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SYNTHETIC PEPTIDE VACCINES FOR THE CONTROL OF ARENAVIRUS INFECTONS

FINAL REPORT

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) Arenaviruses are endemic on both the African and South American continents and represent significant health hazards. Precise diagnostic methods and effective treatment protocols are not currently available. We are using genetic cloning methods to develop an effective vaccine against arenaviruses. Developmental studies are being carried out and techniques established with the prototype arenavirus lymphocytic choriomeningitis virus (LCMV), and these techniques will be applied for rapid future development of vaccines against the pathogenic arenaviruses Lassa, Junin and Machupo. Using techniques of protein chemistry we are identifying and mapping important immunogenic regions within the LCMV glycoproteins. The LCMV genomic RNAs have been cloned and the primary sequence of the viral polypeptides deduced from the cloned viral cDNAs. Short peptides corresponding to the immunogenic regions will be synthesized chemically and assayed for ability to induce protective neutralizing antibody responses in experimental animals. Experimental approaches to therapy for acute arenavirus infections will also be explored. These will include using cloned cytotoxic T-lymphocytes and antisera against components of the major histocompatibility complex (H-2 in mice) in attempts to modify the progress of viral disease in mice.

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Summary

In our original research contract proposal C3013 entitled "Synthetic Peptide Vaccines for the Control of Arenavirus Infections," we proposed to develop synthetic approaches to vaccination against arenavirus infections emphasizing in particular synthetic peptide based approaches. In addition, we proposed to investigate the potential of immune reagents, particularly monoclonal antibodies to control and modify the course of ongoing disease in animals using lymphocytic choriomeningitis virus infection as a safe and accurate paradigm for the pathogenic human arenaviruses.

During the term of this contract, 1 December 1982 - 31 July 1986, we have accomplished the goals set forth in the original proposal. The cDNA sequence of the S RNA segment of LCM virus has been determined and the protein sequences of the structural proteins NP, GP-1 and GP-2 have been deduced. Synthetic peptides corresponding to sequences within the structural proteins have been made and corresponding antisera prepared against them in rabbits. These antisera have been used to map topographically and structurally important domains on the viral glycoproteins. A large panel of monoclonal antibodies to the LCMV glycoprotein has been mapped topographically on the LCMV glycoproteins and both unique and conserved neutralizing epitopes defined. Finally, the sequences of LCMV and other arenaviruses have been aligned and comapred and common structural features of each determined in order to define regions of functional and/or immunological homology.

It is the goal of such studies to derive information useful in targeting specific structural regions of arenaviruses of human disease potential for the development of improved vaccines and diagnostics.

Foreword

In conducting the research described in this report, the investigators 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 (DHEW Publication No. (NIH) 78-23, Revised 1978).

The investigators have abided by the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (April 1982) and the Administrative Practices Supplements.

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Summary Progress Report

A. Introduction and Overview

The results described in the following final summary progress report cover the term of the contract 1 December 1982 through 31 July 1986 inclusive. During this period we have focused on four primary goals as described in the original proposal. These were:

Identification and mapping of Antigenic Determinants on the LCMV glycoproteins.

Cloning the genomic S RNA of LCMV which contains the structural protein genes.

Determination of the amino acid sequence of the viral polypeptides from the cDNA sequence.

Synthesis of peptides corresponding to regions of the viral polypeptides of predicted structural and/or antigenic importance.

We have accomplished the stated goals and in addition have performed comparative analyses of LCMV, Lassa related and Pichinde viruses at the protein level by peptide mapping and at the sequence level. These studies are important to assess the degree of structural similarity among viruses of the group and to predict areas of potential functional or immunological interest. These studies, as well as others enumerated in this progress report, are described in detail in 24 accompanying publications (Appendix 1).

In other studies of interest to this contract we have developed techniques for detecting viral genetic material and gene products in sectioned organs and tissues of whole animals and in collaboration with Dr. Oldstone have followed the expression of these viral "footprints" during the course of persistent LCMV infection. The clearance effect of virus-directed immunomodulation on infection was also studied using cloned T-lymphocytes.

In collaboration with Yves Riviere and M.B.A. Oldstone, genetic reassortant strains of LCMV were made using the mouse neurovirulent Arm strain and the guinea pig lethal WE strain in order to study the viral genes involved in virulence. The L-RNA encoded gene product, presumably the viral RNA polymerase, was found to be required to produce fatal hemorrhagic fever-like disease in the guinea pig, while the S-RNA was shown to determine neuro-endocrine virulence in the mouse. Using these same reassortant viruses it was also shown that virus specific cytotoxic T lymphocytes (CTL) recognize determinants coded on the S-RNA of the virus.

Finally, using sequence information which has become available for several arenaviruses during the past 2 years (8,9,10), we are able to make precise comparisons between virulent and avirulent

and Old and New World strains, and will propose in this contract unifying approaches to their diagnosis and control.

An important consideration in a project such as this is whether the basic information we derive in the laboratory will be applicable to studies of the epidemiology and pathogenesis of the relevant human pathogens. To increase the probability of success of this cross-fertilization we have hosted a senior USAMRIID investigator, Dr. Peter B. Jahrling, in our laboratory on a working sabbatical leave during 1985. Dr. Jahrling has worked actively on several of the projects to be described in the detailed report presented below, but more imporant to the long term goals of USAMRIID is the fact that Dr. Jahrling has acquired significant skills in the areas of molecular immunology (i.e., antipeptide and anti ID approaches), and molecular biology (DNA and RNA analysis and in situ hybridization with oligonucleotide probes). We are confident that Dr. Jahrling will incorporate these new skills and approaches into his work at USAMRIID, and we will welcome proposals for additional sabbatical or exchange visits of this kind during the next contract period.

B. Molecular biology of LCM virus: studies based on cloning and sequencing the LCMV RNAs

During the previous contract period we have made significant progress in the following areas:

cDNA cloning and sequencing of LCMV (Armstrong strain) genomic L and S RNA segments. The entire S segment has been cloned and sequenced and approximately 50% of the L segment has been cloned and sequenced.

Identification of protein coding regions and development of new highly-specific antibody reagents based on synthetic peptide technologies.

Development of region specific and strand specific hybridization probes to monitor replication and transcription in both acute and persistent infections.

Analysis by in situ hybridization to whole body sections, of the distribution of LCMV genetic material in persistently infected mice.

Construction of complete cDNA genes for LCMV NP and GP-C and construction of LCMV-vaccinia recombinant viruses.

Use of genetic reassortants of LCMV to analyze molecular basis of disease.

1) cDNA Cloning and Sequencing

We have generated overlapping cDNA clones that cover the entire genomic S-RNA segment of LCMV and have determined the nucleotide sequence of the S RNA segment from these clones (Appendix 2).

Much of the sequence has actually been derived from independent, overlapping clones and this provides a high degree of confidence that the composite sequence is truly representative of the population of viral RNAs.

In the course of characterizing cDNA clones to cover the carboxyl terminus of GP-2, we examined one clone that appeared to include the GP-C translation termination signal and the intergenic hairpin region. However, nucleotide sequencing revealed that this clone contained a small deletion that removed the A residue from the UGA termination codon and part of the hairpin region. This clone results, most probably, from an in vitro cloning artifact but does have several interesting properties that merit further investigation. As a result of the deletion, an additional twenty amino acids would be added to the terminus of GP-2. The normal terminus of GP-2 contains the amino acids Lys, Arg, Arg COOH, and we have previously shown (Buchmeier et al., J. Virol., submitted) that the cleavage of the GP-C precursor to the mature GP-1 and GP-2 glycoproteins occurs at adjacent Arg, Arg residue, position 262-263 in GP-C. There are no other pairs of adjacent Arg residues within GP-C. Therefore, we might predict that if the extended primary translation products were synthesized, then cleavage could occur by a process equivalent to the GP-1/GP-2 cleavage that would remove the extra 20 amino acids.

We have cloned the extended GP-C gene (called GP-X) into SV40 based plasmid vectors and are about to initiate gene transfer experiments. We are synthesizing a peptide that corresponds to the additional amino acids at the carboxyl terminus and antibody to this peptide will be helpful in monitoring expression of this gene. This antibody will also be used to examine acutely and persistently infected cell cultures to explore the possibility that our cDNA clone may represent a normal (minor) mRNA and that different forms of GP-C and, depending on cleavage, GP-2 may exist during virus infections.

The reconstructed cDNA genes will also be useful for in situ hybridization studies as they will provide long, gene-specific probes which should increase the sensitivity of detection for viral nucleic acids.

The general organization of the LCMV-Arm genome is similar to that reported for Pichinde virus (PV)(3), LCMV-WE (10) and Lassa and the ambisense character of the arenavirus genomic S segments first proposed by Auperin and Bishop (8,11) seems now to be firmly established (Figure 1). All the information so far available for the L segment suggests that this is a conventional negative-sense genomic RNA. We have cloned and sequenced 3.4 kb from the L segment and are currently screening an extensive collection of new cDNA clones for additional L sequences. Different estimates for L put the length between 7 and 9 kb indicating that our available sequence information covers 30-50% of the L sequence.

A number of potential Lassa cDNA clones have been prepared but none has yet been definitively identified. Dr. Kathryn Wright (postdoctoral fellow with Dr. Buchmeier) has been learning the techniques of RNA electrophoresis, transfer and hybridization and is now ready to begin screening these clones. The approach will be to label plasmid DNAs in vitro by Nick translation and hybridize dot blots and RNA gel filters prepared with the Lassa L and S genomic RNA segments. LCMV probes and purified viral RNAs will be used as appropriate for positive and negative controls.

