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DIAGNOSIS AND PREVENTION OF INFECTION BY PHLEBOTOMUS FEVER GROUP VIRUSES

ANNUAL REPORT

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Punta toro M and S and cDNA probes for hybridization to virus infected cells have been produced in plasmids that can be transcribed by T7 and RNA polymerase. The probes react specifically with PT virus infected cells. The PT S cDNA (N, NSs) were expressed to high yield (10-40% total cellular protein) in baculovirus vectors. The M RNA segment of Rift Valley fever virus mutant M 12 has been cloned into DNA and approximately one-half of the M cDNA has been sequenced. Within this sequence there are 18 differences by comparison with RVFV ZZ 501. Clones representing the RVFV S sequence have also been obtained.

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TABLE OF CONTENTS

I. SUMMARY 2

II. REPORT 2

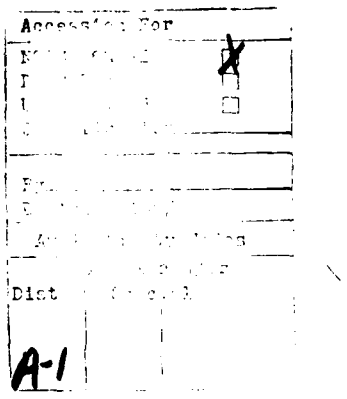
A. Introduction 2

B. Results from this Reporting Period 4

C. Summary of Progress Report 5

D. Literature Cited 6

Distribution List 8



I. SUMMARY

The objectives of the contract are to develop new diagnostic procedures and new vaccine strategies pertinent to selected Phlebotomus fever (PHL) group viruses, viruses that constitute the *Phlebovirus* genus of the negative-sense RNA virus family Bunyaviridae.[Bishop *et al.*, 1980]. This genus of viruses includes members that are known animal [including human] pathogens and that are therefore of consequence to military and civilian personnel in particular regions of the world [Bishop & Shope, 1979].

The development of new diagnostic tools for the specific identification of PHL group viruses is being investigated using eukaryotic expression vectors and cDNA clones of particular phlebovirus genes that are being prepared or that are available to us. Two expression systems are under study, vaccinia and the insect specific baculovirus expression vectors. So far, it has proven difficult to express the complete M RNA gene products of Punta Toro (PT) phlebovirus using either vector. Recombinant vaccinia viruses have, however, been made that express part of the PT M RNA, producing the PT glycoproteins G1 and G2.[Matsuoka *et al.*, 1988]. The expressed products have been characterized. The expression of PT S RNA gene products [Overton *et al.*, 1987] using *Autographa californica* nuclear polyhedrosis virus [AcNPV] baculovirus vectors also been reported.

The objective of developing new vaccine strategies for PHL group viruses is being realized through the characterization of an attenuated, candidate vaccine derivative of Rift valley fever [RVF] phlebovirus [the M12 mutant of RVFV isolate ZZ548]. This candidate vaccine was previously developed by staff of the Principal Investigator working at USAMRIID [Caplen *et al.*, 1984]. The complete sequence of the viral M RNA of the M12 RVFV derivative has been obtained [Takehara *et al.*, 1989]. The M RNA of the RVFV M12 derivative has been expressed in baculovirus vectors and the products characterized [Takehara *et al*, manuscript in preparation].

II. REPORT

A. Introduction

Our analyses have been directed towards developing new strategies for the development of phlebovirus vaccines and diagnostic reagents. Initial studies, supported by prior U.S. Army Medical Research and Development contracts, were aimed at characterising the genome and structural components of phleboviruses.

With the demonstration of a tripartite RNA genome for phleboviruses, genetic analyses were then undertaken to delineate the coding strategies of the viral RNA species and to determine if recombinant viruses could be obtained and used for vaccine purposes. By analyses of intertypic reassortant PT viruses we initially showed that the viral $7x10^5$ dalton small [S] RNA species codes for the viral $26.9x10^3$ dalton nucleocapsid [N] protein. The results of cloning and sequencing the S RNA of PT phlebovirus [Ihara *et al.*, 1984] confirmed these data and showed that an open reading frame in the *viral-complementary* S RNA sequence coded for N [S mRNA]. A second open reading frame in the *viral-sense* strand, coding for a $29.1x10^3$ dalton non-structural protein, NS_S [Ihara *et al.*, 1984], was also identified. The two subgenomic PT S induced mRNA species [one viral-complementary, the other viral-sense] were characterized with regard to their 3' and 5' end sequences and rates of synthesis in infected cells [Ihara *et al.*, 1985a; Emery & Bishop, 1987].

The results of cloning and sequencing the $2x10^6$ dalton middle [M] size RNA of PT virus [Ihara *et al.*, 1985b] demonstrated that the PT M RNA codes for the $50\text{-}70x10^3$ dalton viral glycoproteins, G1 and G2. A $30x10^3$ dalton non-structural protein, NS_M, that constitutes the amino terminal end of the PT glycoprotein precursor was also identified. The data showed that the order of the PT M gene products was NS_M-G1-G2. The PT M viral-complementary mRNA species were characterized [Ihara *et al.*, 1985a]. The results obtained for the PT M RNA correlated with the data reported by Collett and associates [1985] for RVFV M RNA and its gene products [RVFV M gene order: NS_M-G2-G1]. Although not proven, it is assumed that the phlebovirus $3x10^6$ dalton large [L] RNA species codes for the $200x10^3$ dalton large protein [a putative transcriptase component] found in viruses.

