Hawaii Biotechnology Group, Inc.

26 April 94
Re: Contract Number N00014-93-C-0019

Commanding Officer
Naval Medical Research and Development Command
Scientific Officer: LT CDR P. Knechtges
National Naval Medical Center, Bldg. 1, 12th Floor
8901 Wisconsin Avenue
Bethesda, MD 20889-5606

Dear Lt. Commander Knechtges:

Enclosed is the progress report for the fifth quarter (1/1/94-3/31/94) of the contract period. The accompanying report describes our current progress for each portion of the contract. If you have any questions, please do not hesitate to contact me (ext. 380) or the project's lead scientist, Dr. Ogata (ext. 399).

Sincerely,

John M. Ivy, Ph.D.
Scientist, Group Leader
Molecular Genetics

4/7/28

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I. Overview

The following progress report covers the fifth quarter (1/1/94-3/31/94) for contract number N00014-93-C-0019. Four topics are included in the contract; each topic's goal and current status are as follows:

1. Production of anti-ferret IgA antibodies. Status: **Rabbit immunization completed. Immune serum collected. Currently cross-adsorbing serum with ferret IgG.**

2. Purification of lipopolysaccharide from *Shigella* and *Campylobacter* species. Status: **Preparation of *Shigella* LPS completed. Material delivered to Navy. Production of *Campylobacter* LPS on hold.**

3. Development of an enzyme immunoassay for the detection of enteroaggregative *Escherichia coli* heat-stable toxin. Status: **This portion of the project has been suspended.**

4. Production of monoclonal antibodies against strain-specific antigenic epitopes on *Campylobacter coli* flagella. Status: **Have completed screening hybridomas from two fusions. Flagellin reactive MAbs produced; no strain-specific MAbs identified. Preparing for third fusion.**

II. Current Progress

1. Production of Anti-ferret IgA Antibodies

Production of rabbit α-ferret sIgA. In the progress report for the first year, we reported that the immunization of rabbits and collection of their sera had been completed at the beginning of this quarter. During the current quarter, we have attempted to cross-adsorb the rabbit antisera, using a Affi-gel column containing ferret IgG, to remove cross-reactive antibodies. The column had originally been used to cross-adsorb mouse ascites containing α-ferret sIgA antibodies and was very effective (First Year Report). However, we obtained very poor results when using the same column to cross-adsorb two milliliters of rabbit sera. We believed that the difference in efficiency was due to a combination of the high antibody titer in the rabbit sera and an insufficient amount of ferret IgG on the column. Repeated cross-adsorption of the sera produced a visible reduction in the amount of cross-reactive antibodies, thus supporting this hypothesis.

Although repeated cross-adsorption had reduced the amount of cross-reactive antibodies, the final product still possessed significant cross-reactivity. For this reason, we concluded that a column with greater capacity (i.e. - more ferret IgG) would be necessary. Therefore, we purified additional ferret IgG from size-exclusion chromatography fractions that were collected during the purification of the ferret sIgA. Based upon our yields, the amount of IgG that we have at our disposal (chromatography fractions and clarified milk combined) is probably inadequate (~6mg). For this reason, we contacted Captain L. Bourgeois of NMRI and requested ~10mls of ferret serum which should contain ~10mg of IgG per milliliter. This amount of serum should contain sufficient IgG for the preparation of a column that will have the requisite capacity to effectively cross-adsorb the rabbit sera. At present, we are waiting to receive the ferret serum from NMRI.

2. Extraction and Purification of Lipopolysaccharide

The extractions of lipopolysaccharide (LPS) from *Shigella sonnei* 53LB and *Shigella flexneri* 2a have been completed. The final product was sent to Captain Bourgeois in January 1994. Due to the reduction in funding for this contract, the extraction of LPS from *Campylobacter jejuni* 81176 has been postponed.

3. Enteroaggregative *Escherichia coli* Heat-Stable Toxin 1 (EAST1)

All work on this portion of the project has been suspended indefinitely due to reductions in funding.