2) Identification of Protein Coding Regions

Computer analysis of the nucleotide sequences has identified potential protein coding regions (i.e., translational open reading frames) and predicted the amino acid sequences for these proteins. We have synthesized short peptides (typically containing 12-25 amino acid residues) from different regions of these potential proteins and used polyclonal anti-peptide antisera raised in rabbits to determine reactivities with purified virion proteins in Western blotting experiments (12). In this way, we have assigned protein coding regions to actual viral proteins and established that the gene order on the S-RNA segment is 3' NP, GP-2, GP-1 5' (Figure 2). We have also developed the first reagents to recognize the L protein. We have characterized antipeptide antisera raised against predicted amino acid residues that detect a single high molecular weight protein (approximately 200,000 daltons) in Western blots of purified virion proteins (Figure 3). A number of other L-derived antipeptide antisera are being characterized with respect to viral protein recognition, but we will have to wait for completion of the cloning and sequencing to resolve whether any other proteins are encoded by the genomic L segment.

3) Region and Strand Specific Hybridization Probes

We have used cDNA clones derived from the NP and GP coding regions to analyze RNAs from acutely and persistently infected cells. The nick-translation procedure (13) for labelling DNA results in equal incorporation of radioisotope into both DNA strands - such probes detect all viral RNA homologous to the probe but yield no information on polarity (Figure 4). We have therefore sub-cloned many of the LCMV cDNAs into SP6-plasmid vectors in order to synthesize strand-specific (RNA) probes (14,15). This recently described technique works with good efficiency to generate in vitro labelled RNA probes which can be used to examine RNA polarities. We have been able to verify that the NP mRNA is complementary to the genome and that the putative GP mRNA is in the same reading sense as the genomic RNA (Figure 5). During acute infection of tissue culture cells we can also demonstrate significant amounts of the full-length genomic complementary (GC), replication intermediate RNA. We estimate that there may be approximately equivalent amounts of this full-length genomic complementary RNA and full-length genomic RNA at the time of peak virus production (Figure 5). The relative abundance of the full-length GC RNA can be probably explained by

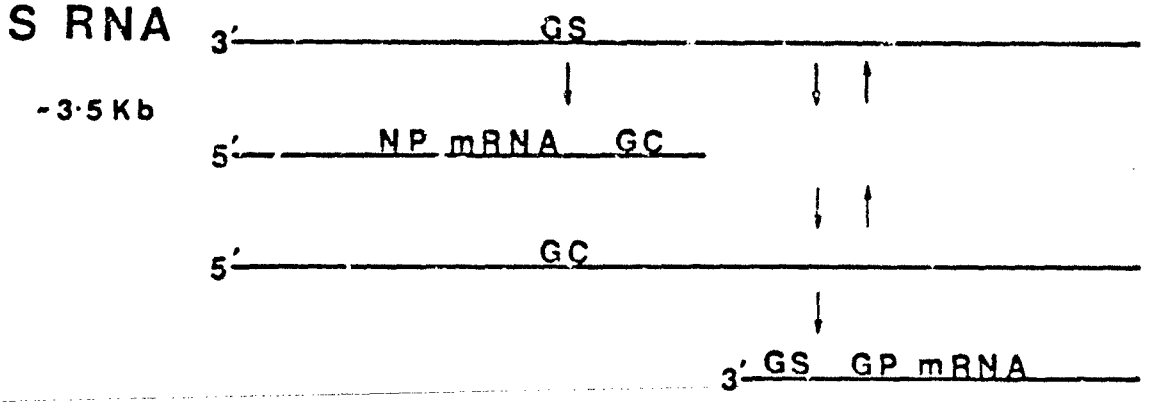

Figure 1

Proposed Transcription and Replication Scheme for the LCMV Genomic S RNA Segment. The genomic sense (GS) RNA from the virion is transcribed into a genomic complementary (GC) NP mRNA which apparently terminates in a hairpin region between the NP and GP coding regions. De novo synthesis of NP may melt the hairpin structure to allow synthesis of a full-length GC RNA which would then function as a replication intermediate for the synthesis of progeny GS RNAs. Transcription of the GC RNA generates the genomic sense GP mRNA which again apparently terminates in the central hairpin region. The two sub-genomic mRNAs and the full-length GC RNA are readily detectable in acutely infected cells by hybridization with nick translation probes or with strand-specific riboprobes (see Figures 4 and 5). We do not yet know whether the 5' ends of the mRNAs coincide with or lie internal to the 3' ends of the full-length GS and GC RNAs.

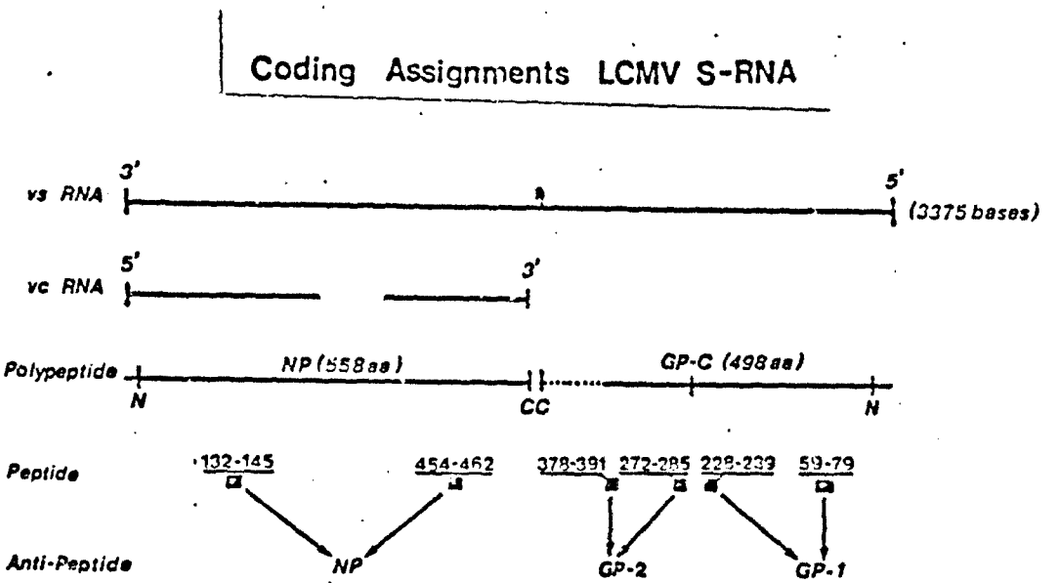

Figure 2. Summary of strategy which has resulted in definitive assignment of the gene order of the LCMV S-RNA. Peptides were synthesized which corresponded to the amino acid sequences of NP and GP-C as indicated. Antisera were raised to each peptide in rabbits and the reactivity of each with bonafide viral proteins is shown.

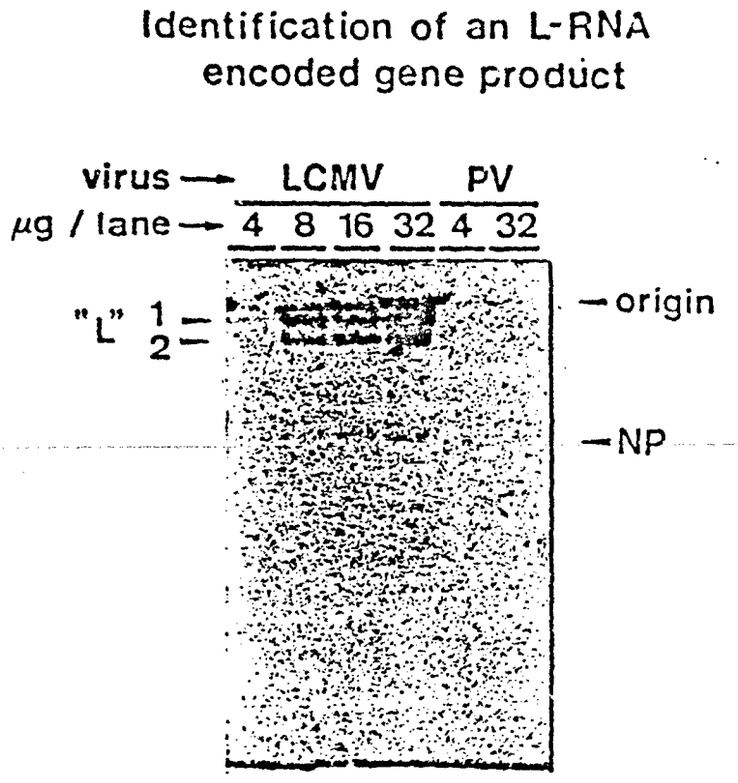

Figure 3. A sequence representing amino acids 174-187 of the LCMV L-RNA open reading frame was synthesized and antibody made in rabbits. The resulting antibody reacted specifically with two polypeptides or approximately 165 and 180 kd. The antibody was specific for LCMV and did not react with Pichinde virus (PV). Reactivity was blocked by incubation in the presence of 1 ug of free peptide. This is a direct demonstration of an LCMV L-RNA encoded gene product and presumably represents the virion RNA polymerase.

