvato and Buchmeier, 1989) (Figure 11). Based on these observations we propose the model for inter- and intramolecular disulfide bond interactions illustrated in Figure 12.

Model of the LCMV GP-2 Monomer
(GP-2 exists as a tetramer)

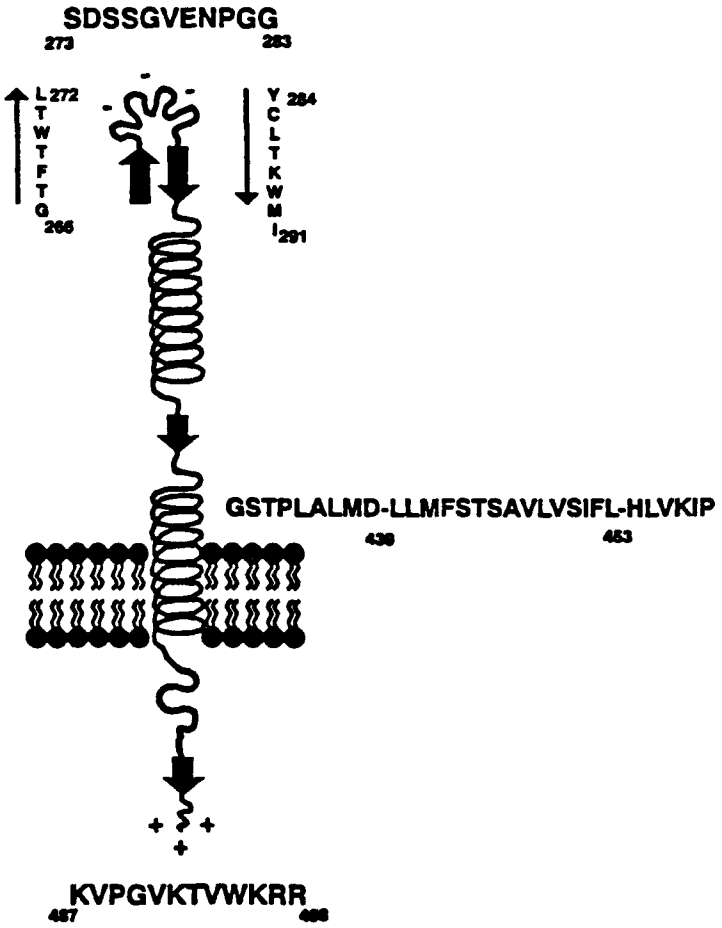
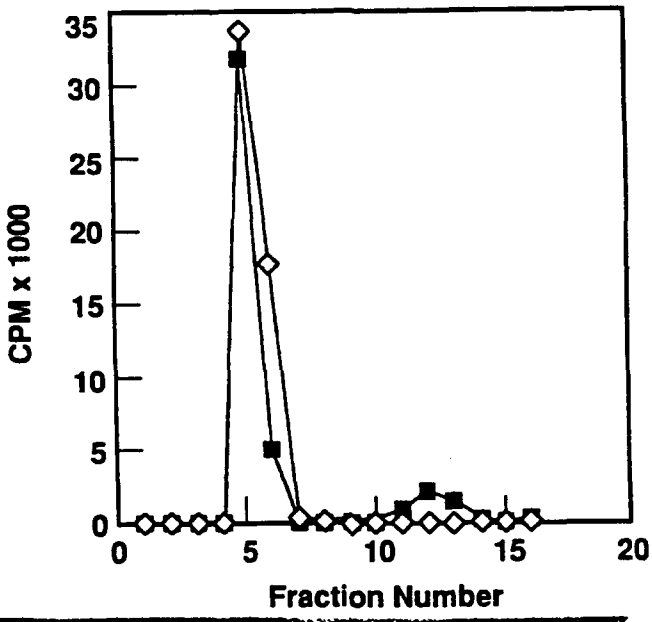


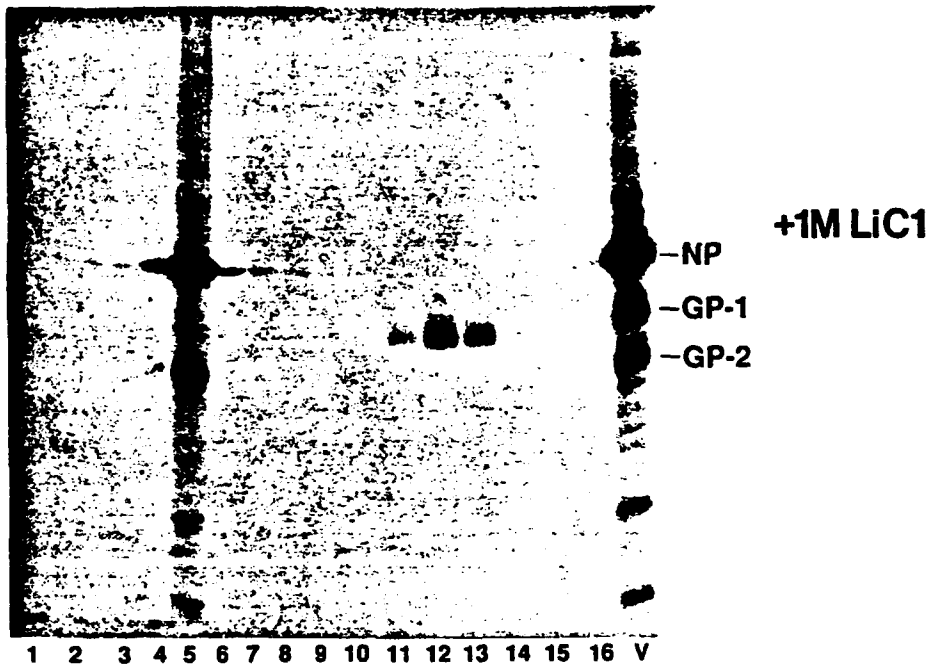
Figure 7. Schematic representation of the structure of an isolated GP-2 monomer. Working model of an isolated GP-2 monomer (the native structure of GP-2 is a homotetramer) illustrating the amino-terminal hairpin loop structure, the alpha-helical (coiled coil) domain, alpha-helical membrane spanning domain (sequence indicated adjacent to the lipid bilayer) and highly basic cytoplasmic tail domain. Single-letter amino acid sequences are included where significant. Alpha helical domains are shown as coils, beta turns are shown as heavy black arrows. Charged amino acid residues, believed to participate in intermolecular ionic bond formation, are indicated (+/-).

