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RESEARCH PROPOSAL

LOS ALAMOS MESON PHYSICS FACILITY

NEGATIVE PION EFFECTS ON IMMUNOCOMPETENT CELLS

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SUMMARY OF EXPERIMENT

A study is proposed to quantitate the effects of negative pions on cells involved in both cellular and humoral immune responses.

Experiments will assay primed and unprimed T cells as well as primed and unprimed B cells. With a hapten-carrier system, T cell function will be assayed by means of carrier recognition, and B cell function will be followed by enumeration of cells that synthesize antibody against the hapten.

For the cellular immune response, the action of negative pions in inactivation of cells capable of mediating cytotoxicity against transplanted plasmacytomas will be studied. Immune spleen cells will be taken from immunized donors and transferred, together with viable myeloma cells to a second recipient. Pion inactivation will be quantitated on the basis of diminished ability of irradiated immune cells to abrogate outgrowth of the transferred myeloma.

Pions from both peak and plateau will be used to generate dose-survival curves for each class of immunocompetent cells. These results will be compared with survival curves generated by conventional Cobalt-60 gamma rays. Data from this study will provide valuable RBE comparisons for the action of pions on immunocytes at the tumor site, as well as for estimating pion effects in intervening tissue included in the radiation field.

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PROPOSAL INFORMATION

Beam Area: Biomedical.

Beam Requirements & Type of Particle: Negative Pions.

Field Size: 5 X 5 cm.

Dose Rate: 30 - 40 rads/min., Minimum.

Other beam requirements are within those necessary for adequate radiotherapy.

Running Time Required: Intermittent usage is requested, comprising 50 hours.

Scheduling: Beginning October, 1975.

Major LAMPF Apparatus Required: None.

Shielding and Enclosures Required: NONE.

Special Services Required: Animal care for approximately 150 mouse cages. (This is a maximum estimate, and no more than 50 cages need be maintained at the laboratory site.)

Space required: Laboratory space in the Biomedical Facility will be adequate.

SPECIAL APPARATUS TO BE FABRICATED BY LAMPF: None.

IDENTIFICATION OF HAZARDS ASSOCIATED WITH THE EXPERIMENTAL APPARATUS AND PRECAUTIONS TO BE TAKEN: None.

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RESEARCH PLAN

A. Introduction:

1. Objective: To obtain quantitative information about effects of negative pi mesons on cell populations involved in immune responses.

2. Background: a. Negative Pi Mesons. Because of their unique distribution of dose with depth, negative pi mesons (negative pions or pions) hold promise as an improved modality for treatment of human neoplasms (1). The depth-dose profile consists of an entrance plateau of relatively constant dose with depth, followed by a dose peak which may be 2½ to 3 times greater than the entrance plateau, depending on the momentum spread of incident particles. For monoenergetic pions the entrance plateau results principally from ionizations produced by repulsion of orbital electrons (2). The dose peak, on the other hand, results from capture of pions by nuclei of the absorbing material. The nuclear cross section is quite low for pions in flight, but increases as they come to rest (3). The negatively charged pion is attracted to a positively charged nucleus, displaces an outer orbital electron of the capturing atom, cascades downward and is ultimately captured by the atomic nucleus (4). Of the pion's 140 MeV rest mass, about 40 MeV is expended overcoming the binding energy of the nucleus, thus about 100 MeV is deposited within the capturing nucleus, causing it to virtually explode into several densely ionizing fragments (5). These fragments are called stars from their appearance in photographic emulsion.

Nuclear fragments in the stopping or star region, may have associated LET values of as great as 1,000 keV/u--calculations indicate that 25% of the dose results from events with associated LET's of 20 keV/u or greater (4). Therefore, not only is the physical dose maximized at depth, but the mode of its distribution provides a differential RBE at depth. The RBE of plateau dose is assumed to be about 1, whereas in the peak, RBE values in the range of 2.5 have been reported (6). Previously, pure high-intensity pion beams have not been available for determining killing effects on cells, a necessary experimental base for beginning clinical trials. The Los Alamos Meson Physics Facility (LAMPF) is now in final construction and includes a biomedical beam line with necessary facilities for carrying out clinical pion therapy, as well as radiobiological experimentation. At this writing beam intensity from the biomedical beam line is anticipated to be 50-60 rads/min for a 3 x 3 cm field during the July-August 1974 run cycle for the accelerator.