4. Type-Specific Campylobacter Flagellin Epitopes

Production of α-Flagellin Monoclonal Antibodies. During the fifth quarter, we completed screening and characterizing the hybridomas from the first and second fusions. We have produced a number of MAbs which react with, but are not specific for, the T2 flagellar antigenic type of *Campylobacter coli* strain VC167. One such MAb which we have characterized is CCA 79. This MAb is an IgG_1 isotype and reacts equally with whole cells, as well as crude flagellin extracts, from both antigenic types T1 and T2. The MAb does not react with *C. jejuni* 81176 cells, thus suggesting that it is directed against an epitope that is species specific. We intend to continue to use MAb 79 as a positive control for future screening. Additionally, the MAb may be useful for immunoaffinity purification of flagellin.

Neither the first nor the second fusion has produced MAbs which are specific for the T2 antigenic type. We had tentatively identified MAb CCB 227 to be T2-specific due to its greater

reactivity with whole-cells of the T2 antigenic type than the T1 type in ELISA and flow cytometry. Further characterization of the MAb for reactivity against flagellin in a sandwich ELISA demonstrated that MAb 227 reacts with flagellin; unfortunately, the MAb recognized both T1 and T2 flagellin equally. In addition, the MAb reacted with whole cells of a T2 mutant which does not possess the T2-specific, post-translational modifications, thus supporting the finding that the MAb is not T2-specific. Immunofluorescence microscopy revealed that the greater reactivity to *C. coli* VC167 T2 in the whole cell ELISA was due to reactivity of MAb 227 with an epitope which is present on the cell surface of VC167 T2 but not on the surface of VC167 T1. Therefore, this MAb does not recognize the epitopes of interest.

The reactivity of MAb 227 with an epitope that appears to be present upon both the flagella and cell surface is intriguing. We believe that MAb 227 is analogous to MAb 1B4 that was reported by Konkel et al (1990). MAb 1B4 labelled the cell surface and flagella of low-passage *C. jejuni*, but only the flagella of high-passage *C. jejuni*. In addition, MAb 1B4 inhibited invasion of HEp-2 cells by virulent *C. jejuni*, thus suggesting that the MAb recognizes an invasin. The low-passage *C. jejuni*, which were more virulent than the high-passage *C. jejuni*, may be analogous to *C. coli* VC167 T2 which is the flagellar antigenic type that preferentially colonizes the host in animal models (Logan et al, 1989). Based upon the similar distributions of the epitopes for our MAb and MAb 1B4, it is possible that ours is against an invasin as well. We have discussed our findings with Dr. Guerry of NMRI who has made arrangements with Dr. Trevor Trust of the University of Victoria, Canada, to further characterize the MAb by immunogold electron microscopy. Prior to completion of this report, MAb 227 was sent to Dr. Trust.

III. Plans for Present Quarter

Having been informed of the allocation of an additional $60,000 to this project during the past quarter, we have sent inquiries to Capt. Bourgeois regarding reinstatement of suspended portions of the project. At present, we are awaiting his reply.

1. Production of Anti-Ferret IgA Antibodies

Obtain ferret serum, purify IgG, then complete cross-adsorption of rabbit α-ferret sIgA.

2. Extraction and Purification of Lipopolysaccharide

Remaining work suspended.

3. EAST1

No work planned due to suspension of this portion.

4. Type-Specific Campylobacter Flagellin Epitopes

Perform third fusion.

IV. References

Konkel, M.E., F. Babakhani, and L.A. Joens. 1990. Invasion-related antigens of *Campylobacter jejuni*. J. Infect. Dis. 162:888-895.

Logan, S.M., P. Guerry, D.M. Rollins, D.H. Burr, and T.J. Trust. 1989. In vivo antigenic variation of *Campylobacter* flagellin. Infect. Immun. 57:2583-2585.