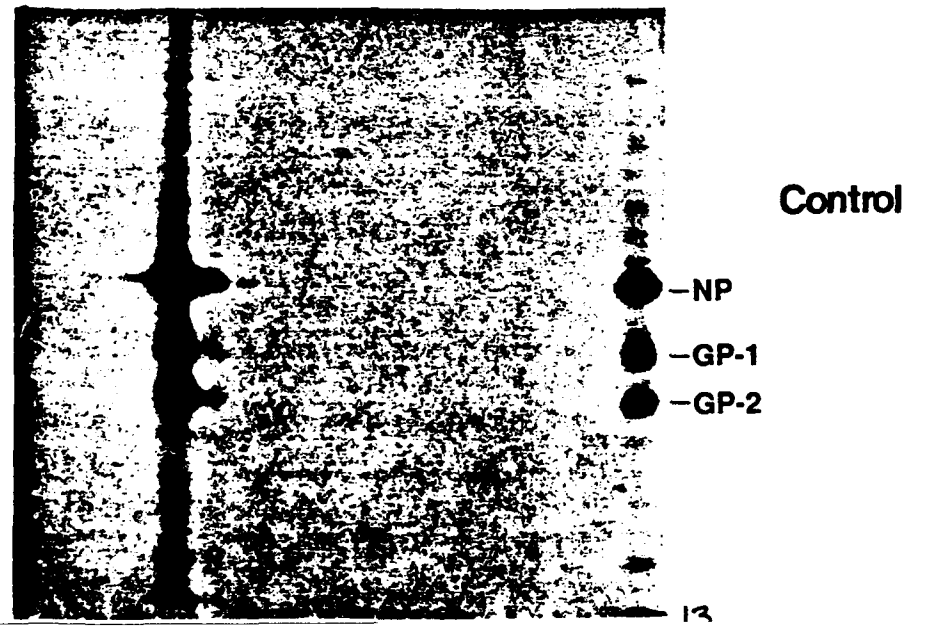
Figure 8 (following page). Typical radioactivity profiles from sucrose gradient fractions of salt treated and control Arm 4 preparations. Two samples of purified LCM virus (60 ug) were pelleted in an Airfuge rotor, resuspended in 1 M LiCl (salt-treated) or TNE (control) and incubated at 37C as described in Materials and Methods. The preparations were then centrifuged for 18 hours on parallel 5-50% continuous sucrose gradients in an SW 50.1 rotor. Each sucrose gradient was fractionated into 300 ul fractions and aliquots (50 ul) were counted in 3 ml Hydrofluor (panel A, black squares- LiCl treated virus, white diamonds-control virus). Fractions from each sucrose gradient were electrophoresed on 10% Laemmli gels following heating for 3 minutes in 2% SDS, 20mM DTT and 500mM Urea (panel B: 1 M LiCl treated virus, panel C: control virus) . Fractions are numbered 1 to 16 from the bottom to the top of each gradient. A sample of the starting virus preparation (V) was included as a marker.