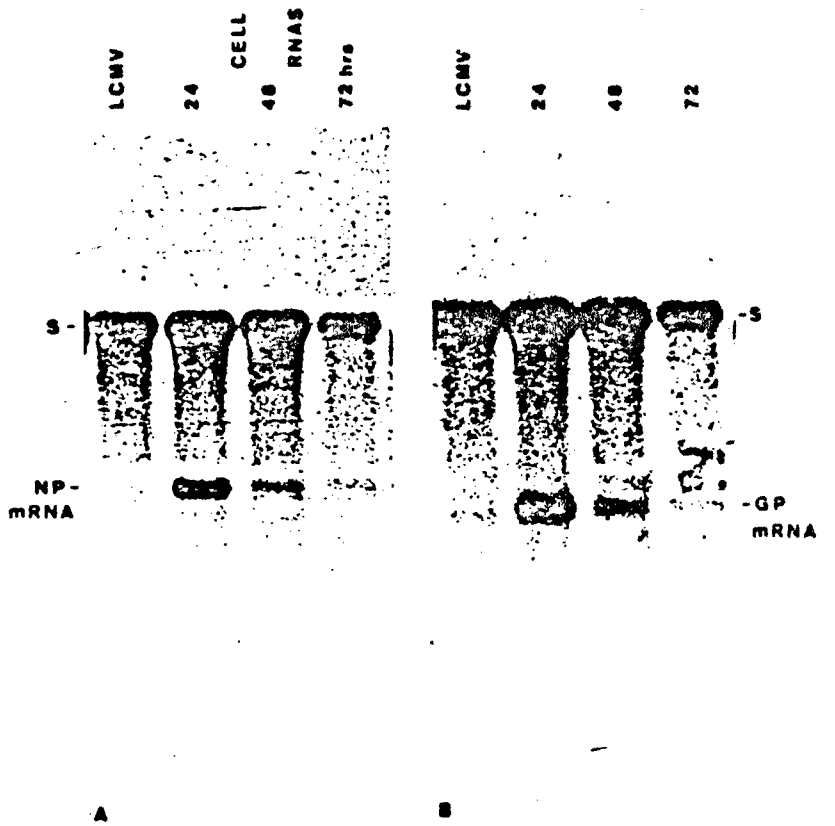

Figure 4. Hybridization analysis of LCMV S-RNA species produced in short term, high multiplicity virus infections. Total cellular RNA was extracted with guanidinium thiocyanate at the indicated times post infection, denatured with glyoxal and separated on the basis of size by electrophoresis in an agarose gel. RNA extracted from purified LCMV virions was electrophoresed in an adjacent lane on the agarose gel. RNA within the gel was transferred to a nitrocellulose filter and then the filter was hybridized sequentially with cloned LCMV S cDNA probes that had been labeled with ^{32}P *in vitro* by nick translation. The figure shows strong hybridization to genomic sized S-RNA but there is also clear hybridization to smaller RNA species in the whole cell RNA samples that diminishes as the infection progresses. Probes from the nucleoprotein (NP) and glycoprotein (GP) coding regions hybridize to two different sized S sub-genomic (mRNA) species. There is a faint indication of mRNA species in the purified virion RNA sample.

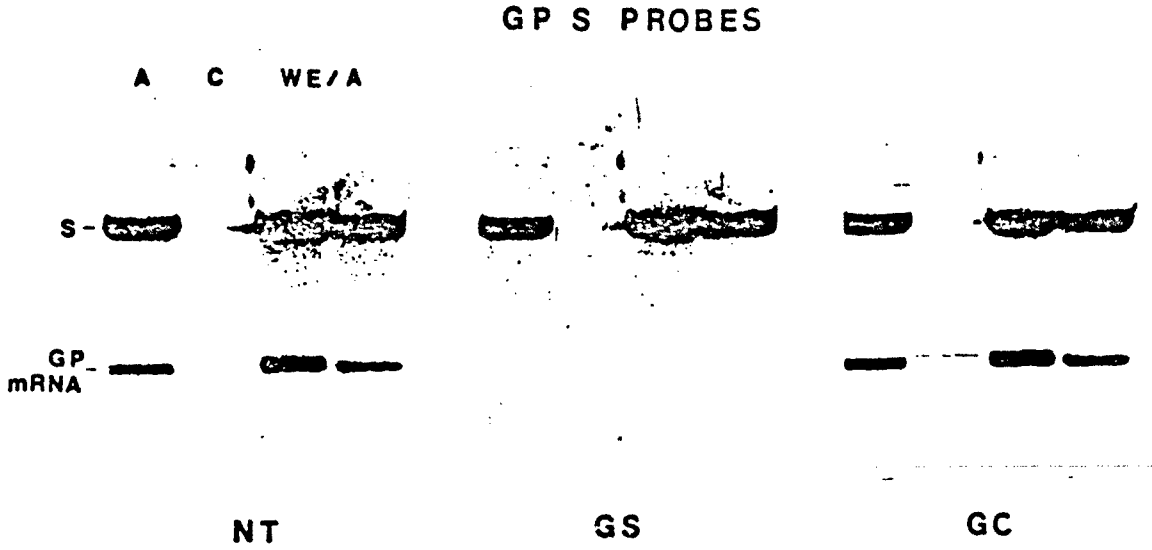

Figure 5. Hybridization analysis with strand-specific probes to LCMV RNAs in acutely infected tissue culture cells.

A LCMV-Arm infected total cell RNA

C Control uninfected BHK cell RNA

W/A Two independent reassortant viruses with the genotype WE-L and Arm-S

The filter was hybridized sequentially with a double-stranded nick translated probe (NT), a strand-specific genomic sense probe (GS) and a strand-specific genomic complementary (GC) probe. All the probes cover the same region of the GP gene. The GS probe only detects the full-length genomic complementary (replication intermediate) RNA, whereas the GC probe detects both the full-length genomic S-RNA segment and the presumptive GP mRNA.

the requirement for this RNA to serve as a template for glycoprotein C mRNA synthesis (11).

To date, we have not been able to detect any L derived RNA species that are shorter than the full-length, but, until we have cloned probes representative of the entire L segment, we cannot completely rule out the possibility that small RNAs may be present. We have established from sequencing and hybridization experiments that the portion of the L segment, linked by anti-peptide antisera to the 200 kd protein, generates a transcript which is complementary to the genomic L RNA.

4) In situ Hybridization

We have developed a technique for in situ hybridization to whole mouse sections in order to monitor the distribution of LCMV genetic material during the establishment and maintenance of persistent infections in vivo. A detailed account of this work has been published (Nature 312, 555-558, 1984) and we have gained substantial new insight into the process of persistence. Individual animals show remarkable similarity in the distribution of LCMV genetic material and a general trend emerges that, with increasing age, the animals show reductions in titers of infectious virus that can be recovered from serum or disrupted tissues but show tremendous increases in LCMV RNA levels in tissues (Figure 6). The sectioning technique has the potential to reveal viral RNA in unexpected locations and by cutting sections from a given animal at a variety of depths we can insure sampling of all major organs (Figure 7). In studies beyond the scope of this contract, we are characterizing the sequence content of the RNAs that accumulate in vivo during persistent infections and should eventually be able to formulate a molecular explanation for persistence and estimate the involvement (if any) of defective interfering viral genomes.

5) Construction of Complete NP and GP cDNA Genes and Subsequent Expression from Vaccinia Virus Vectors.

Using restriction sites that are common to overlapping clones we have begun the process of joining different clones together to create complete genes. For GP, we have joined 3 different clones to form a continuous sequence that covers all of GP-1 and 60% of GP-2. For NP, two clones have provided the complete gene sequence and these have been combined in a straightforward manner.

In collaboration with Drs. Lindsay Whitton and Michael Oldstone at Scripps, we have inserted the partial GP-C gene (aa 1-360) of LCMV-Arm and the complete GP-C of LCMV-WE into a vaccinia virus expression vector (16,17). Following infection with the LCMV-GP-C/vaccinia recombinant virus there is detectable synthesis of a GP-C related protein but immunofluorescence analysis shows substantially more cytoplasmic than surface accumulation of this protein, suggesting that inefficient transport of the recombinant GP-C to the cell surface occurs. Nonetheless, the recombinant vaccinia infected cells were lysed efficiently in vitro in a

Figure 6. Accumulation of viral RNA sequences during the course of LCMV persistent infections in BALB/w mice. All animals were infected at birth with LCMV (60 PFU given by intracerebral injection) and then were sacrificed at the indicated times. The in situ hybridization with an S specific probe was performed as described previously. At day 5, four individual animals showed no detectable hybridization signal despite having high titers of infectious virus in most tissues (e.g., brain, mean titer at day 5: 6×10^6 PFU/g). By day 15 and onwards, all three animals at each time point show significant and highly reproducible accumulation of viral nucleic acid. At day 180 the mean titer in brain: 1×10^4 PFU/g.

BALB/W MICE

S RNA

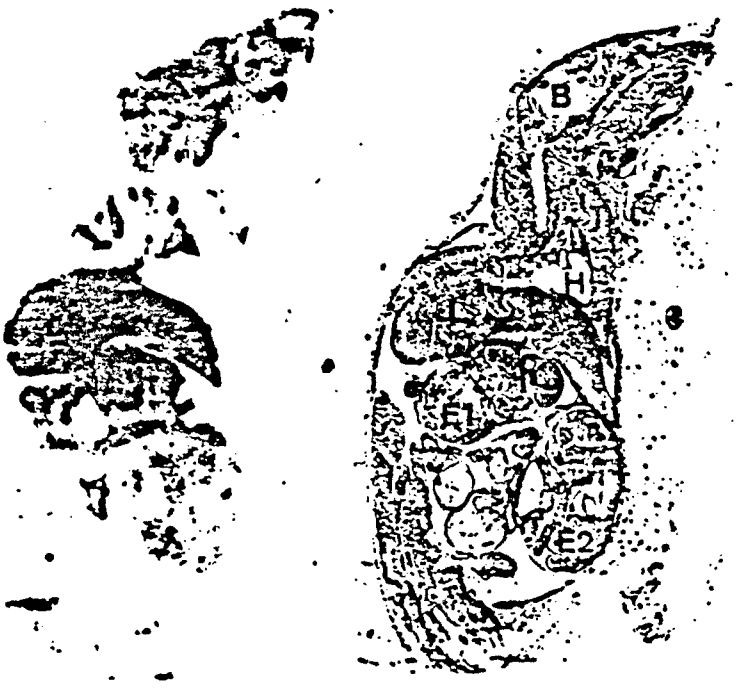

Figure 7. Whole body *in situ* hybridization and histopathologic analysis of a single section taken from a 19-20 day pregnant adult BALB/WEHI mouse persistently infected with lymphocytic choriomeningitis virus (LCMV). This female mouse had been mated with a persistently infected BALB/WEHI male. *In situ* hybridization was performed using a cloned 589-bp cDNA probe derived from the L-RNA segment of the viral genome (4×10^6 cpm of ^{32}P-labeled nick-translated probe, specific activity of 1×10^8 to 5×10^8 cpm/ug DNA was used). Other experiments showed that the hybridization signal was specific for LCMV nucleic acid and could be eliminated by RNase pretreatment; there was no signal with hybridization to tissues from uninfected mice. Two embryos can be observed in the stained section. There is a strong hybridization signal from the central nervous tissues of embryo 1 (F1) and the liver (L), brain (B), and thymus (T) of the pregnant mouse. (H) Heart; (L) liver; (E1) embryo 1; (E2) embryo 2.

