PD-ABB-913

AID PROJECT

A. TITLE PAGE

MOREHOUSE SCHOOL OF MEDICINE
Name of organization submitting proposal

720 Westview Dr., S. W.
Atlanta, Georgia 30310
Address

TITLE OF PROPOSAL: Changes in T lymphocytes of progeny and mothers after exposure to benzo(a)pyrene during pregnancy (midgestation)

PAUL URSO, Ph. D.
Name of Principal Investigator

Assistant Professor
title

11/27/84
Date submitted

Paul Urso
Signature of Principal Investigator

Walter W Sullivan
Signature of authorized official

ASSOCIATE VICE PRESIDENT
SPONSORED PROGRAMS
title

Submission to other sponsors: Although this specific project is not duplicated elsewhere, experiment i, page 4, described herein is common to support from an NIH/MBRS grant entitled, "Immune Cell Changes After Benzpyrene Insult In Utero".

B. RELEVANCE OF PROPOSED WORK TO A.I.D.

1. STATEMENT, PERTINENCE AND IMPACT OF THE PROBLEM

A major problem to be addressed more critically is the influence of environmental pollutants, implicated in carcinogenesis, on the cellular components of the immune system, particularly T lymphocytes, which develop their potential during ontogenesis for reacting against undesirable agents. In addition, since the maternal environment influences development of progeny immunity (see e.g., Beer, 1977), the health consequences of maternal exposure to carcinogenic pollutants during pregnancy, associated with the establishment of adequate defense mechanisms in the progeny, should also be seriously considered. Thus, more attention is needed with regards to the growing health problems in developing countries as a result of exposure to environmental pollutants, concerning immunologic factors that may play a role in the disposition toward tumorigenesis.

A pollutant of major concern is benzo(a)pyrene (BP), a potent carcinogen whose qualities include severe disruptions in the immune system resulting in an inadequate defense mechanism a situation which is believed to provide favorable conditions for tumor growth. In developing countries, urbanization (increased use of motor vehicles and in tobacco consumption (e.gs., Republic of South Africa, Latin America) (Liddell, 1971; Halliday, 1972; WHO, 1979; Haddad, 1975) and industrialization, (conversion of coal into fuel) (e.g. South Africa) (Funke, 1983) can be considered factors for the increasing concentrations of airborne BP although even in traditional domestic situations (e.g. cooking) relatively high concentrations of this carcinogen are encountered especially in more densely populated communities (e.g., Ahmedabad, India) (Aggarwal, 1982). Over the last 50 years incidences of lung tumors have increased 2 to 3-fold in Third World Countries where the incidence of cigarette smoking has correspondingly increased (e.gs., Costa Rica, Thailand) (WHO, 1979). In some developing countries the epidemic of smoking related diseases (e.g., lung cancers) is already of such magnitude as to rival infectious diseases or malnutrition as a public health problem (WHO, 1979; Doll, 1970). In context, this is especially critical in the mother and her offspring in the aftermath of daily exposure during, and even before, pregnancy. For example, in communities where use of fuels for cooking leads to relatively high concentrations of BP in the immediate area (e.g. 9.32 ug/m^3) (Ahmedabad) (Aggarwal, 1982). It has been reported that progeny of mice exposed to methylcholanthrene(MCA) or the carcinogen o-aminoazotoluene before and/or during pregnancy show increased tumor incidence (Shay 1952; Gelshtein, 1961). In view of the potent carcinogenic and immunosuppressive action of BP, a dissection of the cellular elements affected by this pollutant will be extremely helpful in characterizing the necessary components of the defense mechanism that must develop for combating undesirable conditions (i.e., tumorigenesis). Information gained should eventually lead to more effective measures for controlling tumor appearance and growth.

C. SCIENTIFIC ASPECTS OF THE WORK

1. STATEMENT OF THE PROBLEM

The problem to be addressed focuses on disruptions in cellular components, especially T lymphocytes, of the immune system developing during fetogenesis, exposed to a carcinogenic environmental pollutant and how maternal factors following insult may affect the establishment of the progeny defense mechanism.