IONIC BONDS ARE ESSENTIAL FOR THE GP-1:GP-2 INTERACTION

FIGURE 8







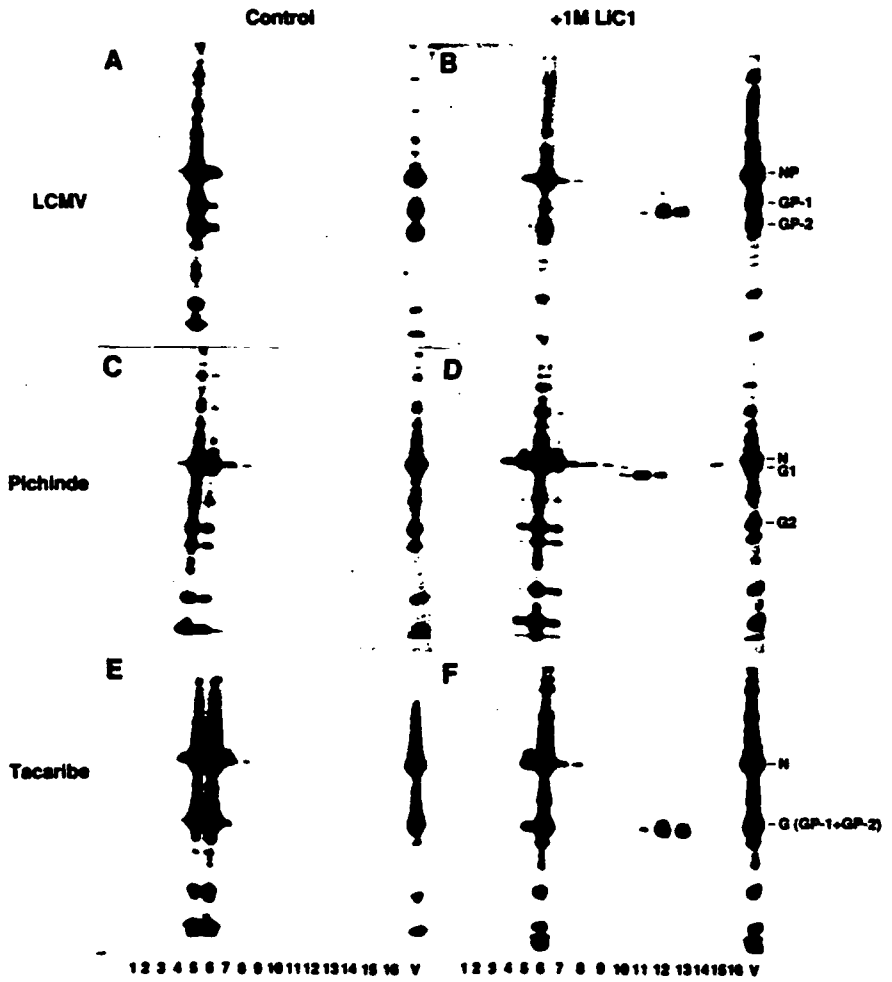
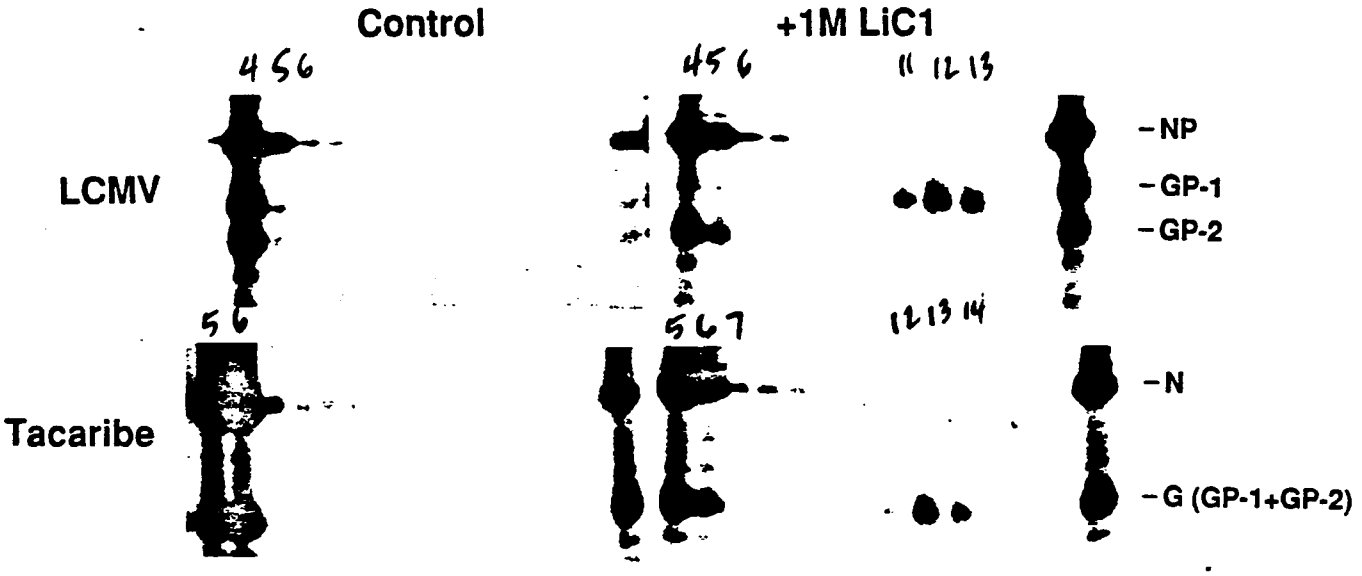
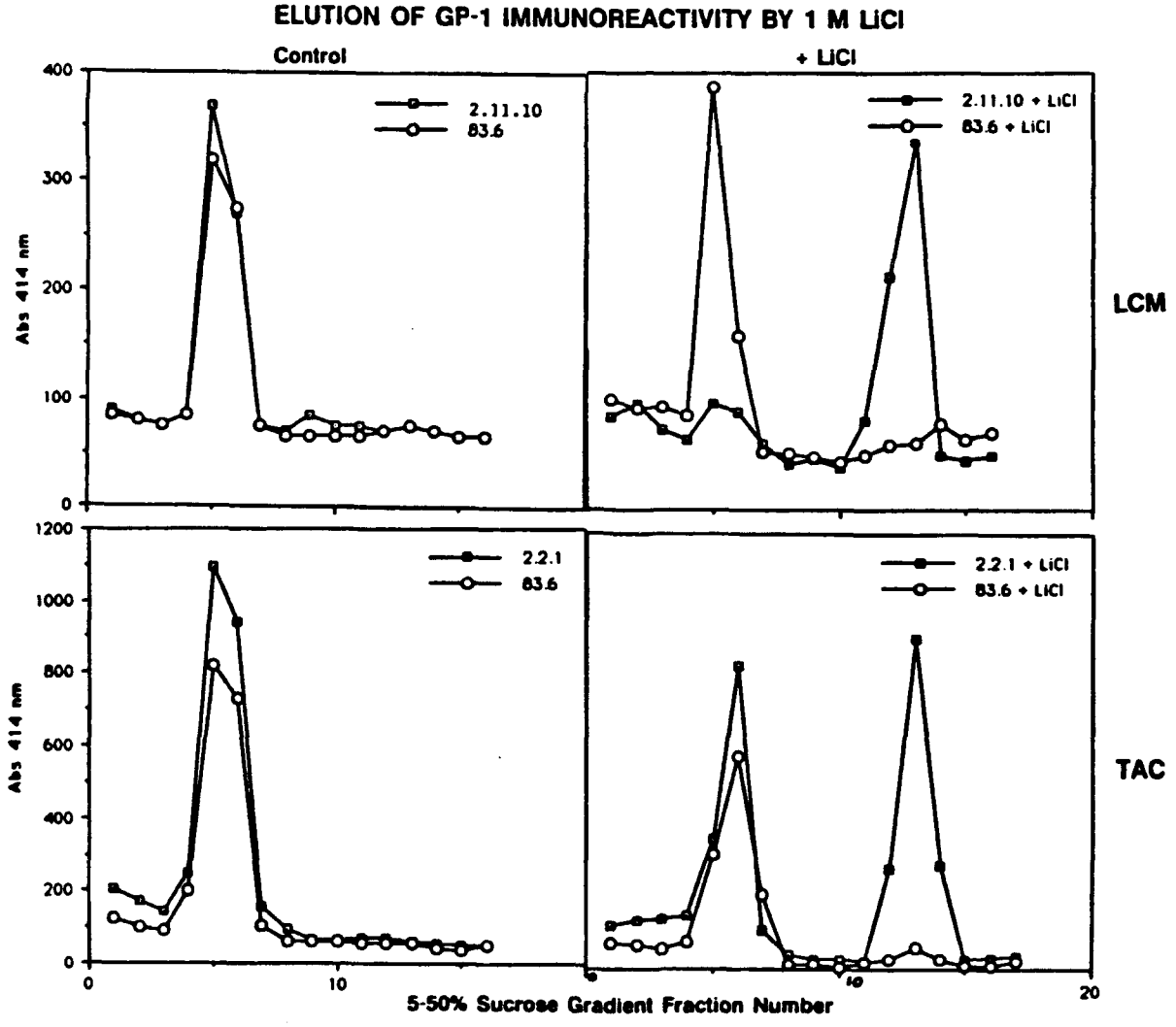


Figure 9. LiCl extraction and isolation of GP-1 glycoproteins from LCMV, Pichinde and Tacaribe viruses. See legend to Figure 7 for details.