standard ^{51}Cr release assay by populations of H-2 restricted, LCMV-specific cytotoxic T lymphocytes (CTLs). This result suggests that although we did not use a complete GP-C gene the sequences contained within the partial gene included a major recognition site for LCMV specific CTLs and that surface detection of antigens is much more sensitive in CTL killing assays than in immunofluorescence studies. Using the truncated GP-C gene (containing amino acids 1-363) and further carboxyl-terminal truncations, Dr. Lindsay Whitton has begun to map epitopes recognized by CTLs using populations of spleen cells and also with H2 restricted, LCMV-specific CTL clones that have been derived in Dr. Oldstone's laboratory. We have performed a first series of pilot experiments to determine whether protection against lethal LCMV-WE challenge is conferred by immunization with the LCMV-Vacc. recombinant. Nine guinea pigs were inoculated intradermally with 10^5 pfu of two different vaccinia constructs. Three weeks after inoculation representative animals were bled, and four of each group as well as two vaccinia wild type and LCMV-Arm wild type infected guinea pigs were challenged with 200 pfu of LCMV-WE. All of the vaccinia construct and wild type infected animals died 11-14 days later of typical LCMV-WE disease. As in previous experiments, the LCMV-Arm immunized controls survived WE challenge and had high titers of neutralizing antibody. The second half of the LCMV-Vacc. inoculated groups have been reimmunized with a second dose of each of their appropriate constructs and will be followed to detect rise in anti-LCMV antibody for an additional month prior to challenge. We will continue to assess additional LCMV-Vaccinia constructs for protective efficacy and are at the present time with Drs. Whitton and Oldstone preparing additional recombinants containing full length and a series of truncated lengths of LCMV-Arm GP-C. These recombinants will be made available to Fort Detrick investigators if we are able to show protection in the WE challenge protocol.

C. Use of Genetic Reassortants of LCMV to Analyze Molecular Basis of Disease

We have collaborated with Drs. Yves Riviere and Michael Oldstone to produce genetic reassortants of LCMV which have been used to analyze the genetic basis of pathogenesis. Although these studies were not formally a part of this contract, the findings impact on our approaches and our thinking with regard to arenavirus virulence. These experiments are documented in the accompanying manuscripts (Riviere et al., J. Immunol. 136:304; Southern et al., Cold Spring Harbor Vaccines 86, in press; Riviere et al., Virology 142:175; J. Virol. 53:966; J. Virol. 55:704), therefore the findings will merely be summarized briefly here.

Genetic reassortants between the LCMV-Arm and WE strains were made by mixed infection of BHK cells. Selected isolates were cloned, and their genotypes with respect to the L and S segment derivations were characterized by monoclonal antibody and cDNA hybridization analysis. Clones of the genotype Arm/Arm, WE/WE,

Arm/WE and WE/Arm (where the notation indicates L/S RNA segments respectively) were identified and re-cloned to ensure homogeneity. Only isolates of the genotype WE/WE (parental) and WE/Arm (reassortant) were found to cause lethal disease in the guinea pig. The Arm/Arm (parental) and Arm/WE (reassortant) clones caused no disease. Thus in the guinea pig model the L segment is associated with virulence. Production of disease is also dependent upon host species. It was shown that the S segment of LCMV-Arm is responsible for determining neuroendocrine tropism in the C3H mouse. Cytotoxic T-lymphocytes which are important in acute LCMV CNS disease apparently recognize a product of the S-RNA since CTL with specificity for LCMV-Arm were only able to kill cells replicating the S segment of that virus. Whether the target of CTL is a viral glycoprotein or nucleoprotein was not resolved by these experiments since both are encoded on S.

The implication of these studies to this contract is twofold. First, any vaccine should strive to maintain high levels of expression of the S encoded gene products because of their demonstrated importance in both CTL and neutralizing antibody responses. Second, any effort to control or systematically attenuate virulent Lassa virus must address changes in the L-RNA segment. These could conceivably be accomplished through targeted or site directed mutagenesis or as we have demonstrated with LCMV, through the construction of reassortants containing the surface proteins of virulent Lassa and the L gene of an attenuated strain or of another arenavirus. Clearly a detailed knowledge of the L gene and its products is important to understand virulence and attempt through rational means to modify it.

D. Structural Analysis of LCMV and Other Arenaviral Polypeptides

1. Epitope mapping of the LCMV glycoproteins: Identification of a type common neutralizing antigenic site.

We have used a panel of over 40 monoclonal antibodies against the LCMV glycoproteins (and over 100 against NP) to characterize the viral structures targeted by neutralizing antibody. Surveys of a large number of the monoclonals in our library were done in order to identify those with neutralizing capacity in the absence of additional effectors such as the complement system (C') or anti-Ig reagents. Six such neutralizing monoclonals were found, all of which reacted with GP-1. Using these and other MAb against GP-1 and GP-2, topographical relationships were established among antigenic sites (epitopes) on the envelope glycoproteins of LCMV. Fourteen purified MAb were radiolabeled and used as probes in a solid phase competition binding assay (Table 1, Figure 8). Epitopes on GP-1 were found to cluster in four antigenic domains as summarized in Table 2. Five neutralizing MAb raised by immunization with the WE strain of LCMV reacted within a single epitope termed GP-1A. This epitope was present on all of the 4 commonly used laboratory strains of LCMV (Armstrong, WE, Traub and Pasteur). A second epitope, termed GP-1B, was characterized

Table 1

Monoclonal Antibodies Selected for Use as ^{125}I-labeled Probes

Designation	Immunizing Virus	IgC Subclass	Polypeptide Specificity	Virus Strain[1] Reactivity	Western Blot	EIA Titer[2] WE	EIA Titer[2] Arm	Neut Tit WE	
WE-36.1	WE	IgG1	GP-1	A,W,P,T	-	501,187	630,957	>10,000	
WE-258.4	WE	IgG2a	GP-1	A,W,P,T	-	794,328	281,838	>10,000	5
WE-197.1	WE	IgG2b	GP-1	A,W,P,T	-	63,095	19,953	3,980	
WE-6.2	WE	IgG2a	GP-1	A,W,P,T	-	8,912	8,912	2,238	
WE-40.3	WE	IgG2a	GP-1	W,P	-	354,813	112	400	
2-11.1C	Arm	IgG2a	GP-1	A	-	158	1,778,280	<20	10
WE-327.3	WE	IgG2a	GP-1	W,P	-	158,489	35	64	
WE-2.9	WE	IgG1	GP-1	A,W,P,T	-	1,412	7,943	20	
WE-18.8	WE	IgG1	GP-1	A,W,P,T	+	56,234	14,125	<20	
WE-67.5	WE	IgG2a	GP-1	A,W,P,T	+	1,260	1,024	<20	
WE-33.6	WE	IgG2a	GP-2	A,W,P,T	+	562,341	83,175	<20	
WE-83.6.6	WE	IgG2a	GP-2	A,W,P,T	+	16,384	32,768	<20	
9-7.9	Arm	IgG2a	GP-2	A,W,P,T	+	6,208	28,526	<20	
WE-11.4	WE	IgG2b	GP-2	A,W,P,T	+	457,088	7,943	<20	

1 Armstrong = A, WE = W, Pasteur = P, and Traub = T.

2 ELISA immunoassay titer of MAb represents reciprocal dilution of ascites giving positive reaction on appropriate substrate.

3 Neutralizing titer is reciprocal of the dilution of ascites yielding 50% reduction in plaques on Vero cell monolayers.

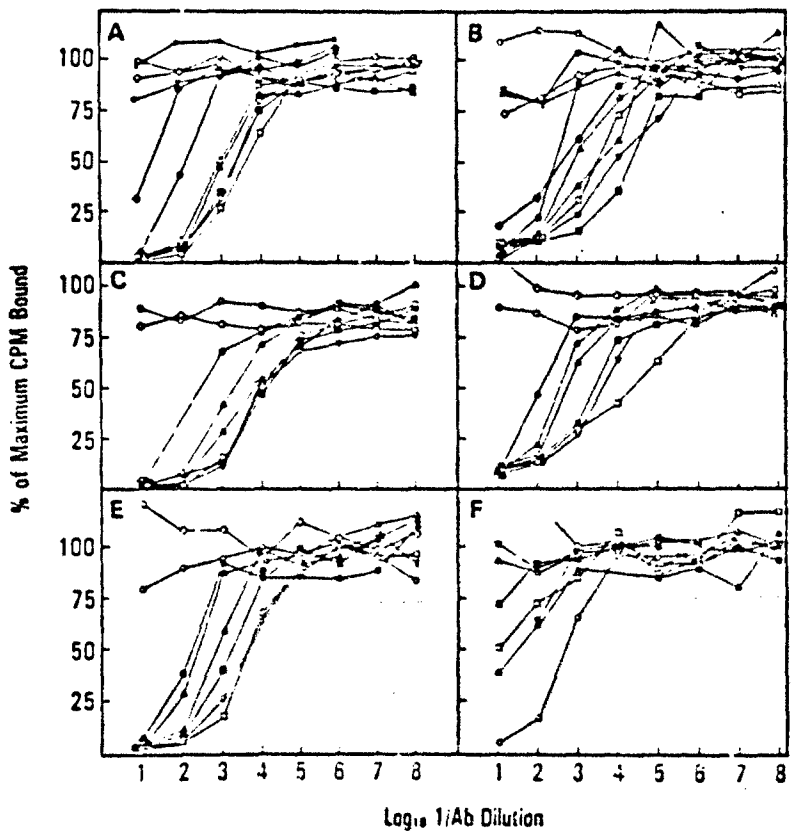

Figure 8. Competitive binding assays with LCMV neutralizing monoclonal antibodies. The following MAb were iodinated for use as probes, Panel A, WE-258.4; panel B, WE-197.1; panel C, WE-36.1; panel D, WE-6.2; panel E, WE-40.3 and panel F, 2-11.10. Radiolabeled monoclonals were mixed with tenfold serial dilutions of unlabeled competitors and binding measured on LCMV-WE (panels A-E) or Arm (panel F) coated wells. Unlabeled competing antibodies were WE-258.4 (□), WE-197.1 (▭), WE-36.1 (▲), WE-6.2 (△), WE-40.3 (▽), 2-11.10 (○), WE-327.3 (◗), WE-2.9 (×), WE-67.5 (◇), WE-18.8 (▽), and guinea pig-antiLCMV (●).

