March 13, 1991

A.J. Melaragno  
Captain, Medical Corps  
United States Navy  
Director of Research and Development  
National Naval Medical Center  
Bethesda, Maryland 20814-5044

RE: Status Report for Grant # N00014-90-J-1847  
Entitled "Development of Hematopoietic Growth Factors for Use in Military Personnel"

Dear Dr. Melaragno:

This report delineates our accomplishments from October 1, 1990 through February 28, 1991.

Project I: Human Erythropoietin

We have completed our studies of the first series of mutant erythropoietins described in our previous report. Our preliminary results were presented at the American Society for Hematology meeting in Boston in December. A copy of the abstract and its complete reference is enclosed. Since that time we have completed a full manuscript on these studies and are just now submitting it. A copy of this manuscript is also enclosed.

In order to focus on the putative active site of erythropoietin more carefully, we have begun to develop a series of single amino acid substitution mutants. Since our last report we have succeeded in deriving five new mutants, with six more to be produced. The mutants already in hand are currently being subjected to DNA sequencing to confirm their structure, and within the next two to three months the cDNAs will be transfected into the mammalian cells and the specific activity of these mutants will be assessed.
Although not previously proposed, we have obtained a series of monoclonal antibodies through collaboration with investigators at the Incstar Corporation in Minnesota. These antibodies are directed toward the 99-129 amino acid sequence of erythropoietin. We have found that these antibodies recognize specific features of the hormone’s active site differentially. Thus they will be quite useful in our characterization of the mutant erythropoietins. This work was presented in abstract form at the same ASH meeting. The abstract and complete citation is enclosed. Additionally, a complete manuscript is just now being submitted. A copy of this manuscript is enclosed as well.

In order to produce a recombinant human erythropoietin with increase in vivo half-life, we have proposed to derive crosslinked higher molecular weight erythropoietin species. We have begun to reduce this overall strategy to practice by designing a method to produce erythropoietin-erythropoietin dimers, which, by virtue of increased molecular weight, should exhibit a markedly higher in vivo half-life. Our strategy is to modify one sample of erythropoietin with the chemical modifying reagent SPDP which imparts a free SH group to the protein. Similarly, a second sample of erythropoietin is modified with another reagent, called SMCC, which imparts a maleimido group to the protein. This group reacts specifically only with SH groups. Thus, by combining the SPDP-erythropoietin and the SMCC erythropoietin in solution, we expect to derive erythropoietin-erythropoietin dimers, covalently attached by a SPDP-SMCC bridge. In the past grant period we have succeeded in the first phase of this strategy. Specifically, we have modified one sample of erythropoietin with SPDP and another sample of erythropoietin with SMCC. We use these reagents at varying concentrations in order to characterize their products more fully. The modified erythropoietins exhibited some reduction in biological activity, but overall, we believe that it is not unreasonable. Additionally, we have measured the degree of modification of each sample of erythropoietin by the respective modifying group. In the next grant period we plan to scale up this modification, combine the SMCC-erythropoietin and the SPDP-erythropoietin to produce dimers, separate the species by preparative chromatography, and determine the biological activity of the resultant products in vitro. If these experiments are successful, we plan to move to in vivo studies of experimental animals, determining the respective half-lives of dimers versus the native erythropoietin product.
Project II: Erythroid Burst Promoting Activity

The complete abstract and reference citation of the work described in the previous report is included. Additionally, a completed manuscript has been submitted. A copy of this is enclosed as well.

We have continued our studies of BPA from animal sources. The results of these studies continue to confirm that BPA and erythropoietin act synergistically on red cell precursors.

We have also begun screening lymphoid cell lines in order to secure a continuous source of BPA. Preliminary results indicate that of several lymphoid cell lines tested, one cell line produces a biological activity similar to B-BPA. In the next grant period, we plan to begin to confirm this finding and to attempt to characterize this product and compare it to that of authentic lymphocyte derived BPA.

Summary

Our progress on both Project I and Project II continues with only minor problems. On the whole, we have met or exceeded each of our targets in terms of achievement and time.

Truly yours,

Arthur J. Sytkowski, M.D.
Director, Laboratory for Cell and Molecular Biology

AJS: rck

enclosures

cc: Dr. Laurie Feldman
Research Administration