Figure 10 (following page). Preservation of immunoreactivity in GP-1 species isolated by LiCl extraction of LCMV and TAC. Monoclonal antibodies used were 2-11.10 (anti LCMV GP-1), 83.6 (anti GP-2 panspecific) and 2-2.1 (anti TAC GP-1). Individual gradient fractions illustrated in top panel were assayed by ELISA in bottom panel.

FIGURE 10


Control
+1M LiC1
LCMV
Tacaribe
-NP
-GP-1
-GP-2
-N
-G (GP-1+GP-2)


ELUTION OF GP-1 IMMUNOREACTIVITY BY 1 M LiCl
Control
+ LiCl
2.11.10
83.6
2.11.10 + LiCl
83.6 + LiCl
2.2.1
83.6
2.2.1 + LiCl
83.6 + LiCl
Abs 414 nm
LCM
TAC
5-50% Sucrose Gradient Fraction Number

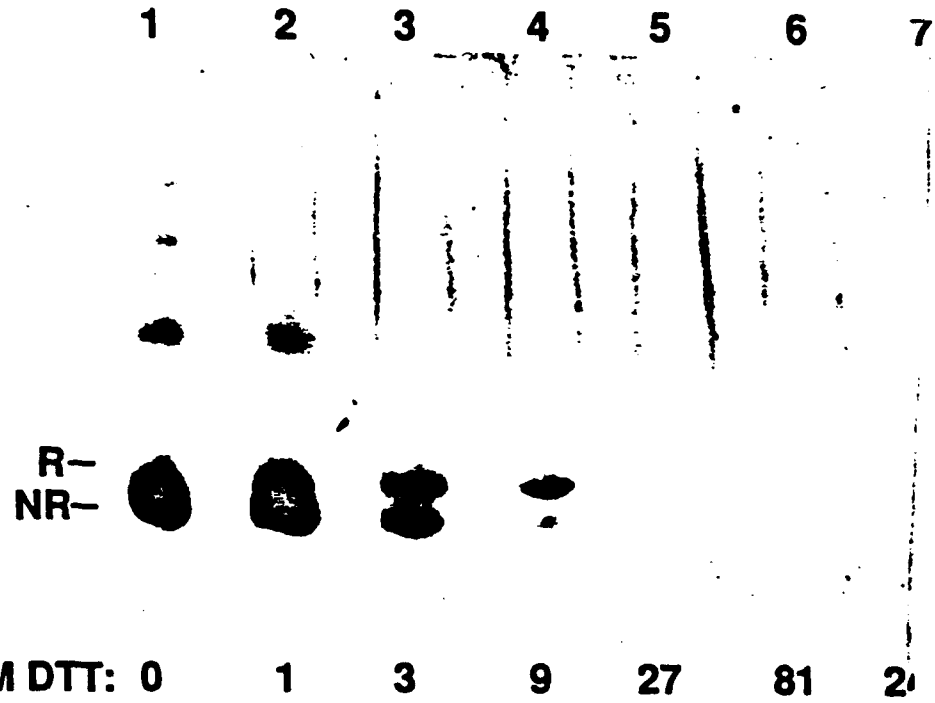


Figure 11. Immunoblot of non-reduced and reduced CHAPS disrupted Arm 4 probed with MAb 2-11.10. Purified LCMV (11 ug) was solubilized by incubation on ice for 5 minutes with 10 mM CHAPS under non-reducing conditions (lane 1) or in the presence of increasing concentrations of DTT, as indicated. These disrupted viral preparations were loaded onto 10% Laemmli gels, without heating or further reduction, electrophoresed at 4C and immunoblotted using the conformation dependent neutralizing anti-GP1 monoclonal antibody 2-11.10.

D. Purification of Native GP-1.

We have established that we can purify native GP-1 molecules from Old and New World arenaviruses by salt stripping and sucrose gradient centrifugation, however the low concentration of GP-1 (ca. 10% of total virion protein) in the virus requires that we explore alternative methods to prepare GP-1 for purification. Consistent with this aim we have obtained Vaccinia constructions expressing LCMV GP-C and Lassa GP-C. These will be used in a transient expression system to produce larger quantities of protein than recoverable from virions. In our coronavirus work we have used the late cowpox promoter CAE I, which drives substantially higher levels of protein synthesis than does the 7.5 promoter more commonly used, and we will attempt to engineer the LCM and Lassa GP-C genes into that vector (p1246 and derivatives). One unexpected problem which we have encountered in using Vaccinia is the apparent failure of the cell to fully process GP-C. Figure 13 illustrates one such experiment, in which cells were infected with a vector expressing full length LCMV WE GP-C, then immunoprecipitated with MAb 33.6 which

