PN-ABB-110

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON D.C. 20523

DATE: 7/25/88

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
PR - 1/1/88 - 6/30/88

Attachment

7 354

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

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Reporting Period: 1 January 1988 through 30 June 1988

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. Based upon this sequence data we have identified a tandemly repeated peptide composed of the primary structure: S-A-G-G-Q-Q-Q-E-S-S-V-S-S-Q-S-D-Q-A-S-T-S-S-Q-L-G-A-D-S-S. The first repeat is a variant of this structure with an E-A insertion for D at the 16th position. The

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subsequent 7 repeats have the D at the 16th position. Importantly as the expressed polypeptide from the pAMT1 clone contains only repeat segments plus 11 amino acids at the N-terminus, this suggests that the epitope recognized by the neutralizing monoclonal antibody is contained within the repeat. We have constructed a 29-mer peptide based upon the D containing repeat and have tested its ability to bind the neutralizing monoclonal antibody using an inhibition radioimmunoassay. The monoclonal antibody binds the 29-mer effectively. Therefore, to date we have cloned and expressed the gene coding for the AmF 105 protein in E. coli, identified promoter and ribosomal binding sites in the sequenced gene, identified the correct reading frame, obtained the primary structure for AmF 105, and localized the neutralization-sensitive epitope to a 29 residue repeated peptide. Currently, we are synthesizing small (6-10) residue peptides in order to further localize the monoclonal antibody binding site.

Collaboration with Zimbabwe: Dr. Palmer travelled to Harare for a collaborative exchange from 9 February to 3 March 1988, in order to help set up the research program in Zimbabwe. During the visit the principal goals were: 1] Finalize the budget with Dr. W.N. Madzima, Deputy Director of Veterinary Services in Zimbabwe, for final approval by the Ministry of Agriculture, Lands, and Rural Resettlement, 2] Arrange laboratory space and animal facilities at the Veterinary Research Laboratory with Dr. George Stewart, Assistant Director of Veterinary Services (research), 3] Develop a research strategy with Dr. Ntando Tebele, project research officer in Zimbabwe, to obtain and characterize Zimbabwean strains of Anaplasma marginale (a copy of the project strategy is attached), and 4] Develop a training program for Dr. Tebele based upon obtaining a M.S. at a U.S. College of Veterinary Medicine. All these goals were successfully met. In addition, a seminar, "Testing enzootic stability as a control measure against tick-borne hemoparasitic diseases", was presented 2 March at the Veterinary Research Laboratory to the research officers of the Protozoology section.

Publications resulting from project:

1. Oberle SM, Palmer GH, Barbet AF, McGuire TC. 1988. Molecular size variations in an immunoprotective protein complex among isolates of Anaplasma marginale. Infect. Immun. 56:1567-1573.

References

1. Ristic M, Carson CA. 1978. Methods of immunoprophylaxis against bovine anaplasmosis with emphasis on the attenuated *Anaplasma marginale* vaccine. p. 151. In Miller LS, Pino JA, McKelvey JJ (eds), Immunity to Blood Parasites of Animals and Man. Plenum Press, New York.

2. Callow JJ. 1978. Vaccination against bovine babesiosis. p. 121. In Miller LH, Pino JA, McKelvey JJ (eds), Immunity to Blood Parasites of Animals and Man. Plenum Press, New York.

3. Redfield RR, Wright DC, James WD, Jones TS, Brown C, Burke DS. 1987. Disseminated vaccinia in a military recruit with Human Immunodeficiency Virus (HIV) disease. New Engl. J. Med. 316:673.

4. Zavala F, Tam JP, Barr PJ, Romero PJ, Ley V, Nussenzweig RS, Nussenzweig V. 1987. Synthetic peptide vaccine confers protection against murine malaria. J. Exp. Med. 166:1591-1596.

5. Palmer GH, Barbet AF, Davis WC, McGuire TC. 1986. Immunization with an isolate-common surface protein protects cattle against anaplasmosis. Science 231:1299-1302.

6. Palmer GH, Waghela SD, Barbet AF, Davis WC, McGuire TC. 1987. Characterization of a neutralization-sensitive epitope on the Am 105 surface protein of *Anaplasma marginale*. Int. J. Parasitol. 17:1279-1285.

7. Anderson BE, Regneny RL, Carlone GM, Tzianabos T, McDade JE, Yuan Fu Z, Bellini WJ. 1987. Sequence analysis of the 17-kilodalton-antigen gene from *Rickettsia rickettsia*. J. Bact. 169:2385-2390.

8. Cold L, Pribnow D, Schneider T, Shinedling S, Singer B, Stormo G. 1981. Translation initiation in prokaryotes. Ann. Rev. Microbiol. 35:365-407.

9. Hawley DK, McClure WR. 1983. Compilation and analysis of *Escherichia coli* promoter DNA sequences. Nuclear Acids Res. 11:2237-2255.

[illegible] farming systems, and it is expected that these differences will have a bearing on the A marginale isolates. The areas to be sampled are the following :-

Place	Region	Rainfall
Chinhoyi	IIa	750-1000mm
Mutare	III	650-800 mm
Bulawayo	IV	450-650 mm
Chiredzi	V	600 mm

Commercial dairy farms with very good tick control and most preferably free from Theileriosis and Babesiosis will be visited and blood samples collected. Heparinised blood, about 10ml per cow, will be collected from 20 animals per farm. Blood from 5 animals will be pooled, and, from this, a single stabilate will be made.

The blood, after collection will be kept on ice and transported to the laboratory the same day. Once in the laboratory, the following procedure will be followed :-

- make blood smears, stain and look for parasites. If Babesia or Theileria parasites present, discard the blood.
- Centrifuge and remove the buffy coat.
- wash three times in PBS (without sodium azide)
- resuspend packed cells 1:1 in 31.2% (v/v) DMSO in PBS.
- make up 3.6ml stabilates of each pool in cryo-tubes.
- freeze in liquid nitrogen - fluid phase.

The stabilates will be stored in liquid nitrogen until they are ready for use.

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