AD NUMBER
ADB259954

NEW LIMITATION CHANGE

TO
Approved for public release, distribution unlimited

FROM
Distribution authorized to U.S. Gov't. agencies only; Proprietary Information; Aug 1999. Other requests shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, MD 21702-5012

AUTHORITY
USAMRMC ltr, dtd 15 May 2003

THIS PAGE IS UNCLASSIFIED
Award Number: DAMD17-98-1-8094

TITLE: Endothelial Cell-Based Gene Therapy of Breast Cancer

PRINCIPAL INVESTIGATOR: John O. Ojeifo, Ph.D.

CONTRACTING ORGANIZATION: Georgetown University Medical Center
Washington, DC 20057

REPORT DATE: August 1999

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary information, Aug 99). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
NOTICE

USING GOVERNMENT DRAWINGS, SPECIFICATIONS, OR OTHER DATA INCLUDED IN THIS DOCUMENT FOR ANY PURPOSE OTHER THAN GOVERNMENT PROCUREMENT DOES NOT IN ANY WAY OBLIGATE THE U.S. GOVERNMENT. THE FACT THAT THE GOVERNMENT FORMULATED OR SUPPLIED THE DRAWINGS, SPECIFICATIONS, OR OTHER DATA DOES NOT LICENSE THE HOLDER OR ANY OTHER PERSON OR CORPORATION; OR CONVEY ANY RIGHTS OR PERMISSION TO MANUFACTURE, USE, OR SELL ANY PATENTED INVENTION THAT MAY RELATE TO THEM.

LIMITED RIGHTS LEGEND

Award Number: DAMD17-98-1-8094
Organization: Georgetown University Medical Center

Those portions of the technical data contained in this report marked as limited rights data shall not, without the written permission of the above contractor, be (a) released or disclosed outside the government, (b) used by the Government for manufacture or, in the case of computer software documentation, for preparing the same or similar computer software, or (c) used by a party other than the Government, except that the Government may release or disclose technical data to persons outside the Government, or permit the use of technical data by such persons, if (i) such release, disclosure, or use is necessary for emergency repair or overhaul or (ii) is a release or disclosure of technical data (other than detailed manufacturing or process data) to, or use of such data by, a foreign government that is in the interest of the Government and is required for evaluational or informational purposes, provided in either case that such release, disclosure or use is made subject to a prohibition that the person to whom the data is released or disclosed may not further use, release or disclose such data, and the contractor or subcontractor or subcontractor asserting the restriction is notified of such release, disclosure or use. This legend, together with the indications of the portions of this data which are subject to such limitations, shall be included on any reproduction hereof which includes any part of the portions subject to such limitations.

THIS TECHNICAL REPORT HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION.

[Signature]  10/24/20
Endothelial Cell-Based Gene Therapy of Breast Cancer

John O. Ojeifo, Ph.D.

Georgetown University Medical Center
Washington, DC 20057

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

Distribution authorized to U.S. Government agencies only proprietary information, Aug 99). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

To determine the feasibility of endothelial cell-based gene therapy for metastatic breast cancer, we investigated the optimal dose, toxicity, and efficiency of incorporation of intravenously (IV)-administered, human interleukin-2 gene-modified murine microvascular endothelial cells (hIL-2/MECs) into individual metastatic foci of breast cancer. Following IV injection of one or three doses of $10^5$, $10^6$, or $10^7$ hIL-2/MECs to BALB/c mice bearing pulmonary metastasis of breast cancer, various tissues from the animals were examined at varying intervals for the presence and expression of hIL-2 gene by DNA polymerase chain reaction (PCR) and reverse transcriptase (RT)-PCR techniques.

In mice treated with a single IV injection of $10^5$ hIL-2/MECs, 2, 5, and 2% of lung metastases obtained were hIL-2 positive by both DNA PCR and RT-PCR on day 7, 14, and 21, respectively. Animals given a single or multiple IV injections of $10^6$ or $10^7$ hIL-2/MECs died from toxicity. In contrast, three sequential IV injections of $10^5$ hIL-2/MECs (at 3-day interval) had no deleterious effects in the animals. Eighty, 90, and 30% of lung metastases recovered from these mice were hIL-2 positive on day 7, 14, and 21, respectively. No hIL-2 gene was detected in all other tissues of these mice or in control tumor-bearing mice.
Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

N/A Where copyrighted material is quoted, permission has been obtained to use such material.