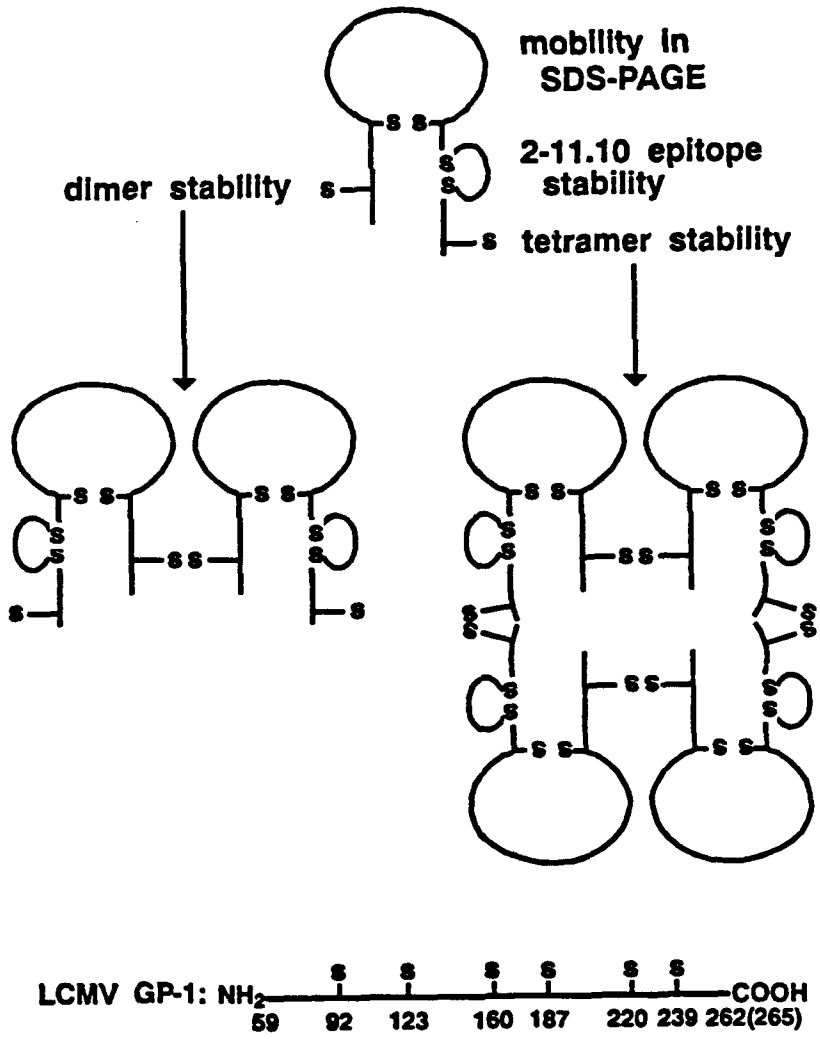


Figure 12. The six cysteine residues in GP-1 form two intramolecular and two intermolecular disulfide bonds.

recognizes both GP-C and its cleavage product GP-2. While cleavage of GP-C to GP-2 was clearly evident in the virus control (lane 15), no cleavage was seen with the Vaccinia vector (lane 12). Instead, an accumulation of uncleaved GP-C was observed. We will explore the basis of this to determine whether it represents a general phenomenon or is specific to this vector (VVB5). We will also attempt to engineer soluble glycoproteins by deletion of the transmembrane domain.

While we have demonstrated that we can isolate native antigenic GP-1, we are currently immunizing animals to confirm that this

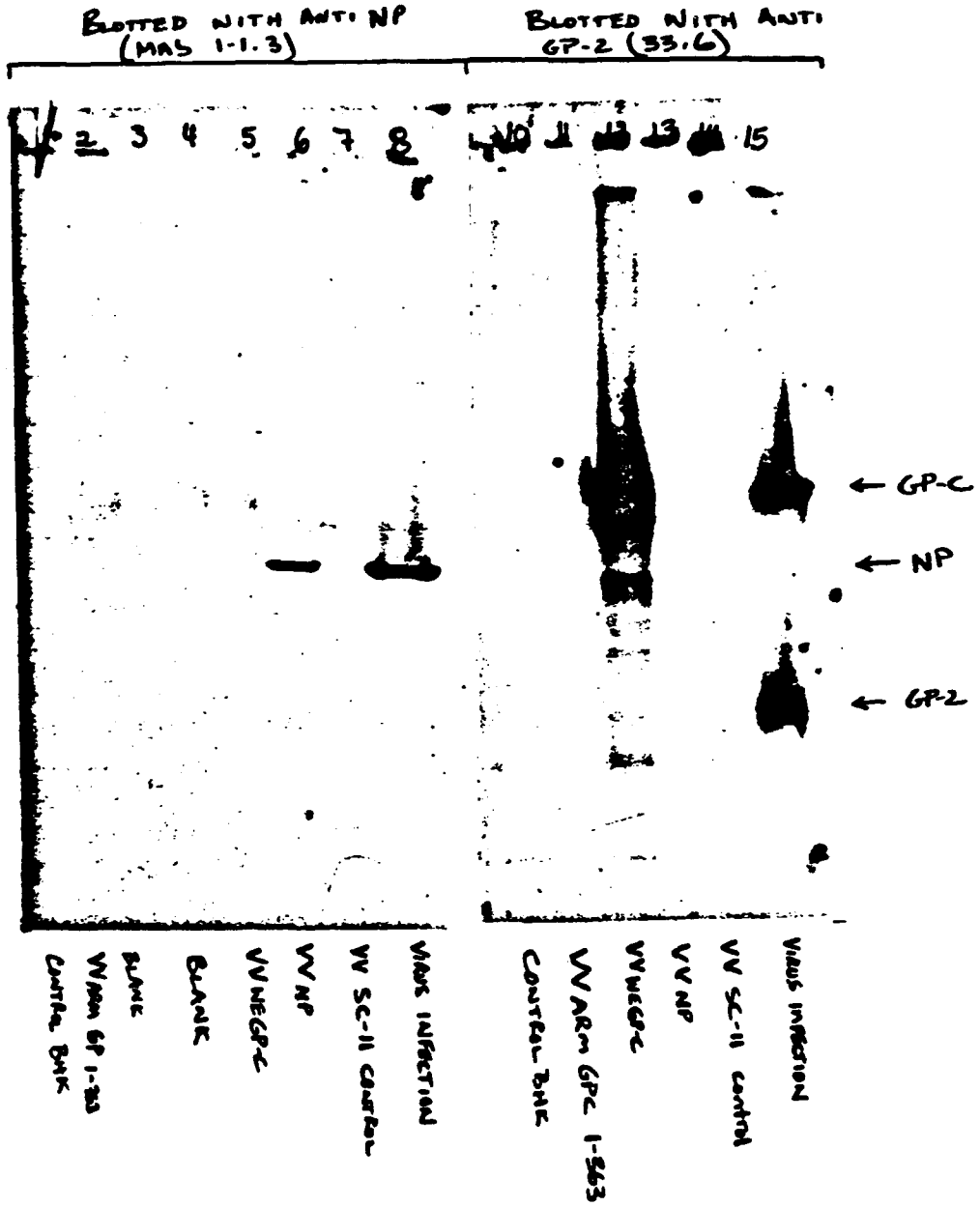


Figure 13. Immunoreactivity of LCMV glycoprotein produced by Vaccinia expression vector (see text for details).

material is immunogenic. An extension of these experiments will be to determine whether GP-1 immunization elicits protective levels of humoral antibody using the model of antibody mediated protection we have recently described (Wright and Buchmeier, 1991).

Summing up the state of our understanding of the structures of the arenavirus glycoproteins, as it stands now we have prepared a schematic model of the glycoprotein spike (Figure 14).