A variety of dangerous pollutants in the environment which are becoming increasingly prevalent in developing countries, include BP a polycyclic aromatic hydrocarbon (PAH). As a by-product of incomplete combustion, it is found in diverse domestic and industrial sources such as gasoline exhaust, cigarette smoke, burning of cooking fuel, soil and vegetable matter, and synfuels produced by liquefaction and gasification of coal (i.e., coal conversion for sources of energy) (Liddell, 1971; Halliday, 1972; Wynder, 1967; Carrier, 1976; Griciute, 1979; Aggarwal, 1982). In South Africa, coal conversion is a large scale industry (Funke, 1983). A ubiquitous environmental toxicant and one of the most dangerous constituents of cigarette smoke (Huhti, 1981), and gasoline exhausts, it exerts a strong carcinogenic effect in experimental animals and humans, (Talmage, 1976). Mice exposed pre- and postnatally show a high incidence of tumors (Bulay, 1971; Vesselinovitch, 1975), and in those exposed postnatally a marked suppression in humoral immunity (HI) against sheep red blood cells (Stjernsward, 1969). Other PAH's (e.gs., MCA, dimethylbenzanthracene) induce similar changes (Ball, 1970; Stutman, 1975), and, in some cases, cell mediated immunity (CMI) (delayed type hypersensitivity) is also suppressed (Baroni, 1970). Recently, we have shown that mouse progeny exposed to BP in utero demonstrate early postnatal suppression of HI and CMI which persists for the greater part of the animals' life span (up to 18 mos) (Urso, 1980, 1982, 1984). Further, the sensitivity for tumor development and humoral immune suppression in prenatally exposed mice (which receive about 3 ug following injection of the pregnant mother with 150 ug/g BW) increases 2 to 6-fold when compared to a similar or 50-fold higher dose given postnatally (Urso, 1982). Although a cause and effect relationship between immune competence and tumorigenesis has not been definitively established, the results we obtained are compatible with the idea that immune deficiency (suppression) influences the increase in neoplastic expression (i.e., a deficient status provides favorable conditions for tumor growth).

Since destruction of neoplastic cells has been attributed primarily to CMI, a T cell response (Baldwin, 1977; Faanes, 1978; Clark, 1980), the effects of BP on ontogenesis of T lymphocytes would more aptly reflect the events in the differentiation of the subsets that must necessarily occur for the control of tumor appearance and growth.

The influence of humoral and cellular elements of the maternal environment on offspring defense mechanisms has been documented (Field, 1971; Beer, 1977). Several have reported systemic suppression in pregnant mice (Chaouat, 1979; Clark, 1980; Gambel, 1982), and most recently the existence of T suppressor (Ts) cells in their spleens (Masatoshi, 1984). Ts cells also exist in fetuses and neonates (Ptak, 1977; Globerson, 1975; Luckenbach, 1978; Pavia, 1979).

2. OBJECTIVE AND SIGNIFICANCE

From the above considerations, it seems in order to characterize, more precisely, the immunologic status of the T cell compartment of lymphoid tissue, particularly in the thymus, of the mother and her offspring (fetal and early postnatal) after exposure to the biohazard during pregnancy, i.e., at 11 to 13 days midgestation when the fetal lymphoid cells that eventually comprise the immune system begin developing into committed cell lines (Scheid, 1973; Sherr, 1977). Exposure to BP may lead to changes in the maternal environment which also may have strong influences on the establishment of offspring immune status (e.gs., increase in Ts cells, alteration in thymosin production). Therefore, the immediate specific objective of this project is to characterize quantitative and functional consequences on the T cell population (including subsets) after in utero exposure to BP, in the mother and her offspring. Undoubtedly, many factors are operating with respect to immunosuppressive and tumorigenic influences after ontogenic exposure to BP. Quantitative and qualitative characterization of the T lymphocyte populations will lead to a clearer understanding of the development, particularly in the progeny, of adequate defenses to combat undesirable conditions (tumor growth). From these studies, additional insight will be gained on the nature of a cause and effect mechanism implicit in the immune surveillance hypothesis.

