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THE JACKSON LABORATORY
Bar Harbor, Maine 04609

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Comprehensive Progress Report to accompany

Eighteenth renewal proposal to the

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Washington, D.C. 20545

Contract number: AT(11-1)-3264

Title of project: INBORN ANEMIAS IN MICE

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1. MAIN RESEARCH ACCOMPLISHMENTS

A. Delineation of inborn anemias. The basic purpose of this contract is delineation of inborn anemias of the laboratory mouse, carried out by preparation of genetically homogeneous stocks segregating only for anemia-producing genes; by descriptions of each condition at all stages in the life-history; by determination of tissue-sites of primary gene action through transplantation experiments; and by analysis of reactions of normal and anemic mice to a variety of stressful stimuli, including X-irradiation and hypoxia. At present 12 single-gene induced anemias are known in the mouse, plus one with multifactorial inheritance, the autoimmune hemolytic anemia of NZB inbred mice. Effects of anemia-producing mutant alleles at 10 of these loci (an; f; ha; ja; mk; nb; Sl and Sl^d; sla; sph; and W, W^v, W^J) are currently under investigation at the Jackson Laboratory with support from AEC Contract AT(11-1)-3264. We also have established an NZB/BlN colony susceptible to autoimmune disease.

We plan to analyze all presently known hereditary anemias of the mouse and to apply our findings towards an increased understanding of the genetic control of hemopoiesis, regulation of gene action, mechanisms for erythroid homeostasis, and relations between erythropoiesis and myelopoiesis.

The anemias under investigation may be classified as follows:

Macrocytic anemias: dominant-spotting W-locus, (W/W, W/W^v, and other double-dominant combinations); Steel (Sl/Sl, Sl/Sl^d); Hertwig's anemia (an/an).

Hemolytic anemias: jaundiced (ja/ja); hemolytic (ha/ha); normoblastic (nb/nb); sperocytic (sph/sph); (NZB autoimmune hemolytic anemia).

Iron defect anemias: flexed (f/f) transitory siderocytic anemia; sex-linked anemia (sla/sla ♀♀, sla/Y ♂♂); microcytic anemia (mk/mk).

Considerable effort has been devoted to establishing each mutant allele on a genetically homogeneous genetic background that allows some postnatal survival of affected individuals. Wherever possible, all mutant alleles have been transferred (by repeated crosses) to two specific genetic backgrounds, C57BL/6J and WB/Re, so that $WBB6F_1$-m/m individuals, congenic except for the differing mutant allele (m), could be compared with each other and with congenic hematologically normal $WBB6F_1$-+/+ mice.

B. Comparison and contrast of W/W^v and Sl/Sl^d macrocytic anemias. The phenotypic manifestations of W/W^v and Sl/Sl^d mutant mice are nearly identical; they are both black-eyed white mice with severe macrocytic anemia and few if any primordial germ cells. W-anemic mice have a primary defect in their stem-cell compartment which leads to marked erythroid hypoplasia, especially after X-irradiation, and which prevents formation of macroscopically visible splenic colonies when W/W^v marrow cell suspensions are transplanted into lethally irradiated normal recipients. W/W^v mice are known to provide a normal environment for erythropoiesis, since implants of histocompatible normal (+/+) hemopoietic tissues, containing normal stem cells, cure the anemia of W/W^v mice completely and permanently. We speak of W-anemic mice as being "stem-cell deficient."

By contrast, Sl/Sl^d mutant mice have normal numbers of stem cells in their hemopoietic tissues, as determined by the colony forming assay. Implants of marrow cell suspensions from Sl/Sl^d anemic mice form spleen colonies and cure the anemia of W/W^v mice. Injections of normal marrow or spleen cell suspensions are completely without effect in Sl/Sl^d recipients, even following heavy irradiation of the host. Sl/Sl^{d}mice seem to have normal hemopoietic cells, but are anemic because they provide a defective environment for erythropoiesis.