Model of the LCMV Glycoprotein Spike

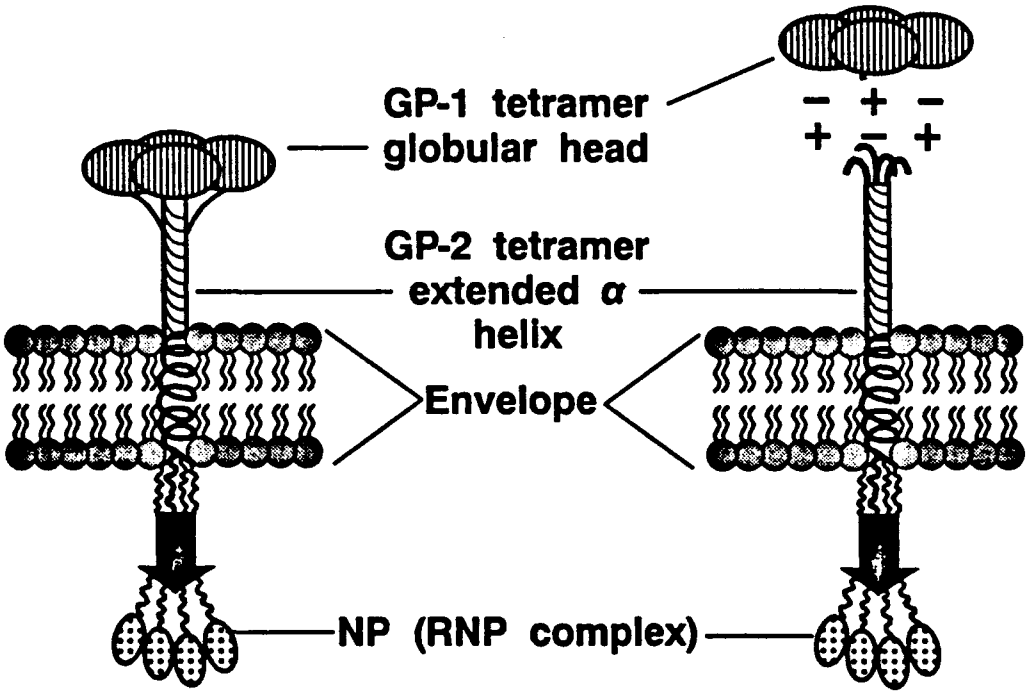


Figure 14. Proposed working model of the LCMV glycoprotein spike. The disulfide linked GP-1 homotetramer forms the crossmember component of the LCMV spike which associates with the amino-terminal hairpin loop of the GP-2 tetrameric stalk via ionic interactions. The alpha helical coiled-coil domain of the GP-2 homotetramer forms the linear region of the stalk. The glycoprotein spike is anchored in the virion envelope by a stretch of 15-25 amino acids and contains, within the virion, a highly charged carboxy-terminus allowing for ionic interaction with the ribonucleoprotein (RNP) complex.

E. Mechanism of antibody mediated protection against lethal arenavirus infection

Three potential outcomes of infection in mice with lymphocytic choriomeningitis virus are possible: (1) an acute asymptomatic infection when immunocompetent adults are inoculated extraneurally, (2) an acute fatal lymphocytic choriomeningitis which develops following intracranial inoculation of immunocompetent mice, or (3) a life-long persistent infection following inoculation of immunocompromised or neonatal mice. It is well established that CD8+ T-cells are required for viral clearance but the supporting role of antibody as well as the relative importance of antibody and T-cells in resistance to reinfection have not been examined fully.

Virus-specific antibodies of the IgG1 isotype are found in the serum of LCMV carrier mice; still the infection is not cleared. In contrast, anti-LCMV antibodies of the IgG2 isotype predominate in convalescent sera following acute infections. These antisera

effectively neutralize virus in vitro and reduce viral titers in vivo in passive transfer experiments. Furthermore, the presence of anti-LCMV monoclonal antibodies can prevent the fatal T-cell mediated lymphocytic choriomeningitis (Wright and Buchmeier, 1991). These studies indicate that antibodies may play an important auxillary role in controlling LCMV infections. Therefore studies were initiated to further evaluate the requirements for and role of antibodies in resistance to LCMV infection.

