MARROW-TUMOR INTERACTIONS; THE ROLE OF
THE BONE MARROW IN CONTROLLING
CHEMICALLY INDUCED TUMORS

Cornelius Rosse, M.D., Ph.D.

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RLO-2225-T16/42

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UNIVERSITY OF WASHINGTON

SEATTLE, WASHINGTON 98195

*The Graduate School
Grant and Contract Services*

June 20, 1980

Ms. Marji W. Parker
Contracts Procurement Division
Richland Operations Office
P.O. Box 550
Richland, Washington 99352

VIA: L. Jon Buttars, Resident Representative
University of Washington

Enclosed is a proposal prepared by Professor Cornelius Rosse, Department of Biological Structure, requesting renewed support of Contract No. RLO-2225-T 16/42, entitled "Marrow-Tumor Interactions, the Role of the Bone Marrow in Controlling Chemically Induced Tumors."

The amount of \$150,412 is requested for the period from January 1, 1981 through December 31, 1981.

It is a pleasure to transmit this University of Washington proposal for your consideration.

Sincerely,

D R Baldwin

Donald R. Baldwin
Director

DRB:gh
Enclosure

MASTER

Please indicate
Principal Investigator's
name on all correspondence.

PROGRESS REPORT: RESEARCH CONTRACT DOE CONTRACT 2225; TASK AGREEMENT EV10270

Title: Marrow Tumor Interactions: The Role of the Bone Marrow in Controlling Chemically Induced Tumors

Report Number: RLO-2225-T16/42

For Period July 1, 1979 - June 30, 1980

Whether or not neoplastic cells induced by environmental agents establish themselves as cancer may be less dependent on the inducing agent than on the effectiveness of host mechanisms capable of controlling tumor growth. For this reason, the experimental program we have proposed in the contract sets out to investigate antitumor mechanisms that operate in the host. We have chosen, rather unconventionally, to focus on the role of the bone marrow in tumor growth regulation, as some of our experiments suggested that the participation of this tissue (which in its total mass may more appropriately be called an organ) plays an important role in the regulation of cancer, and this role has been almost entirely neglected and overlooked in previously published work.

During the first year of the contract our efforts have concentrated on defining the two experimental tumor systems which we have selected for studying marrow tumor interactions, and the fruitfulness of our early efforts bears witness to the importance of bone marrow in tumor growth regulation. The 11 scientific papers that resulted from our work during the first year and are currently progressing through publication channels (see Bibliography and Appendix) document the following significant findings.

1. The bone marrow contains a population of lymphocytes that are capable of developing into cytotoxic T lymphocytes as a result of interaction with alloantigens and acquire the ability to kill tumor targets of the same specificity as the sensitizing alloantigen (1).

2. Precursors of cytotoxic T cells in the bone marrow are unlikely to be immigrant T lymphocytes, but such immigrant cells influence the differentiation of the precursors. The cytotoxic cells generated from the bone marrow are at least as effective in killing tumor targets as are cytotoxic cells generated by splenic T lymphocytes (1). These findings are consistent with the fact that bone marrow cells depleted of T cells can recognize alloantigen and mount a proliferative response (2). The alloantigen responsive cells and the precursors of cytotoxic T cells are bound to be identical with pre-T cells that have been investigated in our previous studies (11).

3. We have discovered in the bone marrow of normal, unsensitized mice a natural regulatory cell (NRC) that is capable of inhibiting both the proliferative and cytotoxic responses of immunocytes that are provoked by alloantigens. NRC effectively blocks the response that induces the generation of cytotoxic T lymphocytes and since their own generation is independent of sensitization by alloantigens, these cells are likely to play a major role in the regulation of responses against self antigens or against responses provoked by altered self. Cancer cells are included in the latter category. NRC are unique to the bone marrow and are absent from spleen, lymph node and the thymus (3-5).

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4. We have investigated the characteristics of NRC quite exhaustively and they do not correspond to any known cell type in the lymphomyeloid system (6).

5. We have discovered that the bone marrow contains natural tumor growth regulatory cells (NTRC). While the exact relationship between NTRC and NRC is being investigated, it is clear so far that NTRC are not T cells, are uniquely present in the bone marrow and not in spleen, lymph node or thymus (7,8).

6. The population of NTRC in the bone marrow does not depend on the thymus or the spleen of the host, and these cells are capable of emigrating from the bone marrow and interacting with tumor specific immunocytes at the site of tumor growth (7,8).

7. NTRC by themselves have no effect on tumor growth, however, in collaboration with immunocytes that have been specifically sensitized by a tumor, they are capable of neutralizing the tumor (7,8).