N/A Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

N/A Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

X In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Bio-safety in Microbiological and Biomedical Laboratories.

[Signature and Date]
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Report Documentation page</td>
<td>2</td>
</tr>
<tr>
<td>Foreword</td>
<td>3</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>4</td>
</tr>
<tr>
<td>Introduction</td>
<td>5</td>
</tr>
<tr>
<td>Body of Report</td>
<td>5</td>
</tr>
<tr>
<td>Specific Aims and Statement of Work</td>
<td>5</td>
</tr>
<tr>
<td>Major Research Accomplishments</td>
<td>6</td>
</tr>
<tr>
<td>Plans for the future</td>
<td>9</td>
</tr>
<tr>
<td>Key Research Accomplishments</td>
<td>9</td>
</tr>
<tr>
<td>Reportable Outcomes</td>
<td>9</td>
</tr>
<tr>
<td>Conclusions</td>
<td>9</td>
</tr>
<tr>
<td>References</td>
<td>9</td>
</tr>
<tr>
<td>Appendices</td>
<td>9</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

Breast cancer is the most common female malignancy in North America (1). This disease is estimated to affect 1 of 9 women (2), and is associated with substantial morbidity and mortality (1). While early detection and treatment have led to significant improvements in cancer-related mortality and quality of life for women with breast cancer, recurrence and metastatic dissemination of the tumors still account for a significant morbidity and mortality in patients. Effective means of treating this subset of patients remains elusive. A novel approach to the problem of recurrent or metastatic cancer involves the activation of potent immune responses that are capable of specifically destroying tumor cells. Transgenic immunotherapy, as the term implies, refers to the insertion of cytokine genes into cells in order to activate anti-tumor immune responses. Moreover, this approach is intended to avoid the dose-limiting toxicities that have impeded the application of otherwise very promising cytokine therapies. The goal of this research is to develop an effective and safe gene therapy for invasive breast cancer. The objectives of the research are (1) to determine whether intravenously (IV) administered endothelial cells expressing exogenous cytokine gene(s) can selectively migrate into pulmonary metastases of breast tumors, express the cytokine transgene at the metastatic sites, and elicit anti-tumor immune responses, and (2) to determine the safety of IV-administered, genetically-modified endothelial cells. This report covers the investigation of (a) the efficiency of hIL-2/MLEC incorporation at multifocal tumor sites, and (b) the optimal dose and toxicity of IV administration of hIL-2/MLECs.

2. BODY

2.1 Specific Aims and Statement of Work

The specific aims of this research are (1) To determine (a) whether IV-injected, interleukin-2 gene-modified murine lung endothelial cells (IL-2/MLECs) can target sites of pulmonary metastases of breast cancer, and (b) how well IL-2/MLECs can express the IL-2 transgene at the metastatic sites; (2) To determine whether the expression of hIL-2 transgene at the local site of pulmonary metastases will induce an anti-tumor immune response. The approved Statement of Work is as follows:-

Task 1: Months 1-24.

Determine (a) whether IV-injected, interleukin-2 gene-modified murine lung endothelial cells (IL-2/MLECs) can target sites of pulmonary metastases of breast cancer, and (b) how well IL-2/MLECs can express the IL-2 transgene at the metastatic sites.

a. Mouse lung endothelial cells (MLECs) will be isolated and enriched using FDG-FACS. The cells will be transduced with a retroviral vector containing human IL-2 gene.

b. Efficiency of IL-2/MLEC incorporation at different tumor sites:
   - Co-localization of IL-2/MLEC and tumor in animals: three experiments; 40 animals per experiment.
c. Determination of toxicity of IV IL-2/MLEC administration:
- Acute toxicity following a single dose of $10^3$ IL-2/MLEC administration
- Cumulative toxicity following 3 IV injections of $10^3$ IL-2/MLECs spaced 3-4 days apart.
Three experiments; 40 animals per experiment.
d. Optimization of IL-2/MLEC incorporation in tumor sites:
- Tumor-bearing animals will receive three IV injections of IL-2/MLECs closely (3-4 days) or widely (5-7) apart. Expression of IL-2 transgene at the metastatic sites determined by RNA PCR amplification of human IL-2 in discrete individual metastases. Four experiments; 40-50 animals per experiment will be performed.
- Comparison of the relationship between different administration schedules with the number of cells incorporated at sites of tumor metastases will be determined. Two experiments; 40 animals per experiment will be performed.