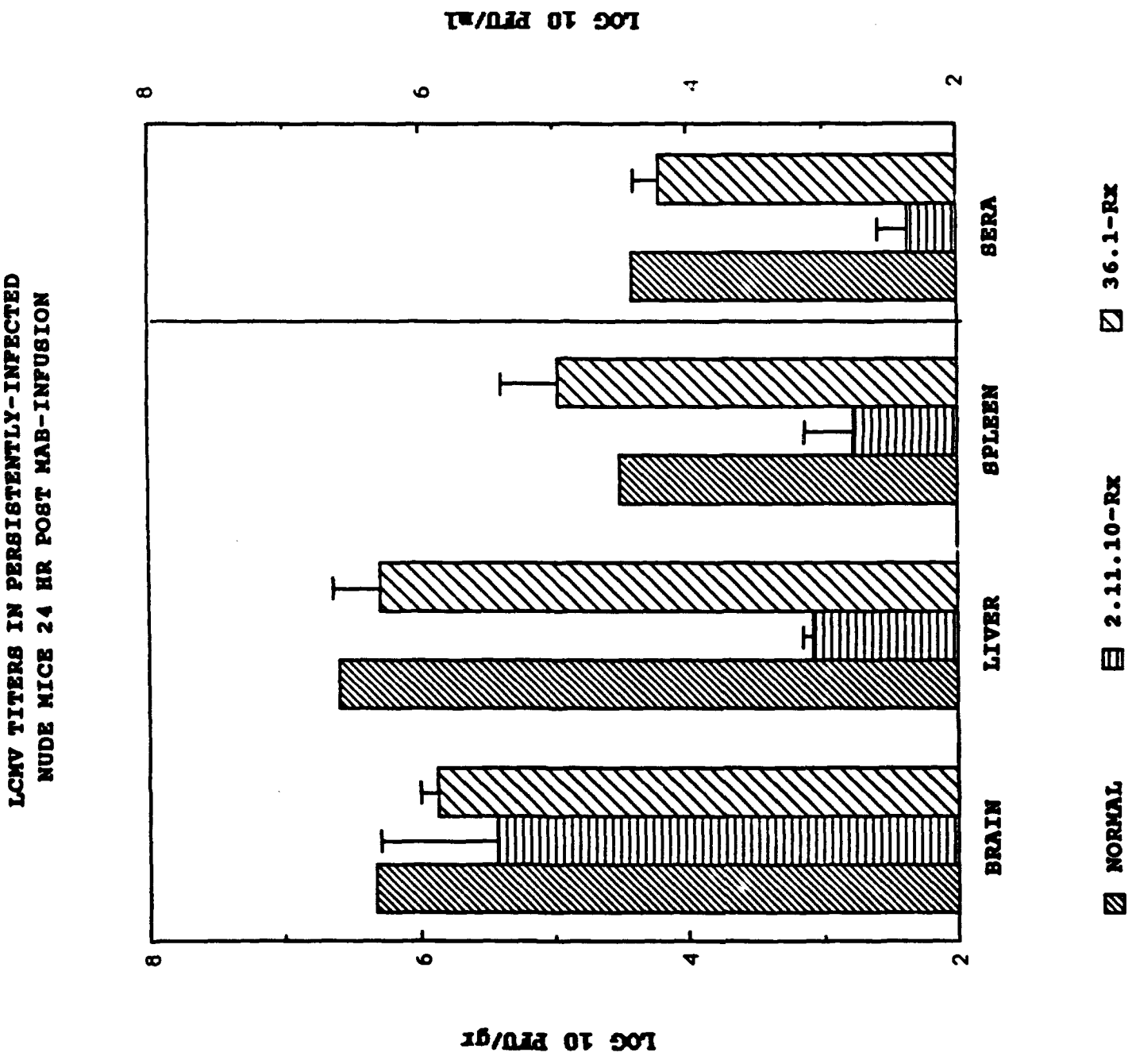
Our success in demonstrating protection of adult mice against lethal LCM disease led us to perform a series of experiments to address the mechanism of humoral protection. The first series of experiments explored the potential for passively acquired immunity among suckling pups born of immune mothers. Dams were immunized by infection with 10^5 pfu of LCMV-ARM 30 days prior to mating, and the litters produced were nursed either on the immune birth mothers or on nonimmune foster mothers, then challenged at 10 or 14 days of age or at 5 weeks. It is evident from the data shown in Table 1 that pups nursed on immune mothers were solidly protected against viral challenge at 10 or 14 days postpartum, but that this protection substantially diminished by 5 weeks. Moreover, nursing of pups born of nonimmune mothers on immune foster mothers and the reciprocal combination established that protection was transmitted in milk. To eliminate the possibility of immune T-cells transferred either transplacentally or in milk, we passively transferred MAb 2-11.10 to nursing mothers postpartum and then challenged the pups at 14 days of age with either ARM-4, which is recognized by 2-11.10, or ARM-5, which is not (Wright and Buchmeier, 1991). Table 2 shows that only mice receiving 2-11.10 and challenged with ARM-4 were protected; ARM-5 challenged mice were not. Thus the specificity of transmammary protection in vivo exactly mirrors that of the 2-11.10 MAb in vitro.

The ability of antibody to clear viral infection was tested by passively treating nude/nude LCMV carrier mice. Figure 15 shows that a single dose of MAb 2-11.10 or 36.1 reduced virus titers substantially within 24 hours. To determine whether MAb could block establishment of a persistent infection in nude mice we transferred antibody to a cohort of nude mice, then challenged with virus. As seen in Figure 16, MAb delayed the rise in virus titer which normally occurs during the first 7 days following infection, but eventually with clearance of the passive antibody titers rose to levels equivalent to those in untreated controls.

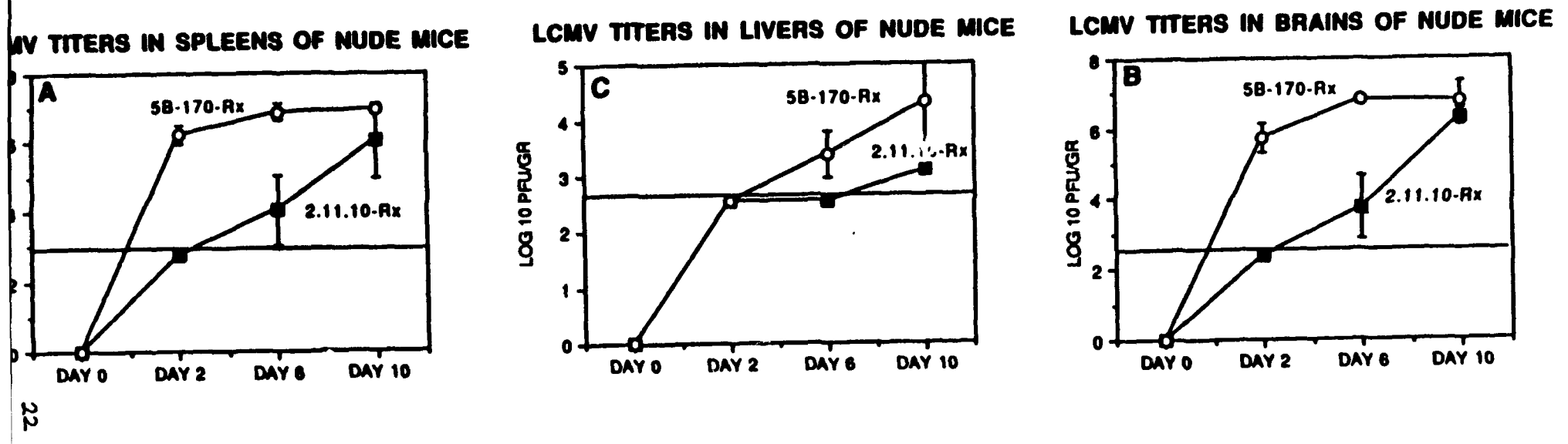
From these experiments it appears that even in the absence of a fully functional T-cell response, antibody provides a potentially useful means of reducing virus burden in an established infection in vivo.

Humoral protection against challenge did not require the complement pathway. Table 3 illustrates that both B10.D2/o SnJ and SWR/J mice which are complement component C5 deficient were protected by MAb. Protection does however require a complete

FIGURE 15


LCMV TITERS IN PERSISTENTLY-INFECTED
NUDE MICE 24 HR POST MAB-INFUSION
LOG 10 PFU/gr
LOG 10 PFU/ml
8
6
4
2
BRAIN
LIVER
SPLEEN
SERA
NORMAL
2.11.10-Rx
36.1-Rx

MAb 2.11.10 RESTRICTS THE SPREAD OF AN ACUTE VIRAL INFECTION IN NUDE MICE



Nude mice were infused with 0.2 ml of either MAb 2.11.10 (anti-LCMV) or MAb 5B-170 (anti-MHV) by ip injection on days -1 and 0. On day 0 the mice were challenged by ic inoculation with 1000 PFU ARM-4. The viral titers in the brain, spleen and liver were detemined on day 2, 6 and 10 following infection.