Pretherapeutic experiments with pions from this laboratory (CA 13371-02, "Preclinical Study of Radiobiology of Negative Pions") will begin at LAMPF at that time. These experiments, part of a planned integrated pretherapeutic effort at LAMPF, will delineate biological depth dose distributions of pions for hematopoietic and immunocompetent stem cells. Experiments outlined below are designed to follow this initial work with quantitative determination of the action of negative pions on the various cell populations that participate in both cellular and humoral immune responses.

b. Immune Responses. Specific immune responses, i.e., those which involve the recognition of foreign antigens, followed by a reaction directed against these same antigens, are mediated by lymphocytes (7). Antigens such as those found on bacterial or foreign erythrocyte surfaces elicit formation of antibody; this is termed the humoral response. In contrast, foreign tissue or organ grafts, including tumors, are rejected by direct participation of activated lymphocytes (8). This response which may involve, but does not require, the elaboration of antibody, is termed the cellular immune response. Although both responses are related in that effector cells may originate from the same stem cell pools, the following discussion will treat them separately.

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b-i. Humoral Immune Response. Two populations of small lymphocytes participate directly in the antibody response--T cells which function under the influence of the thymus, and B cells, derived from bone marrow, which are progenitors of the antibody secreting plasma cells (9). T cells interact specifically with antigen, proliferate, differentiate (10), and interact with B cells to signal their clonal expansion and ultimate differentiation into antibody-secreting plasma cells (11). T cells not only perform this specific helper function, but act to regulate the class of antibody formed (12), the amount of circulating antibody (13), the affinity of antibody produced (14), and participate in immunological memory (15). B cells also exhibit specificity for antigens (11), participate in restriction of antibody class (16), and also participate in immunological memory (17).

Thus four classes of small lymphocytes may exist, viz, virgin T cells, activated (educated or primed) T cells, virgin B cells, and primed B cells. Functional identification of T and B cells has explained in part some of the complexities of immune responses, particularly the observation that a small antigen called a hapten is unable to elicit antibody formation unless it is bound to a larger carrier moiety which is itself immunogenic (18). Elegantly demonstrated for mice by Raff (19) is evidence that the immunogenic carrier is recognized by T cells, the hapten by B cells. Thus for antibody formation against a hapten, the presence of functional T cells capable of recognizing the carrier is necessary. Hapten-carrier systems, such as that described by Kettman and Dutton for mouse responses (20), provide a clear cut means for differentiating between T and B cell function in antibody responses, particularly in regard to ionizing radiation effects.

As early as 1908, Benjamin and Sluka noted the suppressive effect of ionizing radiation on immune function (21). Since that time numerous reports have emerged detailing aspects of radiation-induced immune suppression (compiled in 22). Two studies in which effects of x-rays on immunocompetent cells were quantitated are significant in the context of work proposed in this document. Makinodan, et al. (23), determined survival curves for antibody formation against sheep erythrocytes with primed and unprimed spleen cells. Their results indicated that both the primary and secondary hemagglutinin responses were equally radiosensitive, i.e., data for both responses fit identical survival curve parameters with an extrapolation number of 2.7, and a D_0 of 70 rads. In these experiments antigenic challenge occurred within three hours after irradiation. More recently Zaalberg et al. (24), reported survival curves for primary responses of mice, also directed against sheep erythrocytes. His work showed that the dose survival curve for spleen cells challenged 24 hours after irradiation was purely exponential, i.e., completely lacking a shoulder, whereas cells challenged with antigen 2 hours after irradiation exhibited a shoulder. In both cases the exponential slopes of survival curves, and hence the D_0 values were parallel. Gengozian and Makinodan (25), and Makinodan, et al., (26) investigated the relationship of time between irradiation and antigenic challenge for antibody responses in mice. A consistent feature of these experiments was that the nadir of response resulted when antigenic stimulation occurred either 12 or 24 hours after radiation exposure. These results were confirmed by Carlson (27) who showed in addition that the time-dependent response was differentially exposure-rate sensitive, and that the level of antibody response was less exposure-rate dependent at the nadir than when antigen preceeded irradiation by 12 or 24 hours.