3. DETAILED EXPERIMENTAL AND ANALYTICAL DESIGN, PRELIMINARY FINDINGS, EXPERIMENTAL DESIGNS AND METHOLOGIES AND TIME PHASE OF WORK.

a. PRELIMINARY FINDINGS.

From our earlier experiments in which we demonstrated suppressed T cell proliferation (afferent response) and *in vivo* graft vs host response of effector lymphocytes from BP exposed progeny (Urso, 1984), we were prompted to analyze changes in the T cell compartment to more precisely delineate the modification in T lymphocytes and their subsets. Pregnant C_3H/Anf females were injected with 150 ug BP/g during midgestation and in the mother and her offspring (fetal and early postnatal) changes measured in the following parameters; a) BW, b) lymphoid organ weight (OW) (liver, thymus, spleen), c) organ cell counts (OC), and d) identification of T cells and subsets in lymphoid organs. To identify T cells, specific goat anti-mouse brain associated (θ) antiserum was used while for the Lyt-1^+ and Lyt-23^+ cells the antibodies were monoclonal anti-Lyt 1.1 and anti-Lyt 2.1. The method was to incubate cells from lymphoid tissue in tissue culture medium with diluted antiserum in the presence of C'. Cells positive for the markers were enumerated using the trypan blue dye exclusion test. Three to 4 experiments were performed involving 15 to 20 offspring and 3 to 4 mothers. In some experiments only mother lymphoid tissue was analyzed for a total of at least 5 parents. T cells and subsets were quantitated according to the formula:

$$\% \text{ cytotoxic index(CI)} = \frac{\% \text{ cyt(antibody + C')} - \% \text{ cyt(C' or antibody only)}}{100 \quad - \% \text{ cyt(C' or antibody only)}} \times 100$$

Our results were as follows: 1. Progeny; a) fetal OW and OC of those exposed to BP were not different from controls (corn oil vehicle exposure only) 15 to 19 days gestation), but at 0 to 5 postnatal days significant reduction occurred in the

former ($p < 0.01$ to < 0.001 by analysis of variance) (livers were not done). Postnatal suppression was particularly severe in the thymus, b) The quantity of T cells was decreased in the fetal liver and spleen, but postnatally (0 to 5 days) in the spleen the numbers were enhanced ($p < 0.005$), while in the thymus they were decreased ($p < 0.01$), c) the number of Lyt-23^+ cells were increased in the liver and spleen ($p < 0.01$) at 17 days the only fetal interval studied at this point in time. 2. Mothers; a) from 15 days gestation extending into early postnatal life (0 to 5 days of the progeny) there was a severe reduction of cells in the thymus reflecting decreased numbers of T cells, b) T cell reduction also occurred in the spleen, but by day 5 a significant enhancement was seen, c) the number of Lyt-23^+ cells was enhanced in the spleen but not in the thymus. (This data will be presented at the annual meeting of the Federation of American Societies for Experimental Biology, April 21 - 26, 1985, Anaheim, CA).

The results demonstrate that BP induces severe deficiencies in T cells of lymphoid organs which appear to be a function of time after gestational exposure for a specific organ. Thus, reduction occurred first in the fetal liver and spleen of the progeny, and in the thymus and spleen of the mother, and then in the thymus of the offspring postnatally. The striking enhancement of T cells in the spleen of postnatal progeny dictates that more detailed analysis be done on T subsets in this organ. It may be that this enhancement reflects increase in Lyt-1^-23^+ Ts cells since; a) CTL activity in mouse spleen is low or virtually absent at 0 to 5 days (Widmer, 1979; Pilarski, 1977), and b) Ts cells have been identified at this time interval (Ptak, 1977; Pavia, 1979). Since Lyt-23^+ cells increased at the only time interval we studied thus far, the changes occurring may reflect disproportionate development of the T cell repertoire. At any rate, these findings suggest that BP induces a disorientation (disruption in T cell traffic, distribution and/or accumulation) of the differentiative pathways in the T cell population, a condition which may have profound effects on the development of the necessary T cell subsets vital for combating nascent neoplasms. The reduction in T cells of offspring and mothers thymuses and the increase in Lyt-23^+ cells may also reflect a maternal-fetal communication (direct to indirect) concerning changes essential for the ontogenesis of T cells. Thus, the immediate aims of this project are; a) to measure further the quantity of Lyt-1^+23^- and Lyt-1^-23^+ cells at different time intervals after BP exposure during pregnancy in the progeny and mothers, and b) to assay functional status of these T cell subsets.(see experiments i and ii below).