FIGURE 16

SUCKLING PUPS OF IMMUNE DAMS ARE PROTECTED FROM LETHAL CHALLENGE

BIRTH MOTHERS	FOSTER MOTHERS	AGE OF PUPS	PUPS WEANED	% SURVIVAL (TOTAL MICE)
IMMUNE	-	10 DAYS	NO	100% (6)
NONIMMUNE	-	10 DAYS	NO	0% (4)
IMMUNE	-	14 DAYS	NO	100% (6)
NONIMMUNE	-	14 DAYS	NO	0% (7)
IMMUNE	-	5 WEEKS	YES	29% (7)
NONIMMUNE	-	5 WEEKS	YES	0% (4)
NONIMMUNE	IMMUNE	14 DAYS	NO	73% (15)
IMMUNE	NONIMMUNE	14 DAYS	NO	0% (12)

-- Pups challenged at 5 weeks of age were weaned when three weeks old.

-- Foster-nursed pups were switched to their foster mother within 24 hr of birth.

TABLE 1

SUCKLING PUPS OF 2.11.10-TREATED DAMS ARE PASSIVELY PROTECTED.

EXPT.	MATERNAL STATUS	AGE OF PUPS	VIRAL CHALLENGE	% SURVIVAL (TOTAL MICE)
1	NONIMMUNE	14 DAYS	ARM-4	40% (5)
	NONIMMUNE	14 DAYS	ARM-5	0% (5)
2	NONIMMUNE	14 DAYS	ARM-4	83% (6)
	NONIMMUNE	14 DAYS	ARM-5	0% (3)

-- Nursing mothers were infused with 0.2 ml of 2.11.10 ascites every third day (EXPT 1) or 0.25 ml every other day (EXPT 2) post partum.

-- MAb 2.11.10 recognizes an epitope present on ARM-4 but not ARM-5.

TABLE 2

ANTI-LCMV MAB PROTECT C5-DEFICIENT MICE FROM LETHAL LCM-DISEASE.

MOUSE STRAIN	C5 DEFICIENT	VIRUS CHALLENGE	2.11.10 TREATMENT	% SURVIVAL (TOTAL MICE)
B10.D2/oSnJ	YES	ARM-4	-	0% (4)
B10.D2/oSnJ	YES	ARM-4	+	100% (4)
B10.D2/nSnJ	NO	ARM-4	-	0% (4)
B10.D2/nSnJ	NO	ARM-4	+	100% (4)
SWR/J	YES	ARM-4	-	0% (12)
SWR/J	YES	ARM-4	+	83% (12)
SWR/J	YES	ARM-5	-	0% (12)
SWR/J	YES	ARM-5	+	0% (12)

-- Mice treated with MAb 2.11.10 received 0.2 ml of ascites the day before and the day of viral challenge.

-- MAb 2.11.10 recognizes an epitope present on ARM-4 but not ARM-5.

TABLE 3

antibody molecule. $F(ab')_2$ fragments prepared from MAb 2-11.10 retained essentially full virus neutralizing activity measured in vitro but lost the ability to protect against challenge in vivo (Table 4). Taken with the complement data, these results suggest that Fc receptor- bearing cells may serve as important effectors in humoral protection. We currently investigating the role of antibody dependent cellular cytolysis (ADCC) in protection against arenavirus infection.

These results clearly indicate that preexisting antibody protects against lethal arenavirus infection and that the role of a humoral response has been underestimated. Therefore vaccination strategies which stimulate such a protective antibody response are important goals for future research. Furthermore, the demonstration that passively administered antibody is able to reduce preexisting virus titers in vivo suggests that specific humoral immunotherapy with monoclonal antibodies of human origin or with recombinant human-mouse antibodies is a real possibility.

$F(ab')_2$ FRAGMENTS FROM THE PROTECTIVE MAB 2.11.10 FAIL TO PROTECT MICE FROM LETHAL LCM DISEASE.

GROUP	TREATMENT	SURVIVAL (TOTAL MICE)
RECIPIENTS OF:		
2.11.10	25ug	25% (4)
$F(ab')_2$	25ug	0% (4)
2.11.10	50ug	75% (4)
$F(ab')_2$	125ug	0% (4)

-- MAb 2.11.10 or the $F(ab')_2$ fragments of 2.11.10 were given 1x only concurrently with viral challenge.

-- $F(ab')_2$ fragments had equivalent neutralizing titers as MAb 2.11.10.

TABLE 4

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Appendix 1: Publications supported by this document, 1990-91

Published Papers

*Wright, K. E. and M. J. Buchmeier. Antiviral antibodies attenuate T-cell mediated immunopathology following acute lymphocytic choriomeningitis virus infection. J. Virol. 65:3001-3006, 1990.

*Wright, K. E., C. Schmaljohn, A. Schmaljohn and M. J. Buchmeier. Arenaviridae and bunyaviridae. In Immunochemistry of Viruses, Vol. 2, von Regenmortel and Neurath, eds., Elsevier, Amsterdam, 1990, pp. 237-270.

*Fazakerley, J. K., P. Southern, F. Bloom and M. J. Buchmeier. High resolution in situ hybridisation to determine the cellular distribution of lymphocytic choriomeningitis virus RNA in the tissues of persistently infected mice: Relevance to arenavirus disease and mechanisms of viral persistence. J. Gen. Virol. 72:1611-1625, 1990.

Burns, J.W. and M. J. Buchmeier. Protein-protein interactions in lymphocytic choriomeningitis virus. Virology 183:620-629, 1991.

*previously listed as papers in press.

Papers Submitted

Burns, J. W., R. A. Milligan and M. J. Buchmeier. Proteins of lymphocytic choriomeningitis virus: Structure of the glycoprotein spike. J. Virol., 1991, submitted.

Burns, J. W., T. A. Burke and M. J. Buchmeier. Amino-terminal sequencing identifies two conserved cleavage sites and a long cleavable signal sequence in the glycoprotein precursor (GP-C) of the arenaviruses. J. Virol., 1991, submitted.

Burns, J. W. and M. J. Buchmeier. A unifying model for the structure of the arenavirus glycoproteins and the virion spike. J. Virol., 1991, submitted.