A central question relates to the relative role assumed by T and B cells in radiation-induced suppression of antibody responses. Parameters of radiosensitivity have not been worked out for unprimed B or T cells, primed B cells, nor fully described for activated T cells. Two groups have demonstrated a degree of radioresistance of helper function for primed T cells, although quantitative parameters of radiosensitivity

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have not been described (23,29). Quantitative information relative to B and T cell radiosensitivity for high LET radiations are also lacking.

Experiments in this laboratory have established the feasibility of determining differential B and T cell activity in irradiated mice. Sublethally irradiated mice (400-700 rads) were reconstituted with either thymus cells (50×10^6) or bone marrow cells (10×10^6) and challenged with sheep erythrocyte antigen either 2 hours after irradiation, or at periods of up to 3 days. On the fourth through seventh days after antigen injection, mice were killed and antibody secreting cells were enumerated according to the method of Jerne (30) or Cunningham (31). For primary responses challenged within 2 hours of irradiation the activities of both B and T cells appeared to be relatively equivalent. When groups of irradiated, reconstituted mice were challenged with antigen at intervals of up to three days, data for both direct (IgM) and indirect (IgG) antibody forming cells indicated that the nadir of response which occurs in the mouse 24 hours after irradiation, was due principally to diminished B cell activity. These experiments are being prepared for publication at the present time.

b-ii. Cellular Immune Response. That rejection of foreign grafts is thymus dependent rests on a mountain of both direct and indirect evidence (45). Elimination of T cells from immune lymphoid populations abrogates their ability to display cell mediated toxicity in either graft versus host (GVH) or allograft reactions (32,33). Rouse, et al. (34,35), who investigated a markedly antigenic and spontaneously regressing group of plasmacytomas in NZB mice, found that high concentrations of anti-theta sera abrogated the cytotoxic effects of immune lymphoid cells. Their results indicate that thymus dependent lymphocytes are the primary effector cells in cell-mediated immunity against these tumors. Similar conclusions have been drawn by investigators using both Cr^{51} release and microcytotoxicity as in vitro correlates (36,37). That cytotoxic effector lymphocytes are T-cell dependent is also implied from its parallel to ctyotoxicity in graft rejection, and to the finding that nude or athymic mice (T cell deficient) show a great ability to propogate transplanted tumors. The exact implication of the latter finding is now in some doubt since nude mice reportedly show residual T cell activity, and appear not to be markedly more susceptible to spontaneous (38) or chemically induced oncogenesis (39).

On the other hand, recent reports by two groups suggest participation of B cells in tumor rejection. Investigating Maloney Virus sarcomas in the mouse (37) and bladder carcinoma in man (40) by means of microcytotoxicity test, major B cell components mediating cellular cytotoxicity were found. The exact interpretation of these results is difficult to resolve at this time, but does leave open the possibility that more than one cell type may be involved in antitumor cell-mediated cytotoxicity. Nonetheless care must be taken in interpreting results of any single cytotoxicity method since different tests may give different results when evaluating either allograft transplantation (41) or tumor immunity (42).

Since eventual combination therapy with radiation or chemicals may include immunotherapy or enhancement of host cell-mediated immune mechanisms, interactions of these regimens with host immune function as well as host-mediated antitumor activity is of importance. Although radiation is a common modality for tumor therapy, the effect of such treatment on lymphocyte activity and on host cell-mediated immunity has not been well worked out.