C. High plasma erythropoietin levels in stressed W/W^v and Sl/Sl^d anemic mice. It has long been known that both W/W^v and Sl/Sl^d anemic mice respond poorly to exogenous erythropoietin. To stimulate erythropoiesis in a polycythemic W/W^v mouse requires 150 times as much erythropoietin as is required by a polycythemic normal mouse. No response to erythropoietin has even been detected in polycythemic Sl/Sl^d mice. W/W^v mice cured by implants of +/+ or Sl/Sl^d hemopoietic cell suspensions become normally responsive to erythropoietin.

Capacity for production of erythropoietin seems to be normal in both W/W^v and Sl/Sl^d anemic mice. In collaboration with Dr. Geoffrey Keighley, studies of plasma erythropoietin levels in W/W^v and Sl/Sl^d mice subjected to erythropoietic stress (hypoxia, irradiation), showed that both W/W^v and Sl/Sl^d anemic mice can produce exceedingly large amounts of erythropoietin over prolonged periods while maintained in hypoxic conditions.

The hematocrit levels of hypoxic W/W^v mice become elevated, apparently because their plasma erythropoietin level is high enough to stimulate even their defectively responding erythroid stem cells.

Both W/W^v ($LD_{50/30}$ = 240 R) and Sl/Sl^d ($LD_{50/30}$ = 130 R) are extremely radiosensitive, and show great delay in regeneration of hemopoietic tissue after mid-lethal whole-body irradiation. They become and remain extremely anemic for a considerable period. All through this post-irradiation period of depressed red cell numbers, mice of both genotypes maintain extremely high plasma erythropoietin levels, and even excrete erythropoietin in the urine. Apparently W/W^v mice remain anemic because of a genetic defect in RBC precursors, and Sl/Sl^d mice remain anemic because of a genetic defect in some part of the internal environment in which the RBC must develop (Russell and Keighley, 1972). Both normally responsive stem cells and an environment encouraging erythropoiesis are necessary for an effective response to erythropoietin.

D. Tissue localization of the internal environmental defect in Sl/Sl^d anemic mice. Hemopoietic analysis of Sl/Sl^d anemic mice parabiosed with normal (+/+) partners demonstrated that this fusion neither augmented erythropoiesis in the steel partner nor diminished it in the normal partner. The "environmental defect" in Sl/Sl^d mice thus does not seem to be hormonal, nor due to deficiency of a circulating nutrient essential for erythropoiesis. Long-term treatment of Sl/Sl^d anemic mice, with injections of normal serum fortified with high concentrations of additional vitamins and minerals, was also completely ineffective. In year 16 of this contract, Bernstein (1970) reported that transplantation of intact normal or W/W^v spleens into Sl/Sl^d recipients alleviated their anemia. The "environmental defect" of Sl/Sl^d

mice thus appears to be localized within hemopoietic tissue space, but is extrinsic to erythroid stem cells: a microenvironmental or matrix defect. This defect prevents growth of colonies (following injection of marrow cell suspensions) in $\underline{Sl/Sl^d}$ spleens residing in +/+ or $\underline{W/W^v}$ bodies, but allows their growth in +/+ or $\underline{W/W^v}$ spleens residing in $\underline{Sl/Sl^d}$ bodies. Which particular non-erythroid cells in the spleen are involved in this matrix defect is not yet known, in spite of considerable search for connective tissue or lymphoid cell abnormality.

E. Normal radioresistance in $\underline{W/W^v}$ and $\underline{Sl/Sl^d}$ mice which have been cured with implanted spleens. Both $\underline{W/W^v}$ ($LD_{50/30}$ = 240 R) and $\underline{Sl/Sl^d}$ ($LD_{50/30}$ = 130 R) mice are extremely radiosensitive though for different reasons. $\underline{Sl/Sl^d}$ mice are deficient in their erythroid microenvironment but have normal stem cells. $\underline{W/W^v}$ mice are deficient in their stem cell compartment but have normal erythropoietic microenvironments. Normal +/+ mice have both normal stem cells and a normal microenvironment for erythropoiesis. Since in our stocks, $\underline{Sl/Sl^d}$, $\underline{W/W^v}$, and +/+ mice readily accept hemopoietic grafts from one another, it is possible to analyze the radiosensitivity of specific components of the erythropoietic system through exchange of tissues between mice of different genotypes. Earlier experiments demonstrated that $\underline{W/W^v}$ mice, when cured of their anemia by intravenous injection of +/+ or $\underline{Sl/Sl^d}$ marrow cell suspensions, also became normally radioresistant. Normal radioresistance is also characteristic of $\underline{W/W^v}$ mice whose anemia has been cured by implant of a whole +/+ or $\underline{Sl/Sl^d}$ spleen, which serves as a source of normally functioning erythroid stem cells.