Table 2

SUMMARY OF COMPETITION RIA DATA
Inhibition of Binding by Unlabeled Competing Antibody *

Radiolabeled Antibody	Neutralizing	GP-1 ** 36.1	258.4	197.1	6.2	40.3	GP α CMV	327.3	2.9	2.5	217.7	229.1	18.8	67.5	2-11-10	8-13 (RAT)	8-40 (RAT)	8-55 (RAT)	GP-2 ** 9.7.9	33.6	83.6	57.6	11.4	Site Designation
WE-36.1	+	●	●	●	●	●	●							○	○									
WE-258.4	+	●	●	●	●	●	●	◑	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	
WE-197.1	+	●	●	●	●	●	●	●	○				○	○	○									GP-1A
WE-6.2	+	●	●	●	●	●	●							○	○									
WE-40.3	+(WE)	●	●	●	●	●	●							○	○									
WE-327.3	−	◑	◑	●	●	◑	◑	●	◑	●	●	●	○	○	○									
WE-2.9	−	○	○	◑	◑	○	◑	◑	●	●	●	◑	○	○	○									GP-1B
WE-18.8	−	○	○	○	○	○	○	○	○				●	●	○									
WE-67.5	−	○	○	○	○	○	○	○	○	○	○	○	●	●	●									GP-1C
2-11-10	+(ARM)	◑	◑	○	○	○	○	◑	○				○	○	●	●	●	◑						GP-1D
WE-33.6	−																		◑	●	●	●	○	GP2-A
WE-83.6	−																		◑	●	●	●	○	GP2-A
ARM 9-7-9	−																		●	●	●	●	○	GP2-B
WE-11.4	−																		◑	●	●	●	●	GP2-C

* ● > 80% inhibition by unlabeled antibody

◑ 40-80% inhibition by unlabeled antibody

○ <40% inhibition by unlabeled antibody

** Additional MAb were included as unlabeled competitor antibodies.

by a group of MAb which were very weakly or non-neutralizing. A subset of these GP-1B antibodies partially inhibited binding to site GP-1A, thus these epitopes are probably topographically linked. A third site, GP-1C, was also non-neutralizing, and in contrast to GP-1A and B was a denaturation resistant sequential antigenic determinant. Antibodies to site GP-1B enhanced the binding to site GP-1C presumably through a conformational change in the GP-1 molecule induced by binding of the GP-1B monoclonal (Figure 9). It may prove desirable to build such positive cooperative binding strategies into a potential vaccine provided that the structural perturbations can be well defined, as they are here. In addition to the type common neutralizing site GP-1A described above, we found a second neutralizing site termed GP-1D, which was confined to the Arm strain of virus and mapped near but not at site GP-1A. Site GP-1D appeared on the basis of analysis of Rat-Rat hybridomas to be an immunodominant site on Arm in that animal suggesting that neutralizing antibody response has a component of species specificity.

Analysis of GP-2 revealed 3 poorly resolved binding sites which we termed GP-2A, B and C (Figure 10). GP-2A was defined by two antibodies WE-83.6 and 33.6, while GP-2B and C represented the binding sites of 1 antibody each. These observations are of interest beyond the LCMV system. The 5 MAb defining sites GP-2A, B and C all reacted with the African arenavirus, Mozambique (Mopea) and the 3 MAb against site GP-2A also reacted with Lassa and Pichinde suggesting that this site is conserved on both New and Old World arenaviruses (3,18). We have subsequently tested MAb WE-33.6 on additional viruses by immunofluorescence found that it reacts positively with LCMV, Lassa, Mopea, Pichinde, Tacaribe, Amapari and Parana.

Finally, we have used our competition binding assay to define the epitopes recognized in a polyclonal guinea pig antibody response to LCMV. Guinea pigs were immunized by primary infection with 1000 pfu of LCMV-Arm followed 4 weeks later with a challenge dose of 5 ug purified LCMV-WE i.v. These guinea pigs developed PRD_{50} titers of neutralizing antibody averaging 1/4217 and 1/3543 measured against LCMV-Arm and WE respectively and were solidly immune to WE challenge. Polyclonal IgG was purified from these neutralizing antisera and used as both competitor and ^{125}I probe in the competition assay. As shown in Figure 11 and Table 3, this polyclonal anti-LCMV IgG was directed predominantly against the GP-1A major neutralizing epitope. This observation is of interest for two reasons: 1) because it points out the limited spectrum of antigens on GP-1 that are recognized by the polyclonal antibody response, and 2) because an equivalent immunization schedule with LCMV has been shown by Jahrling and colleagues (personal communication) to result in solid protection against Lassa challenge in guinea pigs and monkeys.

The observation of a major conserved neutralizing determinant on the GP-1 protein of LCMV suggests that this model might be an excellent system in which to explore the feasibility of an anti-idiotypic vaccine approach. We are uniquely prepared to approach

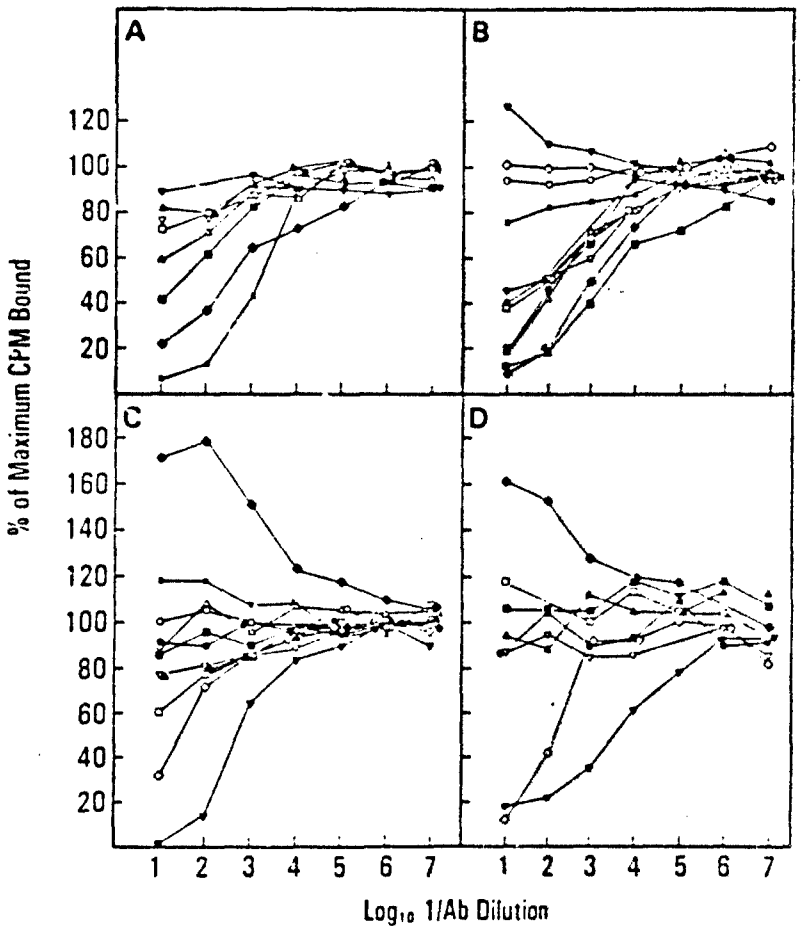

Figure 9. Competitive binding assays with ^{125}I-labeled non-neutralizing monoclonal antibodies WE-2.9 (A), WE-327.3 (B), WE-67.5 (C), and WE-18.8 (D). Assays were performed as described. Unlabeled competing antibodies are shown with same symbols as in Figure 8.