A number of studies have been reported relating radiotherapeutic regimens to lymphocyte activity, measured as responsiveness to mitogens or as changes in numbers of B and T cells. Thus, Stjernsward et al. (47), and Engresset, et al. (48), investigating lymphocyte activity in patients irradiated mediastinally for breast tumors or with

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multiple fields for Hodgkins Disease showed significant and prolonged decreases following irradiation. T cell activity was more markedly depressed than that of B cells. Stratton and Byfield (49) recently reported extremely rapid and significant (although relatively transitory) decreases in both T and B cell activity following irradiation.

Cell-mediated immunity per se has been studied in mice undergoing experimental radiation therapy for transplanted fibrosarcomas (43,44). In these carefully controlled experiments little if any alteration in cytotoxicity occurred in spleen or lymph node cells taken from animals which received single curative doses (as high as 4500 rads) delivered to the thigh. In contrast to these mice in which radiotherapy was successful as measured by tumor size, animals in which therapy was unsuccessful displayed a reduction in cytotoxicity together with development of blocking factors in the serum. The presence of factors, either antibody, antigen or antigen-antibody complexes, which interfere with specific cell-mediated cytotoxicity directed against the host tumor, seems to be a generalized phenomenon in both animals and humans bearing large antigenic tumors (45). The work of O'Toole (46) provides an important step toward relating radiotherapy and cell-mediated cytotoxicity in man. Radiation treatment for bladder carcinoma (4000 to 5000 rads) protracted over weeks, caused relatively rapid decreases in cell-mediated toxicity in the peripheral blood lymphocytes, followed by a rapid recovery of cytotoxicity only in patients destined to show no recurrence. Thus, the time course of cell-mediated cytotoxicity after irradiation may be of prognostic value (61).

In comparing responses of patients (46) and of irradiated mice (44), a marked difference in the time course of cytotoxicity is apparent. In human subjects significant depression in cytotoxicity occurred immediately following irradiation, while this was not seen in the mouse studies. This disparity may have resulted from differences in radiation protocols--fractionated doses were used in the human trials, whereas comparable single doses were used in mice. The fractionated protocol for patients would be expected to irradiate a greater number of lymphocytes during their transit through the radiation fields, Rouse, et al. (34), have shown that effector lymphocytes may be moderately radiosensitive, with LD_{50} values of about 200 rads.

The mouse myeloma presents an attractive model for investigating effects of radiation on growth and host-mediated cytotoxicity: a) tumor growth can be quantitated by measurement of paraprotein concentrations in the sera (50,51). Thus it is possible to follow early changes in tumor growth. b) The plasmacytomas in Balb/c mice are only weakly immunogenic and may more closely parallel the human condition than other more immunogenic experimental tumors. c) The Balb/c plasmacytoma has characteristics of its human counterpart in that it causes kidney dysfunctions similar to that found in man (52), and responsemuch like human tumors to a variety of chemotherapeutic agents (53). d) The classical Winn test can be utilized for quantitating radiation-induced inactivation (54,55), in which graded numbers of immune cytotoxic cells are injected together with known numbers of plasmacytoma cells into sublethally irradiated animals. Absence of tumor growth results from activity of immune lymphoid cells.

At this writing a project is getting underway in this laboratory to evaluate cell mediated cytotoxicity and its enhancement or alteration by various therapeutic regimens, using Balb/c myeloma, MOPC-104. The project is intended in part to provide dissertation research for a Ph.D. candidate, who may also be involved in studies proposed herein.

3. <u>Rationale</u>: Negative pi mesons represent a new modality for the treatment of human malignancy. Because pure high intensity pion beams have not existed previously, the

relative effects of pion inactivation on a wide variety of cells is being determined in a coordinated fashion. Much of this work will be extrapolated directly into planning treatment protocols for patients.