When +/+ or $\underline{W/W^v}$ spleens implanted in $\underline{Sl/Sl^d}$ recipients have cured their anemia by providing a better microenvironment for erythropoiesis, they have also increased the radioresistance of the cured recipient (untreated $\underline{Sl/Sl^d}$, $LD_{50/30}$ = 130 R; $\underline{Sl/Sl^d}$ cured with +/+ spleen implant, $LD_{50/30}$ = 550 R).

F. Curative power of radiated spleens. One of the questions which concerns us is to what extent radiation sensitivity of a given tissue is affected by its immediate surroundings at the time of or subsequent to X-ray exposure. To determine this, spleens were irradiated in the donor and then either removed immediately, or permitted to remain in the irradiated donor for various times (up to 96 hr) before removal and transplantation to unirradiated anemic recipients for assessment of their curative potential.

When a +/+ or $\underline{Sl/Sl^d}$ spleen is transplanted into a $\underline{W/W^v}$ recipient after it has been irradiated in situ in the donor animal, it serves as a source of normal stem cells. Its effectiveness in subsequent cure of the anemia of the $\underline{W/W^v}$ recipient is related to the dose of radiation received before transplantation, and $\underline{Sl/Sl^d}$ spleens radiated in situ in the $\underline{Sl/Sl^d}$ environment are less effective than are +/+ spleens radiated in the +/+ environment (details in Annual Progress Report).

A +/+ or $\underline{W/W^v}$ spleen transplanted into a $\underline{Sl/Sl^d}$ recipient after irradiation in situ provides a favorable site for erythropoiesis. Again its effectiveness in subsequent cure of $\underline{Sl/Sl^d}$ anemia is related to the dose of radiation it received prior to ratiation (details in Annual Progress Report). The effectiveness of the +/+ splenic environment is markedly decreased if it is transplanted immediately after irradiation to a $\underline{Sl/Sl^d}$ recipient, but some of its

curative power is regained if it is allowed to recuperate 96 hrs in situ before transplant. Interestingly, radiated W/W^v spleens may provide a more effective microenvironment than do radiated +/+ spleens.

These studies demonstrating effects of radiation on curative power of the erythroid microenvironment are interesting in relation to Trentin's concept of the hemapoiesis inducing microenvironment (HIM). He envisages the HIM as extremely radioresistant. Either we are talking about two different phenomena, or the approach made possible by intergenotype transplants involving mice with these two complementary hereditary anemias provides a critical evaluation not possible with studies restricted to genetically normal mice.

G. Tissue localization of defects responsible for other hereditary anemias. The macrocytic anemia of W/W^v mice has been changed to the extremely different anemia characteristic of mice with each of three different hemolytic anemias (ha/ha; ja/ja; nb/nb), by implantation of marrow cell suspensions from histocompatible hemolytic, jaundiced or monoblastic donors, demonstrating that the hemopoietic defect responsible for each of these anemias is intrinsic to its hemopoietic tissue.

Similar experiments with implantation of microcytic (mk/mk) marrow showed that hemopoietic precursor cells of mk/mk mice definitely have an intrinsic defect in uptake of iron from the circulation, but in these mice the defect in iron metabolism appears not to be limited to erythroid tissue.

2. PLANS FOR CONTINUATION OF PRESENT OBJECTIVES

We will continue the basic responsibility for preparing and maintaining homogeneous genetic stocks segregating for at least the presently maintained 10 specific anemia-producing genes, and envisage accepting this responsibility for other anemia-producing genes as new mutations occur. Accompanying this will be characterization of each anemia, including establishment of typical hematologic values; determinization of erythropoietin production and capability of the mutant mouse to respond to this hormone; and determinization of tissue site of abnormal gene-action through intergenotype transplantation of hemopoietic tissue.