Task 2: Months 24-36.

Determine whether the expression of hIL-2 transgene at the local site of pulmonary metastases site will induce an anti-tumor immune response.

Groups of experimental and control animals will be sacrificed weekly to monitor hIL-2 expression in the lungs, quantitate metastases, and to assess lung tumor response to IL-2/MLEC treatment. One group of the experimental and control animals will be observed over time for survival. Survivors will receive additional MFP injection of 4T1 cells to determine their ability to reject tumor re-challenge.

2.2 Major Research Accomplishments

Overview

We have made significant progress in studies outlined in task 1 during the past year. Specifically, we successfully generated interleukin-2 gene-modified murine lung endothelial cells (IL-2/MLECs) which were used to determine the efficiency and optimal dose of hIL-2/MLEC incorporation into sites of tumor metastases. We have also evaluated the toxicity of systemic administration of hIL-2/MLECs.

Methods

Endothelial cells harvested from the lungs of BALB/c mice were transduced with a retroviral vector containing the rhIL-2 gene under the transcriptional control of a CMV promoter. These cells (hIL-2/MLECs) secrete 76 ng (1000 IU)/10^6/24h of recombinant human interleukin-2 in vitro.

Tumor-bearing mice were developed by injecting of $10^4$ 4T1 tumor cell line (derived from a mammary tumor in BALB/c mouse) into the mammary fat pad of syngeneic mice. Two weeks later, at the time when the earliest pulmonary metastases are observed, the primary tumors were excised, and one or three doses of hIL-2/MECs were intravenously injected via the tail vein. Multiple injections of hIL-2/MECs were spaced 3 days apart. Beginning on day 7 after the last administration of hIL-2/MECs, at the time when significant accumulation of genetically-modified endothelial cells
(GMECs) and expression of exogenous gene occurs at sites of angiogenesis (3), groups of animals were sacrificed at varying intervals (7, 14, and 21 days), to follow the fate of injected cells over time. Heart, lungs, liver, spleen, long bones, and brain tissues were removed from the animals and examined for the presence and expression of hIL-2 gene using DNA polymerase chain reaction (PCR) and reverse transcriptase (RT-PCR) techniques.

Results and Discussion

In mice treated with a single IV injection of $10^5$ hIL-2/MLECs, 2, 5, and 2% of their lung metastases examined were hIL-2 positive by both DNA PCR and RT-PCR on day 7, 14, and 21, respectively (Table 1).

Table 1: Percentage of pulmonary metastases targeted by hIL-2/MLECs at varying intervals after a single intravenous injection of the GMECs

<table>
<thead>
<tr>
<th>Study group</th>
<th>Treatment</th>
<th># metastases per mouse</th>
<th>% of metastases positive for hIL-2 gene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>1</td>
<td>Tumor + hIL-2/MLECs</td>
<td>&gt;200</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Tumor + Neo/MLECs</td>
<td>&gt;200</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Tumor alone</td>
<td>&gt;200</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>hIL-2/MLECs</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Attempts to improve the targeting efficiency by increasing the dose of the implanted hIL-2/MLECs from $10^5$ to $10^6$ and $10^7$ were unsuccessful. On the contrary, many of the animals that received single or multiple IV injections of $10^6$ or $10^7$ of hIL-2/MLECs became ill and subsequently died, indicating that this large inoculum of GMECs was toxic to the animals. One explanation for this poor targeting efficiency is the heterogenous angiogenic behavior of the individual tumor metastases. That is, in certain instances, the IV-injected hIL-2/MLECs seeded growing tumors at an optimal moment for incorporation and growth within the developing tumor vasculature. Other metastases, perhaps too small to sustain very active angiogenesis, could not promote sufficient GMEC incorporation. In addition, administration of a single bolus injection of GMEC is unlikely to target every tumor deposit present at the moment of injection. Another factor affecting the efficiency of GMEC-targeting of tumor metastases is the ongoing seeding of the lungs with tumor.
cells still in circulation or tumor cells dislodged from other sites at the time of hIL-2/MLECs treatment. This results in repetitive seeding of the lungs, making assessment of the efficiency of GME targeting difficult. For these reasons, we hypothesized that the kinetics of GMEC administration will determine, to a large extent, both targeting efficiency and extent of GMEC incorporation at a particular tumor deposit. We further hypothesized that small multiple injections of small inoculum of GMECs would reduce their toxicity in the animals. To test this hypothesis, we administered 3 injections of $10^5$ hIL-2/MLECs spaced 3 days apart. As predicted, multiple injections of small inoculum of hIL-2/MLECs increased the safety and the targeting efficiency at multifocal tumor sites. Three sequential IV injections of $10^5$ hIL-2/MLECs (at 3-day interval) had no deleterious effects on the animals. Eighty, 90, and 30% of the individual lung metastases recovered from this group of tumor-bearing mice were positive for hIL-2 positive on day 7, 14, and 21 (Table 2).

