MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18
FROM: AID/SCI, Victoria Ose
SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 7, 384

Read 4/7/88

Attachment
Progress Report: Grant No. DPE-5542-G-SS-7008-00, Development of a peptide vaccine for protection against bovine anaplasmosis

PI: Guy H. Palmer
Dept. of Comparative and Experimental Pathology
University of Florida
Gainesville, Florida 32610

Reporting Period: 1 July 1987 through 31 December 1987
note: project implementation was delayed pending final budgetary approval and release of funds.

Goals of the Project: The failure to develop effective immunoprophylaxis against anaplasmosis results from the complexity of the disease, including persistence of the parasite and from lack of application of current research technology to the disease.

We have recently cloned the gene for Am 105 with the objective of deriving the primary structure of the native Am 105 protein. Determination of the structure of Am 105, specifically the protection-inducing epitope, will allow development of synthetic peptide analogs for immunization of cattle. Synthetic peptide immunogens have several unique properties which may be critical to effective use of a subunit Am 105 vaccine: [1] the protection-inducing peptide can be efficiently synthesized in vitro without the requirement for large scale fermentation followed by purification of the single expressed protein from E. coli host cells; [2] the presentation of the key protection-inducing epitopes, especially if linked in tandem repeats, may focus the immune-response more effectively on this isolate-common epitope than vaccines containing extraneous proteins; [3] the peptide based vaccine carries no risk of reversion to virulence or as a threat to immunodeficient animal caretakers.1-3 The recent success of synthetic peptides as a protective immunogen in malaria clearly demonstrates the feasibility of the molecular biology - synthetic epitope approach.4 The identification of Am 105 as bearing an invariant peptide epitope capable of inducing protection in immunized cattle, combined with recent advances in molecular biology, make development of an effective synthetic peptide vaccine an achievable goal.5-6 The research will also provide needed insight into genomic-antigen relationships in rickettsial pathogens and has the potential to demonstrate the effectiveness of a molecular biological approach to solving animal disease problems.

The specific aims to attain these goals are:
1. Sequence the gene coding for AmF 105 and derive the primary structure of AmF 105.
2. Synthesize peptide analogs of the AmF 105 protection-inducing epitope based upon the primary structure of AmF 105.
3. Demonstrate the effectiveness of a synthetic peptide analog of the protection-inducing AmF 105 epitope as a protective immunogen.

Progress to Date: Project progress to date has been strong and has resulted in completion of the gene sequence of the pAMT1 clone expressing the protection-inducing epitope of AmF 105. Briefly, we have developed a restriction endonuclease map for the T1 clone using single and multiple enzyme digests
Interestingly the region bounded by enzymes Kpn I and Pst I has repeated enzyme sites for Bcl I. This observation suggested that a repeat sequence may be present in this Kpn I-Pst I region. Sequencing of the pAMT I has been completed using both strands and is presented in its entirety in Fig. 2. The suggestion of a repeated sequence is confirmed by the actual nucleotide sequence (Fig. 3). Sequence analysis to date reveals both a promoter site and a ribosomal binding site similar to those consensus sequences in E. coli and other rickettsia. These sequences have been used to identify the presumptive reading frame. Currently our efforts are in confirming the reading frame and obtaining the primary sequence of AmF 105 in order to complete Specific Aim 1 in its entirety. We expect to complete this by July 1, 1988.

Collaboration with Zimbabwe: In collaboration with Dr. W.N. Madzima, Deputy Director of Veterinary Services in Zimbabwe, we have selected a post-graduate fellow. Ms. Ntando Tebele received a B.Sc. (Agriculture) from the University of Zimbabwe in 1982 and was a research technician in the Department of Protozoology, Veterinary Research Laboratories 1982-1983. Ms. Tebele qualified as a veterinarian in Zimbabwe in November 1987, and will receive a B.V.Sc. in May 1988. Ms. Tebele will join the USAID Anaplasmosis project full-time in May and will apply to graduate school in the U.S. at that time. Dr. Palmer will travel to Harare in February 1988, to meet with Dr. Madzima regarding project implementation. At this time, a training schedule for Ms. Tebele will be proposed and sent to USAID for approval.

Financial Report: As of December 31 we had not yet expended any funds due to an internal administrative delay in obtaining access to funds. We have corrected this problem and are now proceeding to expend funds.
References


FIG. 1

Anaplasma marginale DNA in pAM T1

Kilobase pairs
TRANSLATED SEQUENCE OF S2

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CAA GCC GCT GTC GTA GGG GCG AAA ATT GTA GCT CGG GAA ACT ATA TCT CAC TAC AGA AAG
Q A A V G A K I V A R T I S P Y R K
K F L - G R K K L - L G K L Y L P T E R
S R C R G E N C S S S G N Y I S L Q K G
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60    70    80    90   100   110
*     *     *     *     *     *
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GAC GTA ACG GCT AAG GTG TAC GGG CGC GAC GTC AGC AGA AAG AGT AAA CTT CTG GAA
D V T A K V Y G R D V T R K M K L L E
T - R L R C T G A T - R E R - N F W K
R N G - G V R A R R D E K D E T S G K
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120   130   140   150   160   170
*     *     *     *     *     *
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ARG CAG AAG AAG GGA AAA AAG AGG TGG CGG TCT ATT GGT AAT GTC AAG GGT GTC GAA
K D D K K G K R L R S I G N V N V P D
S R R R E K R R C G L L V M S T F L K
A E G K K E V A V Y W - C Q R S S K
```

```
180   190   200   210   220
*     *     *     *     *     *
```

```
AGC GCG TTC ATA CAG GCC GCT ARA AAT GAA GAC TGA CGC GCA TCA ATG CAT GAC CCG
S A F I Q A L K M K D - R A - N H E P
A R S Y R P - K - R T D A H E C M S L
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230   240   250   260   270   280
*     *     *     *     *     *
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```
GTT GTC ACT GCG TGC ACA ATC TGC TGC GCT ATG GCT ATC TTA CTT GTG GCG ATT GTG GCC GAC
G V T A C T I W A M L L V A I V A D
V - L R A D E G L W L Y L W R L W P T
C D L V H N L G Y E F T C G D C R H
```

```
290   300   310   320   330   340
*     *     *     *     *     *
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```
ATC GCG CTC ACC CTG TCG TAC TTT CAA GTC CSG TCT ATT ACA TCA GAC AGC TTA CCC AGC
I R L T L S Y F Q V R F T S D S L P S
S G S P C R F F K S D L H Q T A C P A
P A H P V V L S S P I V I R L O A Q K
```

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350   360   370   380   390
*     *     *     *     *     *
```

```
ATT CAG CCA AAG CAC TGC TTT TGC GCT GGA GCT CAG TTA GAC GCC GAC AAG AGG CGC GCA
I Q P R H C F W A G R O L G R K R A
F S G T A F G L D V S - A A G S A H
S A K A L L L G W T S V R R Q E A R I
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This is the Reverse Complement Strand
Three frames translation
Translated to 3' end
Sequence printed from base no. 1 to base no. 2664
Sequence numbered beginning with base no.