Mounting evidence implicates a vital role for immune processes in tumor growth and cure. Furthermore, the immune status of cancer patients undergoing therapy is of concern. Quantitative information about the action of both peak and plateau pions on cell populations participating in immune responses is warranted.

B. Specific Aims: The project outlined below is designed to quantitate effects of both peak and plateau pions on immunocompetent cells that mediate immune reactions. Data from pion exposures will be compared with paired experiments using Cobalt-60 gamma rays. Thus RBE values will be obtained for classes of immunocytes involved in both the antibody and cell-mediated responses.

C. Methods of Procedure: Techniques to be used for the proposed project are straightforward and quantitative. For the most part, these have been employed, or are being used in this laboratory. For both humoral and cellular studies, methods outlined in this section offer the advantage of requiring only a moderate amount of preliminary or baseline work before begining the central experimental protocols. On the other hand, because of necessary controls and quantitation, the numbers of animals required to justify an attempt in this area are moderately large.

Mice: Specific pathogen free CD_2F_1 (Balb/c x DBA_2)F_1 and Balb/c mice will be used for experiments measuring antibody responses and inhibition of tumor growth, respectively. These will be purchased from Cumberland View Farms, Clinton, Tennessee, and used at 12-16 weeks of age.

Antigen: TNP (2,4,6 trinitrophenyl) substituted erythrocytes will be prepared according to the method of Rittenberg and Pratt (56); erythrocytes will be obtained from the Animal Resources Center of this institution.

Irradiation: Recipients in both assay systems, as well as appropriate experimental cell suspensions, will be irradiated from a 3,000 Ci ^{60}Co Theratron. Experimental irradiation will take place at the Los Alamos site using the biomedical beam channel. Cells will be irradiated *in vitro* using a compartmented chamber which has been fabricated for our current pion work.

Antibody Forming Cell Assay: The classical technique of Jerne for enumerating antibody releasing cells will be used (30). In this assay spleen cell suspensions are placed, together with target erythrocytes in a semisolid agar gel matrix, incubated at 37°C, and IgM releasing cells detected after addition of complement. Cells releasing IgG are detected by adding anti-mouse-IgG serum to the preparation to develop hemolysis.

Cytotoxic Lymphocyte Assay: The classical Winn (57) test for measuring antitumor activity of immune spleen cells will be used. Details of this procedure are described below.

Cells in antibody response.

These studies will utilize TNP (2,4,6-Trinitrophenyl) substituted erythrocytes to capitalize on the differential properties of T and B cells to interact with carrier and hapten, respectively. The basic premise is that maximal responses occur when optimal numbers of B and T cells coexist; a suboptimal quantity of one population