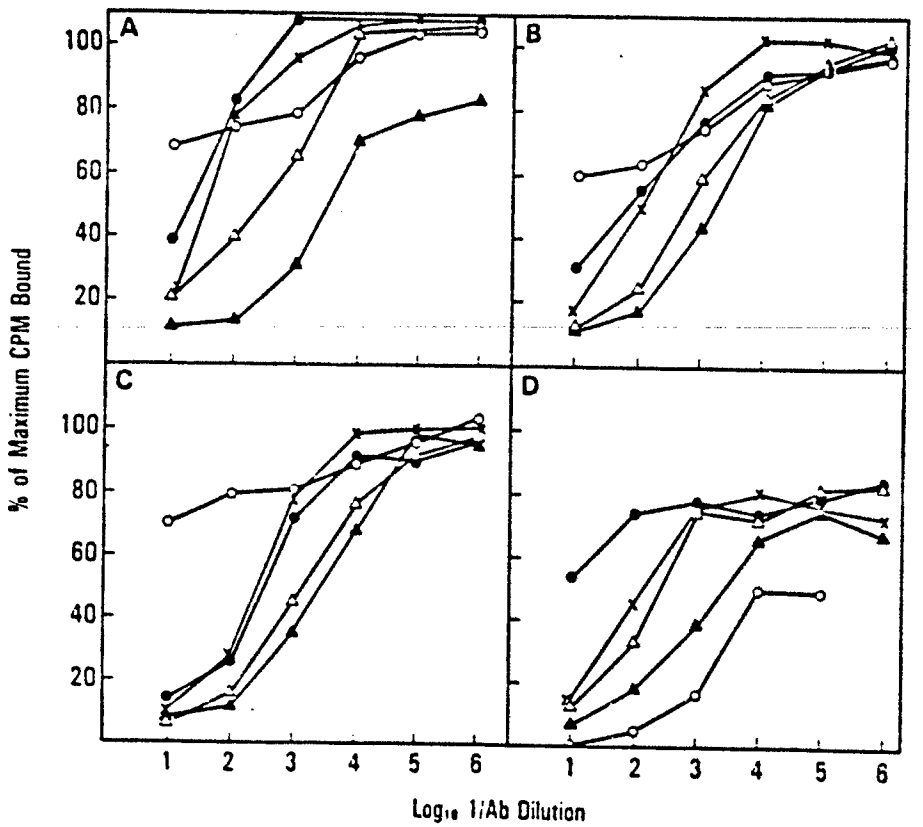

Figure 10. Competitive binding assays with ^{125}I-labeled GP-2 specific monoclonal antibodies WE-33.6 (A), WE-83.6 (B), 9-7.9 (C) and WE-11.4 (D). Unlabeled competing antibodies are WE-33.6 (▲), WE-83.6 (△), 9-7.9 (●), WE-11.4 (○) and WE-57.6 (X) and were used in a serially 10 fold dilution on WE substrate.

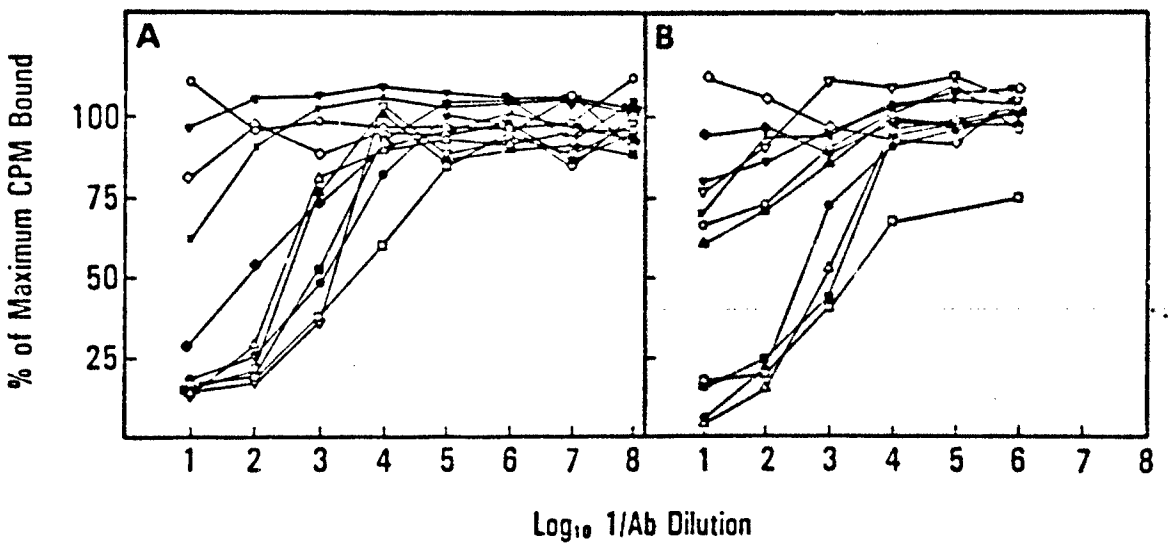

Figure 11. Competitive binding assays using ^{125}I-labeled polyclonal guinea pig-anti LCMV IgG. Assays were performed on both LCMV-WE (A) and Arm (B) substrates without prior disruption. Unlabeled competing antibodies are all GP-1 specific and are represented by the symbols as given: WE-258.4 (□), WE-197.1 (■), WE-36.1 (▲), WE-6.2 (△), WE-40.3 (▽), 2-11.10 (○), WE-327.3 (◆), WE-2.9 (X), WE -67.5 (◇), WE-18.8 (▼), and guinea pig - anti LCMV (●).

this question in the coming project period since we have well characterized mono- and polyclonal anti-LCMV antibodies, and cDNA clones, and are intimately familiar with all of the available LCMV biological model systems. This approach will be discussed more fully in the experimental protocols for the next project period. An effort will also be made in collaboration with Dr. Jahrling to use the techniques we have defined to quantitate the epitope specific responses of humans to Lassa using his collection of human plasmas from convalescent patients and Lassa monoclonals derived elsewhere.

2. Molecular architecture of the GP-C precursor: Use of synthetic peptides and antibodies to define gene order and cleavage.

As mentioned briefly in section B2, we have used antibodies against synthetic peptides corresponding to defined sequences on the NP and GP-C genes to map the genes on the S-RNA segment. We have also used these reagents to precisely identify the GP-1/GP-2 junction on GP-C and to identify the cleavage signal. Additional studies are in progress to define the linear sequences of GP-C which correspond to binding sites of neutralizing and other antibodies and to attempt to define stretches of amino acids which are recognized by cytotoxic and helper T cells.

All of these manipulations have a common basis in our ability to efficiently produce synthetic peptides corresponding to specific sequences deduced from cDNA analysis. Peptides were synthesized by solid phase chemistry using an Applied Biosystems model 430A peptide synthesizer (funded in part by this contract). Coupling efficacy was monitored by ninhydrin analysis at each cycle and homogeneity of the hydrogen fluoride cleaved product was assessed by analytical HPLC on a VYDAK reverse phase C-18 column. Coupling efficiency was usually greater than 98% per cycle and 70% or more of the cleaved product of a typical 12-16 residue peptide eluted as a single HPLC peak.

Peptides were coupled to Keyhole Limpet Hemocyanin (KLH) via free sulfhydryl groups of cysteine using the bifunctional reagent MBS (M-Maleimidobenzoyl N-hydroxysuccinimide ester). KLH-peptide conjugates were emulsified in Freund's Adjuvant, and rabbits were immunized 3 times at biweekly intervals. Ten days after the third immunization a boosting dose of 100 ug of free peptide adsorbed on alum was given intraperitoneally. Sera were obtained starting 1 week later and assayed by ELISA against free virus and against viral proteins. Specificity of anti-viral responses was demonstrated by inhibition of anti-viral reactivity by free peptide.

Initially synthetic peptides were made corresponding to several regions of the S and L-RNAs of LCMV with the aim of first defining the coding assignments of these regions and as a first step toward studying their functional and biological properties. Seven peptides were chosen from the S-RNA (see Figure 2) and one from the L-RNA and antibody was produced. Results of these immunizations and the specificity of the resultant antibodies are

summarized in Table 3.

Table 3

SUMMARY RESULTS OF ANTI-PEPTIDE IMMUNIZATION

Peptide	Rabbit #	Elisa Titer Anti-peptide[1]	Elisa Titer Anti-viral[2]	Polypeptide Specificity (Method)
GP-C 59-79	7542	16,000	64	GP-1 (IP, WB)[3]
	7543	16,000	16	GP-1 (IP, WB)
	8827	16,000	4	GP-1 (IP, WB)
GP-C 62-71	789	75,000	5	GP-1 (WB)
	790	75,000	-	GP-1 (WB)
GP-C 228-239	791	15,000	5	GP-1 (WB)
	792	78,000	-	NIL
GP-C 272-285	797	3,125	25	GP-2 (WB)
	798	3,125	25	GP-2 (WB)
GP-C 378-391	9257	260,000	64	GP-2 (WB)
	9258	16,000	64	GP-2 (WB)
NP 132-145	2159	15,000	ND	NP (WB)
	2160	15,000	ND	NP (WB)
NP 454-462	2165	625	ND	NP (WB)
	2166	3,125	ND	NP (WB)
L 174-187	8821	260,000	64	P 180+165 (WB)
	8822	260,000	16	P 180+165 (WB)

1 Anti-peptide titer measured on microtiter wells coated with 0.1 nmol of peptide.

2 Anti-viral titers measured on microtiter wells coated with 1.0 ug of purified LCMV-Arm.

3 IP, Immune Precipitation, WB, Western Blotting.

It can be seen from these data that generally high levels of anti-peptide antibody were achieved using the immunization scheme described. In subsequent experiments antibody titers measured against peptide have ranged between 1/75,000 and 1/1,000,000. Despite high anti-peptide ELISA titers, anti-viral titers in these assays have been lower and less consistent. For example rabbits 7542, 7543 and 8827 were all immunized in the same manner with the 20 amino acid peptide GP-C 59-79 and all three produced antibody titers of 1/16,000 against peptide. When measured against virus these three sera titered 1/64, 1/16 and 1/4 respectively. Despite the low ELISA titers all three sera were active against native GP-1 in immune precipitation assays and all reacted with denatured GP-1 in Western blots leading us to discount the significance of low anti-viral ELISA results. Similarly sera from rabbits 9257 and 9258 reacted only moderately against virus in ELISA but in Western blots were strongly positive at dilutions of 1/400 and greater (Figure 12A). Also shown is the inhibition of binding activity by the addition of 1 ug of free peptide to the antisera before use in the blot (Figure 12B), thus confirming that the anti-viral activity was due to peptide immunization and not an effect of polyclonal stimulation by or molecular mimicry with the KLH carrier protein.