The genetic studies, including interference assays, reported from the work conducted in the previous contract, indicated that although intertypic reassortant viruses could be obtained, heterotypic virus interactions were not demonstrable (i.e., heterotypic phleboviruses did not interfere with each other and did not reassort their genomes in dual virus infections). Although not all phleboviruses were tested for genetic interactions, the results did not hold out much hope for this approach for vaccine development. Consequently, an alternative approach, that of developing candidate, including subunit, vaccines is under investigation in this contract. These objectives, together with research directed towards the development of new and specific diagnostic reagents for phleboviruses form the principal objectives of this contract.

B. Results from this Reporting Period.

Expression of the M RNA of the M12 candidate vaccine of RVFV using baculovirus vectors

The sequence of the M RNA of the ZZ501 strain of RVFV has been reported by Collett and associates (1985). In order to characterize the candidate RVFV vaccine that was produced from the related ZZ548 RVFV [Caplen *et al.*, 1984], we sequenced the M RNA of the M12 derivative [Takehara *et al.*, 1989]. The sequence was obtained by analyses of λ cDNA clones. The RVFV M12 M RNA was thereby deduced to be 3885 nucleotides long (mol. wt. 1.38×10^6) coding, in its viral-complementary sequence, for a single long gene product [the viral glycoprotein precursor] that is comprised of 1197 amino acids (130,000 daltons). The gene product is initiated by a methionine (AUG) codon at the M RNA nucleotide residues 21-23.

Unlike the RNA of the parent ZZ548 virus or that of ZZ501, there is a second AUG codon [residues 9-11] in the viral-complementary sequence *upstream* of the M12 M gene product. It is followed by valine and histidine codons, then a translation terminator [TAA, residues 18-20]. Of interest therefore to the question of why the M12 mutant is attenuated is whether the presence of this upstream short open reading frame (ORF) inhibits or reduces the expression of the viral glycoprotein. For this reason the M12 cDNA has been inserted into baculovirus transfer vector, pAcYM1 (Matsuura *et al.*, 1987). A variety of cDNA constructions have been made (e.g., with or without the upstream ORF) and recombinant baculoviruses prepared. Four recombinant baculoviruses have been analysed. The M1 baculovirus has both the upstream ORF and the ORF coding for the M glycoprotein precursor. The M2 baculovirus lacks the upstream ORF but contains the entire coding region of the glycoprotein precursor. The M3 recombinant lacks the upstream ORF and the sequences coding for the NS_M gene product. The M4 recombinant lacks the upstream ORF and the sequences coding for the RVFV G1 gene product.

In order to characterize the RVFV proteins that are expressed, the recombinant baculoviruses have been analysed by Western and immunofluorescence analyses using G1 and G2 specific monoclonal antibodies provided to us by Dr Jonathan Smith of USAMRIID. The studies have shown that the RVFV G1 and G2 proteins are made by all the recombinants (except in the case of M4). The glycoproteins are processed into the expected size classes and

appear on the surface of the baculovirus infected cells, although whether these are intrinsic properties of the proteins or related to the course of the baculovirus infection, are not known. From the pattern of surface expression, as analysed by the monoclonal antibodies, it appears that the G2 protein is present all over the cell surface while the G1 protein is segregated into discrete entities. The data show that there is little, if any, difference in the level of expression of the RVFV glycoproteins whether the upstream ORF is present or absent.

Analyses of the phenotypes of the expressed RVFV glycoproteins have shown that they mediate cell fusion at acid pH values. The cell fusion was inhibited by pretreatment with the G1 monoclonal antibody but not after pretreatment with the G2 monoclonal antibody.

The data on the expression of the RVFV M clones is in the process of preparation for publication.

Identification of RVFV S cDNA clones

From the cDNA library that yielded the M cDNA clones, phage containing RVFV S cDNA sequences have been sought. The library appears to contain 95% recombinant phage (based on the recovery of phage and the frequency of M specific clones). Consequently it was considered likely that there should also be S-specific clones in the library. DNA preparations from non-M phage were therefore sought and recovered by their ability to hybridize to RVFV RNA in "Northern" analyses. This procedure identified RVFV S cDNA clones. Several cDNA clones were obtained and the sizes of their inserts determined by restriction analyses of the DNA. The largest DNA inserts have been sequenced. The sequence obtained for approximately 800 nucleotides of S cDNA predict a protein that is comparable to the NS_S protein of PT and Sicilian sandfly fever phleboviruses. Studies are continuing to find cDNA clones representing the rest of the RVFV S RNA.

C. Summary of Progress Report

The sequence of the M RNA of the M12 derivative of RVFV ZZ548 has been inserted into baculovirus expression vectors and the properties of the expressed products have been investigated. The glycoproteins are processed and

transported to the infected cell surface. Cell fusion at acid pH values has been documented. cDNA clones representing part of the S RNA of RVFV ZZ548 have been obtained and their sequences determined.

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