8. In normal, intact animals the tumor neutralization effect of specific anti-tumor immunocytes is dependent on their interaction with NRC derived from the bone marrow.

The findings listed under 5-8 were obtained in our MCA tumor system and assign a major, hitherto unrecognized role to several subclasses of mononuclear bone marrow cells in the regulation of tumor growth. Our experiments with the spontaneous CE mammary carcinoma system illustrate that a rapid growth of certain neoplasms may be due to the fact that through some as yet undefined mechanisms the tumor eliminates mononuclear cells in the bone marrow of the host and stops their production. Findings listed under 9-16 document the evidence that supports this possibility.

9. CE mammary carcinoma that exerts a profound granulocytopoietic effect on the bone marrow and spleen of mice also shuts off concomitantly the production of lymphocytes in the bone marrow (9).

10. In strains of mice which exhibit different tumor growth rates and survival rates with this tumor also show correspondingly accelerated or retarded rates of changes in bone marrow lymphocytes (9).

11. All subpopulations of lymphocytes decrease in femoral marrow including T, B and null cells (9).

12. The tumor causes activation of hemopoietically inert peripheral fatty marrow in bones of the tail and feet (10). This newly established hemopoietic marrow is initially lymphocytic with the predominance of null lymphocytes. However, lymphocyte production in peripheral marrow is inadequate to compensate for the decreased lymphocyte population in central marrow (9).

13. In addition to the bone marrow, the tumor also affects the thymus, producing a transient decrease in the cellularity of this organ. Thus both primary sites of

lymphocyte production, marrow and thymus, are compromised by CE mammary carcinoma (9).

14. The decrease in bone marrow and thymic lymphocyte populations is reflected in the spleen. Despite gross splenic hyperplasia due to extramedullary granulocytopoiesis and erythropoiesis, the total number of lymphocytes falls in the spleen and this decrease involves B and T cells (9).

15. The decrease in bone marrow, thymic and splenic lymphocyte populations is not due to a stress effect that can be ascribed to the tumor. Bone marrow, thymic and splenic lymphocytes, behave the same way in tumor bearing animals that have been adrenalectomized or sham operated (9). An MCA that is without granulocytopoietic effects does not produce changes in bone marrow lymphocytes in intact or adrenalectomized animals (9).

16. The presence of the spleen has no effect on changes induced in the bone marrow by CE mammary carcinoma as bone marrow lymphocytes decreased also in splenectomized tumor bearing animals (9).

The data on which the above summary is based will not be discussed in detail. Their full exposition is to be found in the Appendix with a review of the pertinent literature. A comprehensive review (11) discusses most of the findings, although the review was written before many of the experiments reported in Papers 1-10 were completed.

Since many tumors provoke an immune response to antigenic determinants expressed on their cell membrane, it is important to investigate the potential of the marrow to respond to cell surface antigenic markers. This is best done making use of H-2 histocompatibility antigens and papers 1 and 2 pertain to this issue. Since mononuclear cells of the marrow can control both immune responses (3-6) and directly/ interact with tumor specific immunocytes (7,8); it is important to appreciate the complexity of the cell populations present in the marrow that may influence and regulate tumor growth. Lack of responsiveness on the part of bone marrow cells may be due in many systems to the inhibitory effect of natural regulatory cells. Likewise, since the conditions required for the activation of cytotoxic precursor cells in the bone marrow differ markedly from those required by the spleen (1), it is important to pay rigorous attention to the methodology when studying the potential of various bone marrow cells. In in vivo tumor systems the influence of the tumor on the cell populations under study must not be neglected.

Discovery of a cell type, the NTRC, upon which neutralizing effects of tumor sensitized immunocytes depend is a major contribution to tumor biology (7,8). It is possible that NTRC synergize with antitumor cytotoxic T cells or they may inhibit tumor specific suppressor T cells. Without specific immunocytes NTRC are incapable of affecting the tumor.

Work Proposed for the Forthcoming Year

The experiments planned conform to the original contract proposal. They will

not be outlined in detail. Major areas of activity will include:

1. Testing the immunogenecity of CE memory carcinoma
2. Establishment of in vitro cell lines of both MCA and CE tumors
3. Establishment of in vitro microcytotoxicity assays for both tumors so that our in vivo assays can progress with in vitro studies, necessary for characterizing the mechanisms of the phenomena defined in vivo
4. Characterization of NTRC and testing its relation to NRC and natural killer (NK) cells

A major change in the plan originally proposed is an emphasis on in vitro studies. Dr. Klimpel's activity in the project should be evident from the bibliography. The effective collaboration between Drs. Klimpel and Lee, which is taking to it's wings, is expected to be very productive.

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