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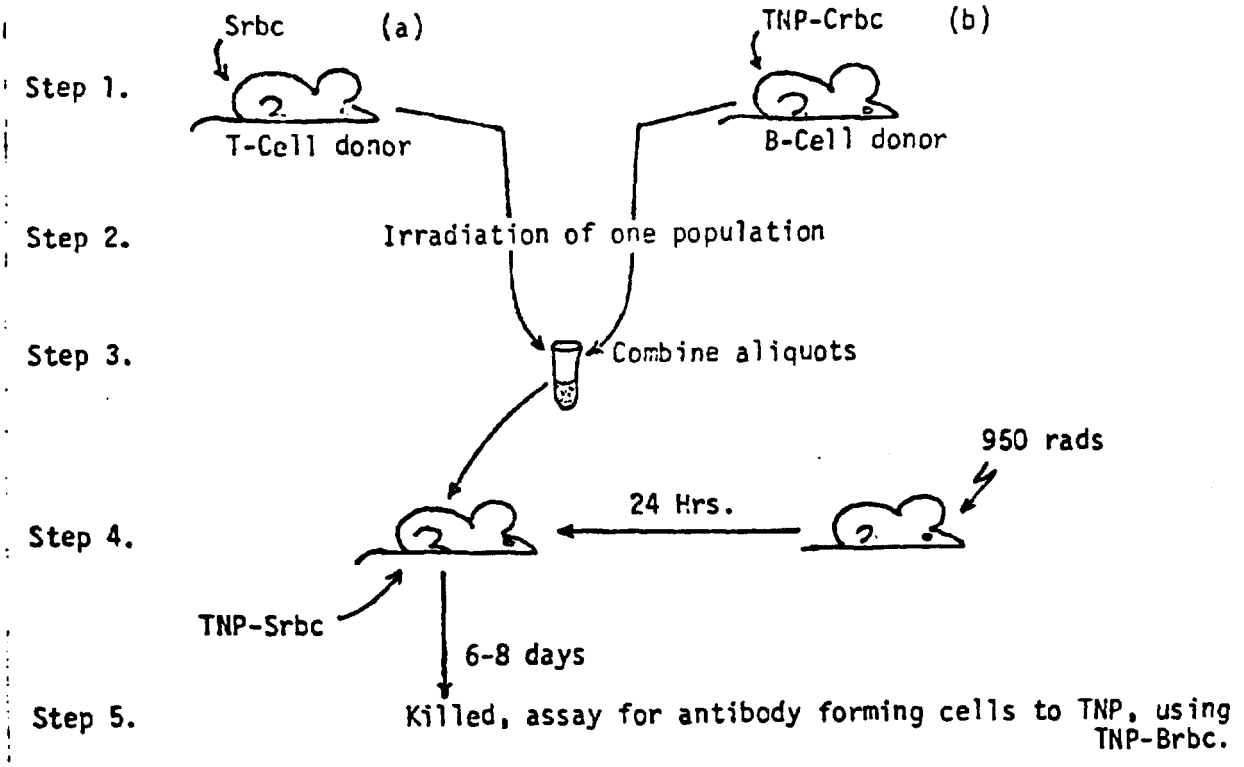
results in diminished responsiveness, and either population may be limiting to the response. Thus decreased antibody forming cell numbers, following irradiation of one population, reflect decrease in cell number of that population.

The general experimental outline is shown schematically below, and will be discussed in relation to individual experiments.



Step 1. Donor mice will be primed either with (a) .2 ml of .5% v/v Srbc (Srbc= sheep erythrocytes), the carrier, or (b) with 10^8 TNP-Crbc (Crbc= chicken erythrocytes), the hapten, coupled to an immunogenic carrier which cross reacts only slightly with Brbc (Brbc = burro erythrocytes). In this way cell populations from donor spleens can be obtained in which (a) T cells are primed against the carrier, but B cells in the suspension are not primed to TNP, and (b) B cells are primed to TNP, but T cells present are not primed to the test carrier Brbc.

Step 2. Primed T or B cells will be irradiated with either pions or gamma rays.

Step 3. Irradiated cells will be combined with unirradiated suspensions in appropriate numbers.

Step 4. The final cell suspension will be injected, together with TNP-Srbc into syngeneic recipients exposed 24 hr previosly to 950 rads.

Step 5. Recipient mice will be killed, spleens surgically excised, cells suspended, and numbers of antibody forming cells against TNP enumerated by the method of Jerne.

Initial experiments will establish the relationship between cell number and PFC responses for the four cell populations to be studied, namely (1) primed T cells, (2)

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unprimed T cells, (3) primed B cells, and (4) unprimed B cells. As an example of this procedure 50 x 10^6 spleen cells from carrier-primed and an equivalent number from hapten primed animals will be combined, injected, challenged and assayed. Graded numbers of e.g. carrier primed cells will be given to other groups of recipients, together with the standard hapten-primed inoculum. These data will be used to construct standard curves against which responses following irradiation can be compared to yield surviving fractions.

Primed T Cells. Carrier primed spleen cells will be harvested, irradiated, combined with 50 x 10^6 unirradiated TNP-primed cells, as outlined in the preceding diagram. For a typical radiation dose at least three doubling dilutions of test cells will be included

Experimental groups will include the following cell populations

1. Irradiated carrier-primed + unirradiated TNP-primed
2. Unirradiated carrier-primed + unirradiated TNP-primed
3. Irradiated carrier-primed
4. Unirradiated TNP-primed

Group 1 is experimental and as indicated above, will include subgroups of graded carrier-primed inocula.