Using these techniques we were able as shown in Figure 2 to definitively map the gene order of the S-RNA of LCMV. On the GP-C precursor molecule, GP-1 occupies the amino terminal and GP-2 the carboxy terminal domain. We wished then to define the limits of each glycoprotein. Sequence comparison between LCMV-Arm and WE and Pichinde GP-Cs revealed several conserved features near the middle of the approximately 500 amino acid molecule. As shown in Figure 13, a sequence homology of 11 of 12 residues appears at amino acids 228-239 (238-249 in Pichinde) and a second stretch of 12 of 15 is conserved at 272-286 (281-295 in PV). Between these conserved stretches there is little significant conservation with the exception of the double basic amino acid sequence-RR- at 262-263 in LCM, and -RK- at 271-272 in Pichinde. We reasoned that since there are a number of biological systems in which double basic residues serve to signal proteolytic processing events that the double basic sequence might serve the same function in arenavirus GP-C processing. Therefore we made peptides corresponding to the two conserved sequences flanking the -RR- putative cleavage site. The results of analysis of these peptides is shown in Table 4 and Figure 14. In the next contract period we will synthesize and study additional peptides representing the major portion of the GP-C gene of LCMV. Complete sequences of GP-Cs of LCM Pichinde and Lassa viruses are shown in Figure 15. Antibody to peptide 228-239 reacted with GP-1 and 272-285 with GP-2. Furthermore, we have synthesized an additional peptide 253-262 which represents the sequence immediately adjacent on the amino terminal side of the -RR- doublet, and this antibody recognizes GP-1. Thus it is unlikely that the cleavage of GP-C to produce GP-1 and GP2 occurs anywhere but at the double basic residue -RR- at positions 262-263. This is the first use of the synthetic peptide approach to precisely localize the cleavage signal for a protein and will undoubtedly

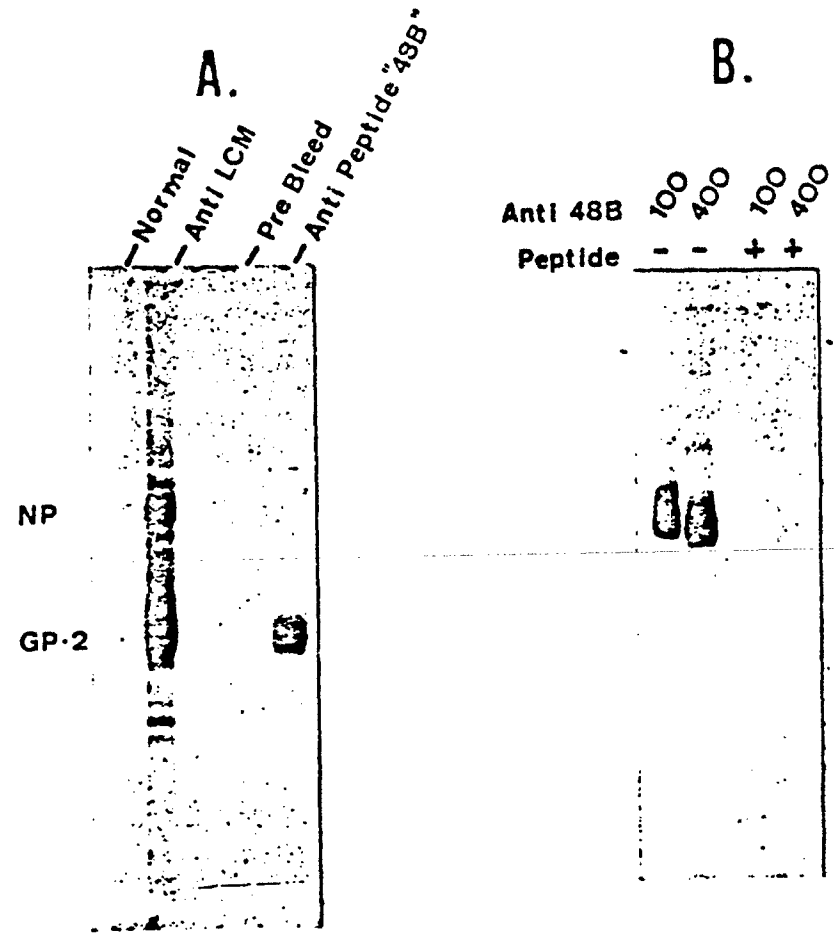

Figure 12. Reaction of rabbit antibody to peptide GP-C 378-391 sequence LEHAKTGETSVPKC. Panel A. Prebleed and post-immunization sera showing reaction with a band migrating in the position of GP-2 indicated by guinea pig anti-LCMV serum. Panel B. Antipeptide serum reacts at 1/100 and 1/400 with GP-2 but addition of 1 ug of free peptide abolishes reactivity.

PUTATIVE CLEAVAGE REGION OF GP-C

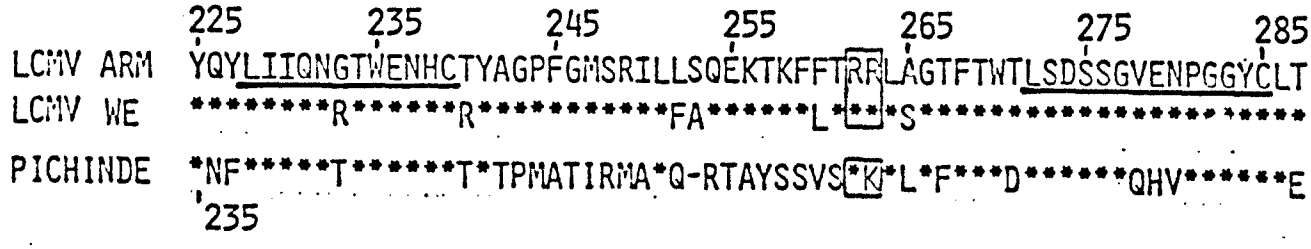

Figure 13. Amino acid sequence around the cleavage site of GP-C. Comparison of LCMV-Arm with LCMV-WE and Pichinde GP-C sequences is shown. Sequences chosed for peptide synthesis are underlined.

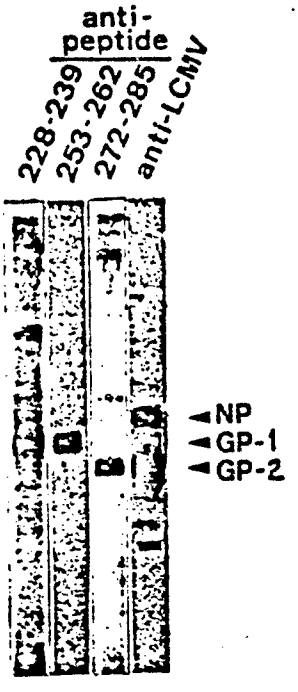

Figure 14. Reaction of anti-peptide antibodies, corresponding to sequences flanking the GP-C cleavage site, with LCMV structural proteins. Reaction of guinea pig polyclonal antibody to the virus is shown for comparison (anti-LCMV).

```
          WE   MGQIVTMFEA LPHIIDEVIN IVITVLIIIT SIKAVYNFAT CGILALVSFL
          LA   MGQIVTFFQE VPHVIEEVMN IVLIALSVLA VLKGLYNFAT CGLVGLVTFL
          PV   MGQIVTLIQS IPEVLQEVFN VALIIVSVLC IVKGFVNLMR CGLFQLVTFL
CONSERVED      MGQIVT      P    EV N    I         K   N    CG   L  FL

               LLAGRSCGMY GLKGPDIYKG VYQFKSVEFD MSHLNLTMPN ACSANNSHHY
               FLAGRSCGMY GLNGPDIYKG VYQFKSVEFD MSHLNLTMPN ACSVNNSHHY
               LLCGRSCT.. ....TSLYKG VYELQTLELN METLNMTMPL SCTKNNSHHY
               ILSGRSCDSM MIDRRHNLTH VEFNLTRMFD NL......PQ SCSKNNTHHY
                L GRSC                V                   P   C  NN HHY

               ISMGTS...G LELTFTNDSI ISHNFCNLTS AFNKKTFDHT LMSIVSSLHL
               ISMGSS...G LEPTFTNDSI LNHNFCNLTS ALNKKSFDHT LMSIVSSLHL
               IMVGNET..G LELTLTNTSI INHKFCNLSD AHKKNLYDHA LMSIISTFHL
               YKGPSNTTWG IELTLTNTSI ANETSGNFSN IISLGYGNIS NCDRTREAGH
                        G  E T TN SI       N

               SIRGNSNYKA VSCDFNNG.. .......... .ITIQYNLTF SDAQSAQSQC
               SIRGNSNYKA VSCDFNNG.. .......... .ITIQYNLSS SDPQSAMSQC
               SIPNFNQYEA MSCDFNGG.. ....K..... .ISVQYNLSH SYAGDAANHC
               TLKWLLNELH FNVLHVTRHI GARCKTVEGA GVLIQYNLTV GDRGGEVGRH
                                                  QYNL

               RTFRGRVLDM F.RTAFGGKY MRSGWGWTGS DGKTTW.CSQ TSYQYLIIQN
               RTFRGRVLDM F.RTAFGGKY MRSGWGWTGS DGYTTW.CSQ TSYQYLIIQN
               GTVANGVLQT FMRMAWGGSY I......ALD SGRGNWDCIM TSYQYLIIQN
               LIASLAQIIG DPKIAWVGKC FNNCSGDTCR LTNCEGGTH. ..YNFLIIQN
                           A  G                            Y  LIIQN

               RTWENHCTYA ..GPFGMSRI LLSQEKTKFF TRRLAGTFTW TLSDSSGVEN
               RTWENHCRYA ..GPFGMSRI LFAQEKTKFL TRRLSGTFTW TLSDSSGVEN
               TTWEDHCQFS RPSPIGYLGL LSQRTRDIYI SRRLLGTFTW TLSDSEGKDT
               TTWENHCTYT ...PMATIRM ALQRTAYSSV SRKLLGFFTW DLSDSSGQHV
                TWE HC       P                   R L G FTW  LSDS G

               PGGYCLTKWM ILAAELKCFG NTAVAKCNVN HDAEFCDMLR LIDYNKAALS
               PGGYCLTKWM ILAAELKCFG NTAVAKCNVN HDEEFCDMLR LIDYNKAALS
               PGGYCLTRWM LIEAELKCFG NTAVAKCNEK HDEEFCDMLR LFDFNKQAIQ
               PGGYCLEQWA IIWAGIKCFD NTVMAKCNKD HNEEFCDTMR LFDFNQNAIK
               PGGYCL  W     A  KCF  NT  AKCN   H  EFCD  R L D N  A

               KFKEDVESAL HLFKTTVNSL ISDQLLMRNH LRDLMGVPYC NYSKFWYLEH
               KFKQDVESAL HVFKTTLNSL ISDQLLMRNH LRDLMGVPYC NYSKFWYLEH
               RLKAEAQMSI QLINKAVNAL INDQLIMKNH LRDIMGIPYC NYSKYWYLNH
               TLQLNVENSL NLFKKTINGL ISDSLVIRNS LKQLAKIPYC NYTKFWYIND
                                 N L I D L   N  L       PYC NY K WY

               AKTGETSVPK CWLVTNGSYL NETHFSDQIE QEADNMITEM LRKDYIKRQG
               AKTGETSVPK CWLVTNGSYL NETHFSDQIE QEADNMITEM LRKDYIKRQG
               TTTGRTSLPK CWLVSNGSYL NETHFSDDIE QQADNMITEM LQKEYMERQG
               TITGRHSLPQ CWLVHNGSYL NETHFKNDWL WESQNLYNEM LMKEYEERQG
                 TG  S P  CWLV NGSYL NE HF          N   EM L K Y  RQG

               STPLALMDLL MFSTSAYLVS IFLHLVKIPT HRHIKGGSCP KPHRLTNKGI
               STPLALMDLL MFSTSAYLIS IFLHFVRIPT HRHIKGGSCP KPHRLTNKGI
               KTPLGLVDLF VFSTSFYLIS IFLHLVKIPT HRHIVGKSCP KPHRLNHKGI
               KTPLALTDIC FWSLVFYTIT VFLHIVGIPT HRHIIGDGCP KPHRITRNSL
                TPL L D     S   Y     FLH V IPT HRHI G  CP KPHR

               CSCGAFKVPG VKTVNKRR
               CSCGAFKVPG VKTINKRR
               CSCGLYKQPG VPVFWKR
               CSCGYYKIPL LPTLWVRLGK
               CSCG  K
```