It must be pointed out here that, in future experiments, analysis of T lymphocytes and their subsets will not be confined to fetal and early postnatal intervals but will be extended up to and including time intervals involving increases in tumor frequency, nor will analysis be confined to T cells only but will also include other cells which have been implicated in tumor control (e.g., Natural Killer cells).

b. EXPERIMENTAL DESIGNS AND METHODOLOGY

i. Measurement of T subsets in offspring and mothers exposed to BP during pregnancy (midgestation).

These experiments will be carried out as described above to further elucidate changes in T cells and their subsets (particularly Lyt-1^+23^- and Lyt-1^+23^+ which as yet have not been analyzed) of lymphoid tissue during fetogenesis and early postnatal life. The relative amounts of the 3 subsets will be calculated according to Shiku (1976). As mentioned, disorientation of the differentiation process may have profound effects on T cell recognition of tumor cells as an initial step

in the control of developing neoplasms.

ii. Functional status of T cell subsets

Quantitative changes observedin T cells including an apparent disorientation of the subsets (see above) may reflect changes in functional capabilities of these cells. Some of the questions to be answered are as follows:

a) Does the reduction of T cells in the thymus of the progeny and mother represent a failure of the cells to recognize allogeneic tissue?

The mixed lymphocyte response (MLR) will be used to measure T cell proliferative capacity (an Lyt-1^+23^- function) of thymus cells cultured with allogeneic targets and incubated with tritiated thymidine. If the quantity of Lyt-1^+23^- cells is changed (increased or decreased) relative to other subsets, changes should occur in the magnitude of the MLR. Further, results of this experiment (and of experiment i above) may explain the subnormal MLR we observed in 1 wk old progeny exposed *in utero* (Urso, 1984).

b) Do the T cells in the fetal liver and spleen and in the spleen of the mother reflect changes in suppressor cells (e.g., Lyt-1^-23^+I-J^+) or in cytotoxic cells (e.g., Lyt-1^-23^+I-J)?

For this experiment, cells from designated lymphoid tissue (of the progeny or mothers) will be cultured with normal syngeneic cells already determined to have given a positive MLR. If an MLR is inhibited, tissue cells will be treated with monoclonal anti-I-J^+ antiserum. If T suppressor (Ts) cells are involved removal of these cells with anti-I-J^+ antibody will result in a positive MLR.

Recently, we have determined that spleen cells from normal C_3H mice immunized with C_3H fibrosarcoma cells induced by BP (BP8 tumor cells) are active against these tumor cells. The following description of the experimental design uses cytotoxic T lymphocytes (CTL) usually identified as Lyt-1^-23^+. However, we are aware that the cytotoxic activity of Lyt-1^+23^- effector cells against BP8 cells may also have to be considered since Perry (1981) has shown, in a Winn assay, that effector cells of this type inhibit the growth of MCA (a PAH)-induced fibrosarcoma cells.

To measure CTL in BP-injected mothers and her progeny, normal spleen cells with known activity against syngeneic tumor will be cultured with spleen cells of mothers or their progeny (from which Lyt-1^+ or Lyt-23^+ cells are removed with anti-Lyt 1.1 or 2.1) in the presence of BP8 target cells labeled with ^{51}chromium (^{51}Cr). This should lead to a normal release of ^{51}Cr from the tumor target (provided Lyt-1^-23^+ cells are not Ts cells). If CTL are removed from the normal spleen and the remaining cells cultured either with spleen cells from the mother or with those from her progeny, cytotoxicity of the labeled target should occur upon removal of I-J^+ (suppressor) cells from both mothers and progeny, and in the case of the progeny, if their Lyt-1^-23^+ cells have cytotoxic ability. If the Lyt-1^-23^+ cells (putative cytotoxic cells) of the progeny are not functional (as may be the case) liver cells of the fetus or spleens of postnatal progeny (up to 5 days) upon removal of I-J^+ cells (suppressors) should not improve the cytotoxic response of normal adult spleen cells from which Lyt-1^-23^+ cytotoxic cells have been removed nor suppress (or improve) the cytotoxic activity of normal adult