The potentiality of circulating blood as a source of transplantable pluripotent or committed erythroid stem cells will be tested extensively, with attempts to identify the circulating pluripotent stem cell morphologically by electron microscopy. Bloods of mice with different hemolytic anemias, which contain many nucleated cells, and which have been shown to be effective in intergenotype hemopoietic transplant, will be invaluable in this project.

We will continue to study the role of the intrasplenic microenvironment in the support of erythropoiesis, making use of the contrasting intrinsic defect in hemopoietic tissue of W/W^v anemic mice vs. the microenvironmental defect inhibiting erythropoiesis in Sl/Sl^d anemic mice. We hope to identify a defective cellular component or metabolic process in the non-erythroid microenvironment in spleens of these Sl/Sl^d mice.

We will exploit these anemias further in studies of the contribution of the hemopoietic microenvironment to total radiosensitivity, including direct tests of the effects of radiation on capacities of the splenic microenvironment.

3. GRADUATE STUDENTS

We have not sponsored any graduate students for the Ph.D. degree under this contract, nor have we supported postdoctoral trainees.

4. BIBLIOGRAPHY

The following is a bibliography of publications during the past three years associated with this contract.

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* Seven reprints of each of these papers enclosed.

5. SIGNIFICANCE AND RELEVANCE OF RESEARCH

Each of the single-gene induced hereditary anemias of mice represents abnormality in a different process important for maintenance of erythroid homeostasis, and determination of the basic nature of each genetic defect contributes information vital for our understanding of hemopoietic control mechanisms. To date we have not discovered hemoglobinopathies in the mouse, a situation rather different from that in human hereditary anemias. Two of our anemias (W/W^v and Sl/Sl^d) are in many ways similar to the human Blackfan-Diamond syndrome, but we know these two mouse anemias have very different basic defects. Although it may work out that no mouse anemia is an exact duplicate of a specific human disease, they definitely provide valuable experimental models with which to attack specific biomedical problems.

One of these is analysis of factors contributing to radiosensitivity. The extreme radiosensitivity of W/W^v and Sl/Sl^d mice, and their normal production of erythropoietin combined with defective response, can be very illuminating. Especially important is the potentiality for testing separately effects of radiation on hemopoietic cells and effects on the microenvironment supporting erythropoiesis. We also have initiated studies of the response of W/W^v and Sl/Sl^d intestinal crypt cells to radiation.

6. DIVISION OF SUPPORT FOR OVERALL RESEARCH PROGRAM

The support from the U.S. Atomic Energy Commission is combined with three grants from two different agencies to cover a broad research program in mouse physiological genetics, with special emphasis upon anemia and other hematological characteristics. NIH grant HD-00254 from the Nationla Institute of Child Health and Human Development to Dr. Seldon E. Bernstein supports studies of inherited hemolytic anemias in the mouse. Support from the American Cancer Society (ACS VC-58-0) continues studies of lethal and other deleterious genes affecting growth and differentiation, and NIH grant CA-01074, from the National Cancer Institute to Dr. Elizabeth Russell, continues studies of the physiological genetics of the mouse. These three grants include funds to supplement studies of inborn anemias of mice.

Ninety per cent of the total research effort of Dr. Russell is devoted to physiological genetics. She is also project director for a Program Project (NIH HD 05523) to which she devotes 10 per cent of her total effort.

Dr. Seldon E. Bernstein now devotes 100 per cent of his research effort to investigation of hereditary anemias, with 25 per cent support from the Atomic Energy Commission, 25 per cent from NIH grant HD-00254, and 50 per cent from NIH grant CA-01074.

Dr. David E. Harrison, Associate Staff Scientist, devotes 75 per cent of his total effort to physiological genetics, with 25 per cent support from CA-01074, and 50 per cent from ACS VC-580. He is also principal investigator on one of the six individual projects that make up the program-project on aging (NIH HD 05523), and devotes 25 per cent of his total effort to that activity.