Group 2 serves as positive control, in which responses are expected to be optimal, and to agree with the previously determined standard curve.

Groups 3 and 4 are negative controls indicating the degree of cross-reactivity between carriers, and will be subtracted from experimental values.

For PFC assay of recipient spleen cells, the following determinations will be made:

1. TNP-Brbc
2. Brbc

Group 1 will measure antibody forming cells to TNP developed as a consequence of T cell - B cell interaction. Group 2 will indicate the level of cross-reactivity between TNP and Brbc; that value should be consistently zero

Primed B Cells. Experiments to assay responses of primed B cells after irradiation will be done similarly to those described above for primed T cells, except that hapten-primed spleen cell population will be irradiated, and will therefore be limiting to the response.

Unprimed T and B Cells. For these experiments cells from unprimed donors will be used, together with primed cells of the complementary cell type. If necessary, T Cells will be obtained from secondary donors in the following way. Syngeneic recipients will be lethally irradiated, and injected with 50 x 10^6 thymus cells. Recipients will be killed on day 7, and cells from these spleens, enriched for T cells (58) will be used. Likewise, populations enriched for B cells can be obtained by injecting bone marrow cells into 950-rad irradiated recipients and harvesting spleens at day 4.

Data Handling and Evaluation. From standard curves generated as described above, expected response values will be available; these values for a given cell inoculum will be treated as "100% survival". Thus following irradiation of a cell population the resulting responses will be used to calculate surviving fractions. As a function of radiation dose, survival curves will be generated by computer, using a standard program in use at this institution. From these survival curves it will be possible to compare parameters of radiosensitivity (defined as $1/D_0$) as well as D_q ("quasi-threshold dose"). Thus a complete picture of differential radiosensitivity for cells involved in antibody responses will be obtained for gamma rays, and both peak and plateau pions

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Cells in Tumor Rejection. To quantitate the role of immune lymphoid cells in the abrogation of tumor outgrowth, the technique described by Winn (57) will be employed. This in vivo all-or-none technique is far more quantifiable than existing in vitro cytotoxicity methods that rest upon quantitation of both lymphoid and tumor cells in culture, or release of radioactive label. To induce immunity to the tumor, the method of Krueger (54) will be used, in which multiple injections of killed tumor cells will be injected into normal Balb/c mice. At intervals to be determined the mice will be killed, spleens removed, cells suspended in Eisens "A" medium, pooled, counted for nucleated cells, placed in the radiation chamber and exposed to either peak or plateau pions, or to gamma rays. Following irradiation and removal of cells from the chamber, cells will be recounted, the percent viability will be determined, and the desired concentration will be mixed with the minimal ED 100/60 of myeloma cells, and injected subcutaneously into sublethally (450 rads) irradiated recipients. Recipient mice will be observed daily for appearance of tumor growth. Animals in which tumor growth does not occur will be scored positive for the presence of a critical number of activated cytotoxic cells against plasmacytoma MOPC194.

For a typical experiment the following groups will be included:

1. MOPC 104 + Irradiated Immune Spleen Cells
2. MOPC 104 + Unirradiated Immune Spleen Cells
3. MOPC 104 + Nothing
4. MOPC 104 + Normal Spleen Cells

Expected results are as follows: Groups 3 and 4 are negative controls, in which 100% of recipients are expected to show tumor outgrowth. Group 2 comprises the positive control in which no growth should occur. Group 1 is experimental, and will include at least four doubling dilutions of spleen cells.

Preliminary experiments will establish the following baseline information:

1. Minimal $ED_{100/60}$ for MOPC 104. The minimum number of plasmacytoma cells that will produce tumor growth in 100% of sublethally irradiated recipients, will be determined by injecting groups of 20 mice with doubling dilutions of MOPC 104 cells, and following recipients to observe tumor outgrowth over 60 days. The smallest inoculum yielding consistently 100% tumor growth will be used as the standard tumor cell dose.

