(12)

January 23 1991

Commander Lyn Yaffe
Naval Medical Research and Development Command
Naval Medical Command
National Capital Region
Bethesda, Md 20814-5044

Dear Commander Yaffee:

The project on polymyxin B(PMB)-specific monoclonal antibodies was initiated in September 1989 and was terminated in June 1990. A previous progress report was written and submitted around January 12 1990. Please forgive me for the tardiness of this final report on this project. As has been reported earlier, PMB conjugated to either bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH) were very immunogenic in both rabbits and Balb/c mice. The latter was not as immunogenic as the PMB-BSA conjugate largely because of it insoluble state. Both preparations have lost significant antibacterial properties suggesting that a significant number of the diaminobutyric acids were substituted by the conjugation process. The conjugated PMB preparations were still toxic to mice when given intravenously in 2 ug quantity. Multiple intraperitoneal or subcutaneous injections of 100 ug of PMB-BSA in the presence of Freund's adjuvants stimulated the production of high levels of PMB-specific antibodies in Balb/c mice.

We went ahead with the fusion experiments to make monoclonal antibodies even though some of the epitopes might have been destroyed by the conjugation method. It was our hope that some intact epitopes would remain on the PMB molecules to allow recognition by specific monoclonal antibodies. Dr. Fei-Xiong Yao was hired to assist in the production phase of the monoclonal antibody work. Approximately 10 fusion experiments were carried out and the resulting hydridoma screened for PMB-specific monoclonal antibodies on PMB-coated ELISA plates using polyclonal mice serum as positive controls. The first four fusions yielded approximately 2500-3000 hydridoma but none were specific for the PMB molecules. The next two fusions yielded essentially no hybridoma and the remaining hydridoma did not survive in culture for more than 10 days. Intracellular inclusions were found in the primary fusion partner, SP2 cell line, and mycoplasma contamination was documented by repeated cultures. A clean-up operation was undertaken and all new cell lines (SP2 and P3X) were purchased from the American Tissue Culture Company. After the new cell lines were passed in culture several times, repeated cultures for mycoplasma were negative. Four additional fusions yielded no viable hybridoma in culture. The remaining immunized mice

all tested for viral infection which would have interfere with successful fusion. Many of the mice had antibodies against parvovirus, a virus known to infect spleen lymphocytes and likely to be the cause of fusion failures.

Attempt were made to conjugate PMB to cyanogen bromide. After discussion with chemists at UCLA, this approach was abandoned since the likelihood of successful conjugation without destroying the native epitopes of PMB was extremely low.

The functional properties of polyclonal anti-PMB serum were examined in a TNF induction assay employing RAW cells. The polyclonal antibodies were incubated with RAW cells for stimulation of TNF production. If there is antibody against PMB which has a Fab fragment structurally similar to the business end of endotoxin, one should be able to induce TNF production by cross-linking endotoxin receptors to activate the stimulation pathway. No TNF was produced in response to anti-PMB polyclonal antibodies. Preincubatin of polyclonal antibodies with RAW cells also failed to block endotoxin-induced TNF production suggesting there is no competition on for a common target molecule on the cell surface.

Because of the lack of neutralizing activity of these polyclonal antibodies, the difficulties encountered in the monoclonal antibody work and the lack of new conjugation method, I have decided to terminate this project in June 1990.

I want to express my sincere appreciation for the generous support from the Naval Research and Development Command. I hoped that project was more successful but the technical problems encountered was more than I have anticipated. Again, I apologize for the delay of this summary report on this short but worthwhile project.

Sincerely Yours

John K.S. Chia M.D.

P.S.: There were no inventions developed during the grant period. In addition, no inventions were conceived by me during the course of this research.