spleen cells against labeled tumor target. In addition, removal of cytotoxic cells from spleens of BP-exposed mothers (or progeny if their cells have cytotoxic activity) with monoclonal antibody should prevent the release of ^{51}Cr from the targets when incubated with normal adult spleen cells from which CTL have been removed, but not when CTL are present in normal spleens.

These experiments may necessitate an initial incubation of spleen cells with BP8 fibrosarcoma cells (live or inactive unlabeled tumor cells) to generate cytotoxic cells to be tested in the design described above.

From the type of experiments described (in ii), information will be gained on how BP influences the changes we have observed in immune cells (see PRELIMINARY FINDINGS). For example, does BP reduce the number of T helper cells (Lyt-1^+23^-) reduce the number of CTL, or increase the number of Ts cells? Two important contributions will be made; a) insight on disruption by BP of the differentiative pathway(s) leading to development of the T cell repertoire, b) the steps needed in the pathway(s) that may be essential for the control of neoplastic development.

iii. Maternal-fetal associations in the development of a functional immune system in the progeny.

We must re-emphasize here that our immediate goal is to determine reasons for the apparent T cell disorientation induced by BP in the progeny and mother after *in utero* exposure.

Concommitant evaluations in the progeny and mothers should reveal further maternal-fetal associations in the development of progeny immunocompetence. Direct and indirect effects on the progeny during fetogenesis or postnatally can be tested in several ways. Some of these are as follows:

a) The effect of mother thymus on the development of the T cell repertoire in the offspring after exposure to BP during pregnancy.

In this experiment, females will be thymectomized (see Urso, 1974) before mating. When mated females plug, they will be injected with 150 ug BP Ip/g BW or with the corn oil vehicle during midgestation. T cell quantity and subsets will be measured in lymphoid organs of the fetus and early postnatal progeny. If no changes occur in the offspring this would suggest that the thymus of the mother influences the behavior of T cell-development of the progeny. If changes do occur, this would suggest that the mother, if playing a role, would do so by some means other than thymic influence (e.g., other hormonal participation), or that BP induces changes directly or indirectly by some other mechanism. If the results suggest a thymic role, implantation of a normal syngeneic thymus (10 to 12 wk old) under the kidney capsule of the mother before injection with BP should provoke changes in the offspring resembling those seen in progeny from mothers not thymectomized. Implantation after BP exposure should restore T cell levels in the progeny.

b) The effect on T cells in pups exchanged between BP- and corn oil-exposed mothers.

In some experiments all the pups from BP-exposed mothers will be exchanged

with those from controls. In others, 1/2 of the litters will be exchanged. Since changes in the thymus cells were seen postnatally (see PRELIMINARY FINDINGS), it would appear that the effects induced in this organ become evident after birth. Influence of the mother on thymus cells should be reflected by differences in T cell changes in the exchanged litters and a similarity in changes in 1/2 of the pups from the natural mother exchanged with 1/2 of the pups from the foster mother. The T cells will be characterized with regard to quantitative profiles and functional activity (see experiments i and ii above).

c) Maternal influence as measured by thymosin production.

Mothers will include BP- and corn oil-exposed; a) non-thymectomized, b) thymectomized, and c) thymectomized and grafted with a normal syngeneic 10 to 12 wk old thymus. Thymosin in the serum of the mother and in the offspring (fetal and early postnatal) will be measured by radioimmunoassay (Goldstein, 1981). Antibody against thymosin will be raised in rabbits. Serum from the mother and tissue and serum from the offspring (comprising the groups indicated above) will be assayed for thymosin. It may also be necessary to analyze tissue (thymus, spleen) from the mother. After exposure to BP, if there is a reduction of thymosin in the mother and her progeny this would suggest an influence of the maternal environment (particularly the thymus) on development of immunocompetence in the progeny. T cell levels in the progeny should be directly related to thymosin levels (i.e., low quantities of T cells will be associated with low amounts of thymosin). Implantation of a normal thymus in thymectomized mothers after BP injection should then restore both T cell and thymosin levels in the offspring (and mothers).

c. TIME-PHASE PLAN OF WORK.