ARM = LCMV Armstrong
WE = LCMV WE
LA = Lassa virus, Josiah strain
PV = Pichinde virus

Figure 15. Amino acid sequences of the GP-C glycoproteins of four arenaviruses.

be useful to others. Moreover, in a number of biologic systems including viruses, enzymes, and the complement system, cleavage activates or regulates biological functions of a molecule. For example in the paramyxo- and coronaviruses proteolytic cleavage activates the fusion function by cleavage of F_o $F_1 + F_2$ and E_2 $E_{2A} + E_{2B}$ respectively (19,20). The anti-peptide reagents we have made representing the cleavage domain will provide useful probes to study the process of cleavage, and potential biological activity of the cleaved glycoproteins. Finally, it is worth noting that sequence data for the Lassa GP-C supplied to us by David Auperin at CDC (personal communication) reveals precisely the same architecture around the cleavage site as we have described for LCM and Pichinde viruses.

Our efforts to define the structural and functional topography of GP-C continue. Currently we are preparing antisera to a series of overlapping peptides spanning the bulk of the GP-C molecule in an effort to 1) define regions accessible at the surface of native GP-1 and 2; 2) to survey for evidence of virus neutralizing activity in association with a specific region or regions of GP-1; and 3) to identify peptides with stimulatory activity for T- helper and cytotoxic cells. We have also made an effort to identify regions which are conserved broadly among arenaviruses with the interest of producing a set of widely applicable antibody probes for the glycoproteins of a number of the viruses.

E. Anti-idiotypic approaches to immunization: Pilot studies

We have begun studies to determine the feasibility of preparing an anti-idiotypic vaccine (21) against arenaviruses. The observation of a single neutralizing determinant shared among multiple strains and immunodominance of that site in polyclonal antibody responses suggested to us that an anti-idiotypic vaccine could be prepared. We purified monoclonals reacting with site GP-1A, the major neutralizing determinant on LCMV, and reduced these to F(Ab) fragments by digestion with pepsin. Purified F(Ab) was used to immunize guinea pigs and rabbits to produce anti-F(Ab) which was then adsorbed with an unrelated IgG_{2A} monoclonal to remove anti-xenotypic and isotypic reactivity. The adsorbed sera react only with the relevant, immunizing monoclonal. We are currently in the process of confirming anti-idiotypic reactivity by showing the ability of the adsorbed antisera to block monoclonal antibody-antigen interaction. An internal image anti-idiotypic antibody would be predicted to block the antigen antibody union. Once specificity has been demonstrated the antibody will be purified by affinity chromatography on a column containing the immunizing GP-1A monoclonal antibody, F(Ab) fragments will be prepared, and guinea pigs will be immunized. The animals will be monitored for production of LCMV neutralizing antibody and will ultimately be challenged with LCMV-WE to test protection. If protection can be demonstrated we will extend these studies in collaboration with Dr. Jahrling, who was a partner in the pilot work, to include anti-Id prepared against monoclonal and polyclonal antibodies to

Lassa virus. If these pilot experiments show evidence of neutralizing response and/or protection we will move rapidly to produce monoclonal anti-idiotypic antibodies which would then supply a virtually unlimited source of a given immunogen of interest. Success of this approach hinges upon identifying the appropriate internal image anti-Id which mimics the conformational structure of the neutralizing site on the virus.

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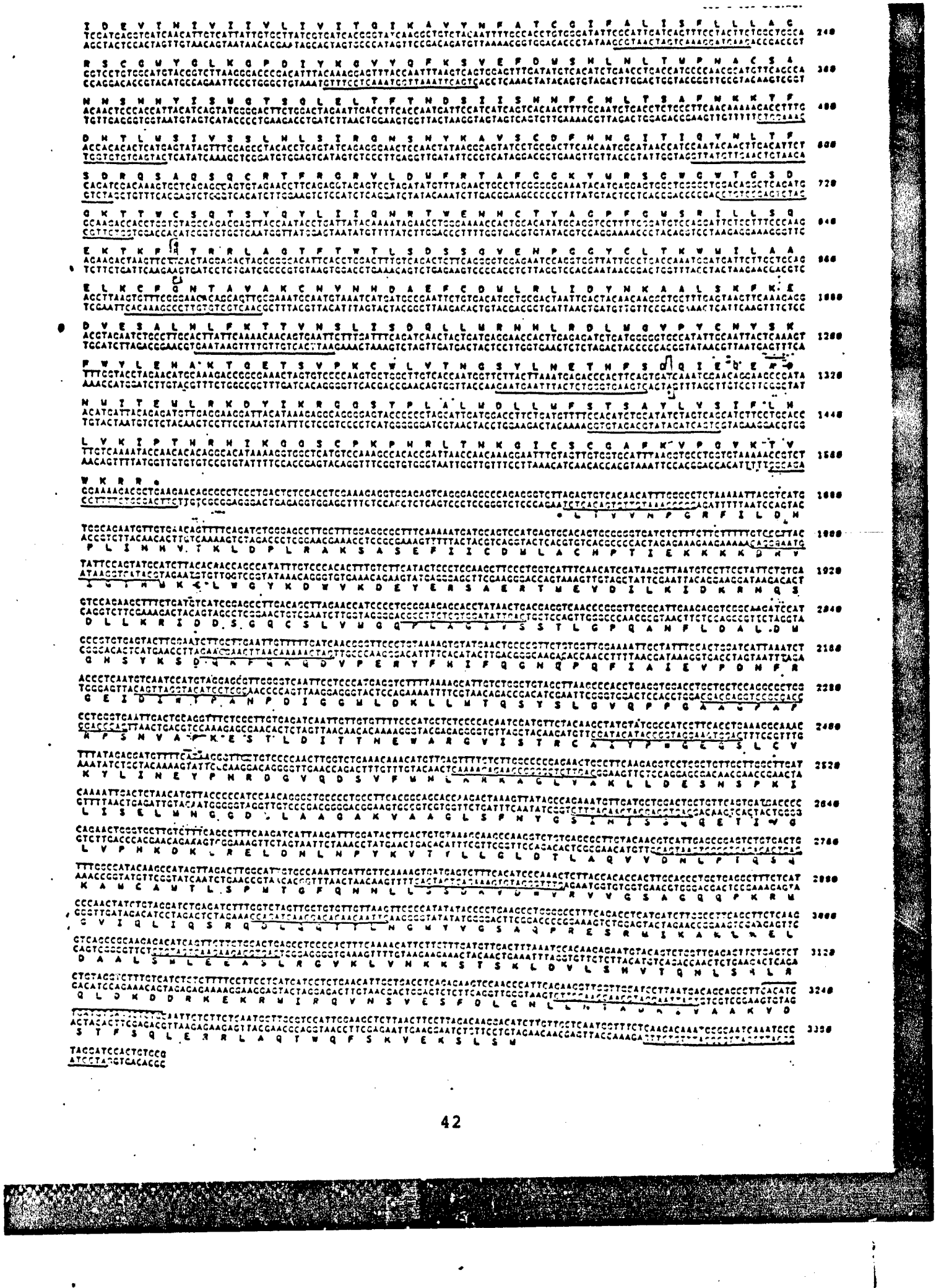
Appendix II

Complete nucleotide sequence of the LCMV Armstrong S RNA. The sequence is shown as duplex DNA running 5' to 3'. The glycoprotein coding region is located at the 5' end of the sequence and the nucleoprotein coding region at the 3' end.



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