Table 2: Percentage of pulmonary metastases targeted by hIL-2/MLECs at varying intervals after three intravenous injections of the GMECs

<table>
<thead>
<tr>
<th>Study group</th>
<th>Treatment/ # metastases per mouse</th>
<th>% of metastases positive for hIL-2 gene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>1</td>
<td>Tumor + hIL-2/MLECs &gt;200</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>Tumor + Neo/MLECs &gt;200</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Tumor alone &gt;200</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>hIL-2/MLECs 0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>None 0</td>
<td>0</td>
</tr>
</tbody>
</table>

We did not detect hIL-2 gene in any other tissue obtained from all hIL-2/MLECs-treated mice. As shown in Tables 1 and 2, all tissues obtained from vector (Neo/MLEC, no hIL-2 gene insert) -treated mice were hIL-2 gene negative. Furthermore, we did not observe any tumor in normal mice which received the same three IV injections of hIL-2/MLECs, nor did we not find any significant statistical difference in the number of metastatic foci seen in the lungs of tumor-bearing mice with and without hIL-2/MLECs treatment, suggesting that systemic administration of gene-modified endothelial cells did not promote the formation of tumors in these animals.
2.3 Plans for the Future

In the coming year, we plan to complete all studies under task 1. Specifically, further experiments will be performed to: 1) compare closely (3-4 days) with widely (5-7) spaced IV injections schedule of hIL-2/MLECs; 2) determine whether IV-injected hIL-2/MLECs can lodge within an inactive or a physiologically active site in mice, and (3) determine whether IV-administration of hIL-2/MLECs can promote tumor formation in mice. Also, we plan to investigate a) whether the expression of hIL-2/MECs at metastatic sites can induced an anti-tumor response that will abrogate tumor metastasis, b) the nature, level, and duration of anti-tumor immune response that is induced at the local tumor site; and c) the ability of microvascular endothelial cells (MECs) expressing interleukin-12 (IL-12) transgene or herpes simplex thymidine kinase (HSV-TK) gene to inhibit the growth of established breast cancer metastases mice.

3. Key Research Accomplishments

We have:

1) Isolated pure population of lung endothelial cells from BALB/c mice. The cells have been transduced with a retroviral vector containing human IL-2 gene and high expressing clones have been isolated and fully characterized.

b) Determined the efficiency of hIL-2/MLEC incorporation into sites of breast cancer metastasis.

b) Optimized hIL-2/MLEC incorporation into sites of pulmonary metastasis of breast cancer.

d) Determined acute and cumulative toxicity of IV-administered hIL-2/MLECs.

4. Reportable Outcomes

None

5. Conclusions

These results demonstrate that 1) three IV injections of $10^5$ hIL-2/MLECs, given at 3-day intervals, efficiently target tumor metastases, 2) genetically-modified microvascular endothelial cells can express interleukin-2 transgene at the local site of breast cancer metastasis, and 3) multiple IV injections of $10^5$ hIL-2/MLECs (given at 3-day intervals) can be safely administered to mice. These results will enable us to proceed to determine whether IV injections of hIL-2/MLECs can abrogate breast cancer metastases and prolong the survival of the tumor-bearing mice.

6. References


7. Appendices

None
MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

[Signature]

Encl

PHYLIP M. RINEHART
Deputy Chief of Staff for Information Management