Experiments i and ii above will be completed at the end of the first year of the grant period. Experiment iii is planned for future endeavor to continue work for measuring maternal-fetal relationships in the development of progeny immunity following *in utero* exposure to this dangerous environmental pollutant.

d. LITERATURE CITATIONS PERTINENT TO THIS WORK.

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D. QUALIFICATIONS OF PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Paul Urso

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Paul Urso	Assistant Professor of Microbiology	August 3, 1925

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
St. Francis College, Brooklyn, N. Y.	B. S.	1950	Biology, Chemistry
Marquette University, Milwaukee, Wis.	M. S.	1952	Zoology
University of Tennessee, Knoxville, TN	Ph. D.	1961	Immunology, zoology

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1952-1953 Cardinal Stritch College, Milwaukee, Wis., Department of Biology and Mathematics, Instructor in Biology and Mathematics.

1953-1955 National Institutes of Health, National Cancer Institute, Bethesda, Md., Biologist, Hempoietic recovery from radiation injury.

1955-1957 Oak Ridge National Laboratory, Oak Ridge, TN., Jr. Biologist, Hemopoietic recovery from radiation injury.

1957-1958 Leave of absence to pursue graduate studies, Oak Ridge National Lab.

1958-1959 Oak Ridge National Laboratory, Oak Ridge, TN., Research Associate, Immunology of radiation chimeras.

1959-1961 Oak Ridge Institute of Nuclear Studies, Predoctoral Fellow. Graduate School, University of Tennessee, Knoxville TN. Research done at the Oak Ridge National Laboratory in Immunology, "differentiation during antibody formation

1961-1971 Seton Hall University, South Orange, N. J., Assistant and Associate Professor of Biology. Immunology, Cellular aspects of antibody formation; radiation chimeras.

1971-1981 Oak Ridge Associated Universities, Oak Ridge, TN., Senior Scientist/Immunologist. Cellular immunology of radiation chimeras, immunology after fetal exposure to chemical and radiation carcinogens.

1981-Present Morehouse School of Medicine, Atlanta, GA., Assistant Professor of Microbiology. Immunology, Cellular changes after fetal exposure to carcinogens (chemical and radiation).

Honors and Awards.

Sum, 1950 Scholarship to Woods Hole Marine Biological Laboratory given by the Mendel Society, St. Francis College.

1959-1961 Predoctoral Fellow, Oak Ridge Institute of Nuclear Studies while a student at the University of Tennessee. Research done at the Biology Division, Radiation Immunology group headed by Dr. T. Makinodan research supervisor.

1964-1966 NIH grant AI-05883, CELLULAR DIFFERENTIATION DURING ANTIBODY FORMATION, Principal Investigator, $32,000, for 3 years.

1964-1965 NIH grant GM-11434. CELL PROLIFERATION STUDIES IN NEWT TISSUES USING H3T. Co-Investigator, $17,000, for 2 years.

1978-1981 DOE/EPA Interagency Agreement 79-D-X0533, Task No. 1931, DEVELOP MARKERS FOR DAMAGE TO IMMUNE SYSTEM; changed to; STATUS OF THE IMMUNE SYSTEMFOLLOWING EXPOSURE TO ENVIRONMENTAL TOXICANTS. Co-Investigator, about$300,000.00/yr.

1983-1987 MBRS/BISRIP/NIH grant (subproject 916-526-229). IMMUNE CELL CHANGES AFTER BENZOPYRENE INSULT IN UTERO. Principal Investigator on subproject, $56,506., first year; total, $200,000.00 (4 years).

Publications. Listed in order of relationship to the project.