2. ED_{50} for Abrogation of Tumor Growth by Immune Spleen Cells. Using the minimal $ED_{100/60}$ determined as described above, doubling concentrations of immune spleen cells will be mixed with MOPC 104 cells, injected into 450 rad irradiated recipients, and observed for tumor growth through 60 days. Results will be plotted on log-probit plots, and the $ED_{50/60}$ determined by the method of Litchfield and Wilcoxon (59).

Once established, these parameters will be used to generate dose-survival curves for immune spleen cell cytotoxicity as outlined.

3. To allege that abrogation of growth by immune syngeneic spleen cells is due to T cell mediated function, it is necessary to confirm the findings of Rouse, et al. (35) that growth abrogation can be suppressed by exposure of spleen cells to anti-theta serum.

Surviving fractions after either pion or gamma ray exposure will be calculated from the ratios of ED_{50}'s for unirradiated to irradiated immune spleen cells. Dose survival curves will be generated by computer, using a standard program in use at this institution.

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These central protocols comprise the principal basis for requesting funding, although it is likely that additional experiments will be carried out, such as the following.

Characterization of radiosensitivity parameters for MOPC-104 to both peak and plateau pions: While not necessarily unique in the coordinated pretherapeutic pion program (60), information on RBE values for plasmacytoma might bear clinical implications. With $ED_{50/60}$ data in hand for MOPC-104, a limited series of experiments in which myeloma cells are exposed to pions in our compartmented chamber, will yield a series of survival curves for pions.

Effect of dose rate on cells mediating growth abrogation of MOPC-104: Experiments in this laboratory have addressed the question of the relative effects of dose rate in combined radium-cobalt therapy, analogous to common treatment for carcinoma of the uterine cervix. (Results are now in preparation for publication). Using this radium-cobalt model it would be interesting to compare the effects of dose rate on cell-mediated cytotoxic activity using the procedures described above.

Because of the moderately long-term nature of the cell-mediated cytotoxicity experiments, a support period of at least three years is necessary. In order to begin preliminary experiments for both cellular and humoral response protocols, a funding date of January 1, 1975 would be optimal. The present pion project will be funded through August 31, 1975, and our work at LAMPF will proceed until that date. Thus portions of the program outlined in this proposal could likely begin at LAMPF in September of 1975.

In any event, experiments with pions are intended to begin toward the end of the first award year. The duration of experiments for humoral ($\leq$9 days) and cellular (60 days) responses are sufficiently different to allow both areas to proceed concomitantly for preliminary as well as for pion experiments.

D. Significance:

Experiments outlined in this proposal will provide information on the effects of negative pi mesons on lymphoid cell populations that participate in immune responses. Information on pion inactivation of T and B cell populations involved in antibody responses will yield RBE values for peak and plateau pions. For this new radiotherapeutic modality, data on destruction of cells that mediate cytotoxicity against tumor growth will relate effects of pions, both entering and at depth, to effects of conventional gamma rays. These data may have clinical usefulness in evaluating immune responses of patients undergoing radiotherapy with pions by providing parameters of radiosensitivity for immunocompetent lymphocytes in the radiation field.

E. Facilities Available:

Within the Radiology Department adequate laboratory, kitchen, and office space is available. Animal quarters, cages, etc., sufficient to house several hundred mice are located near the laboratory. Facilities for cleaning and sterilizing cages are provided by our Animal Care Unit. An irradiation source (3700-Ci Cobalt-60) is available; dosimetric equipment and basic holders for irradiating mice, microscopes, Coulter Counter, centrifuges, water baths, refrigerator and freezer space, and most smaller items are on hand. Capability for handling data and statistical evaluation will be provided through the Scientific Computer Center at this institution.

Facilities at the LAMPF site are discussed in the following section.

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