1. Urso, P. and Gengozian, N. Subnormal expression of cell mediated and humoral immune responses in progeny disposed toward a high incidence of tumors after in utero exposure

to benzo(a)pyrene. J. Toxicol. Environ. Health, In Press, 1984.
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E. BUDGET FOR PROJECT.

1. Total funding: $74971.00

2. Finanacial contributions, cost-sharing arrangements: In considering all the experiments listed in the NIH/MBRS grant, it is estimated that about $1500.00 would be applicable for part of the research described in this proposal, namely; experiment i, page 4.

3. Detailed budget.

a. Personnel.

Name	title	% of time	rate of pay	fringe benefits	total amount
Paul Urso	Principal Investigator	25	$ 8000	$ 1520	$9520
Ray A. Johnson, B.S.	Research Assistant	50	$ 8000	$ 1520	$9520
To Be Named	Laboratory Technician	50	$ 5600	$ 1064	$6664
Totals			$21600	$ 4104	$25704

b. Travel.(for PI) $ 1000

c. Consultants. 0

d. Equipment and supplies.

i. Equipment

a) microdissection apparatus $ 3800

b) Freezer (-80°C) $ 5500

ii. Supplies.

a) animals (mice and rabbits) $10000

b) radioactive compounds (^{51}Cr, ^{3}H-T), waste disposal $ 3500

c) reagents (monoclonal antibodies, tissue culture medium, etc) $ 3500

d) miscellaneous (glassware, plasticware, microtiter plates, automatic pipets, etc) $ 4000

Totals $31300

e. Indirect costs (69.9% of wages and benefits). $17967

f. Other.

i. Justification of budget.

a) Mr. Ray A. Johnson, Research Assistant.

Mr. Johnson is an efficient, conscientious and intelligent assistant who is very much aware of the conceptual nature of the project. In addition, other duties (see Laboratory Technician justification) would make it very difficult to carry out the necessary experimentations so vital to the project. Most of the assays (e.g, ^{51}Cr release from tumor cells) take more than one day to carry out. Consequently, it is important that Mr. Johnson has sufficient time to assist the PI in the experiments described and to be relieved of other duties (see below; duties of Lab Tech) that would hamper the progress of this research.

b) Lab. Technician.

This individual will be responsible for several duties which are now being carried out by Mr. Johnson and the PI. These include accurate recording of stock animals, mating and weaning procedures, washing glassware, sterilization of necessary supplies and reagents, ordering of supplies and maintaining accurate records of experimental animals on hand (e.g., weaned progeny after treatment of the pregnant mother). A part time lab technician will allow Mr. Johnson and the PI to concentrate more on the experimental designs, methodology and conceptual aspects of the project.

c) Equipment.

The micrdissection apparatus is needed for making determinations on developing lymphoid tissue at less than 15 days of gestation. At 15 days, the spleen is difficult to remove from the fetus, including other lymphoid tissue, i.e., the thymus, prior to 15 days without the use of a microdissection apparatus. Determinations will probably have to be made on these tissue before the 15th day of gestation.

The -80°C freezer is needed for; a) keeping in catalog order monoclonal antibodies and rabbit and guinea pig complement for which there is a continuous need, b) for slow freezing of tumor cells, c) for other reagents recommended to be kept at this temperature.

4. FACILITIES AVAILABLE AND EQUIPMENT.

A laboratory of approximately 360 sq ft is available plus office space close to the laboratory for carrying out this project. The equipment available includes; CO_2 incubators (2), freezers and refrigerators (1 each) (2 freezers maintained at -80°C are community property), refrigerated centrifuges (2), ultra and preparative centrifuges (1 each), fluorescence microscopes (3, one in the PI's lab), gamma counters (2), liquid scintillators (2), cell harvester (1), glove box to confine and prepare BP, liquid nitrogen tanks for storage of certain tumor cells, immunoelectrophoresis apparatus (2, one in the PI's lab), fraction collectors (at least 2) and spectrophotometers (at least 2) the last two items in other investigators' labortotries to which the PI has access, autoclaves (2 large ones), and finally a large animal facility (about 5600 sq ft)which is being expanded and is supervised by a qualified veternarian, Dr. E. Stephens, D.V.M.