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TITLE: Innate Anti-Breast Cancer Activity of (Gamma)/(Delta) T-Cells: A Novel Biological and Clinical Approach to the Treatment of Relapsed or Refractory Breast Cancer

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14. ABSTRACT We initially identified and characterized a CD2-mediated, interleukin (IL)-12–dependent signaling pathway which inhibits apoptosis in mitogen-stimulated human $\gamma\delta$ -T cells. We have since exploited this pathway to develop the methodologies allowing the large-scale ex vivo expansion of viable apoptosis-resistant $\gamma\delta$ -T cells – an undertaking until now, not possible. Importantly, we have shown that apoptosis-resistant human $\gamma\delta$ -T cells retain significant innate, major histocompatibility complex (MHC)-unrestricted cytotoxicity against a wide variety of human-derived tumor cell lines, including human breast cancer cell lines. Our efforts related to this proposal have remained focused upon testing the hypothesis that $\gamma\delta$ -T cells – by virtue of their innate ability to recognize and kill epithelial-derived malignancies – play an important role in regulating the initial growth or spread of breast cancer in vivo. In this progress report, we discuss the findings we have made in the first year of this award. Although the human pre-clinical work is too preliminary to report at this point, we have made some important progress in optimizing our animal models to assess the ability of $\gamma\delta$ -T cells to moderate tumor growth in a syngeneic model of breast cancer. Problems encountered in the first year – and their solutions – are discussed in this first annual report.					
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INTRODUCTION

We initially identified and characterized a CD2-mediated, interleukin (IL)-12–dependent signaling pathway which inhibits apoptosis in mitogen-stimulated human $\gamma\delta$ -T cells. We have since exploited this pathway to develop the methodologies allowing the large-scale ex vivo expansion of viable *apoptosis-resistant* $\gamma\delta$ -T cells – an undertaking until now, not possible. Importantly, we have shown that apoptosis-resistant human $\gamma\delta$ -T cells retain significant innate, major histocompatibility complex (MHC)-unrestricted cytotoxicity against a wide variety of human-derived tumor cell lines, including human breast cancer cell lines. Our efforts related to this proposal have remained focused upon testing the hypothesis that $\gamma\delta$ -T cells – by virtue of their innate ability to recognize and kill epithelial-derived malignancies – play an important role in regulating the initial growth or spread of breast cancer in vivo.

BODY

In the initial period of this grant for which this report is generated (2 February, 2006 to 2 February, 2007) our accomplishments are presented in relation to the following tasks as outlined in the approved Statement of Work.

Task 1: Clinicopathologic correlations. To determine the extent to which $\gamma\delta$ -T cell numbers; $\gamma\delta$ -T cell innate antitumor capacity and $\gamma\delta$ -T cell expansion potential vary as a function of breast cancer clinical stage, clinical progression and clinical response to standard therapy.

Task 2: Basic tumor immunobiology. To further refine our understanding of the in vitro biology (recognition and effector functions) of the antitumor cytotoxicity mediated by human $\gamma\delta$ -T cells against human breast cancer cells.

- We have re-prioritized the order in which we are approaching our tasks. All patient-related studies or studies using human materials (patient blood or human cell lines) were moved back 12 months. As such, there are no results to be presented in this report related to **Task 1** and **2**. These studies are now underway and will be reported with the 2nd annual report as they are too preliminary.

Task 3: Pre-clinical models for the adoptive cellular immunotherapy of breast cancer. To determine the extent to which $\gamma\delta$ -T cells can regulate the growth and metastasis of breast cancer cells in vivo using pre-clinical animal models.

1) Treatment of tumor-bearing animals with human $\gamma\delta$ -T cells (xenograft model).

- As noted above, all studies involving the use of human materials (patient blood or human cell lines) were moved back 12 months. Studies in this sub-task are now beginning and will be reported with the 2nd annual report as they are too preliminary.

2) Mouse syngeneic breast cancer model. Tumorigenic mouse breast cancer cell line 4T1 (derived from BALB/c) have been used to establish disease in syngeneic BALB/c animals. Using two approaches (proof-of-principle studies and adoptive immunotherapy studies), we are now examining the extent to which murine gamma/delta T-cells can prevent the growth or metastasis of 4T1 cells in vivo.

1. Proof-of-principle studies. As initially proposed, we predicted that mice depleted of $\gamma\delta$ -T cells using the GL3 anti- $\gamma\delta$ T cell receptor (TCR) antibody would have more rapid disease progression when challenged with tumorigenic 4T1 breast cancer cells.

We have currently been optimizing our model by first determining empirically the fewest number of tumorigenic 4T1 cells to implant in mice so that a proportion – but not all mice – develop tumors. We have discovered that this is key since we are attempting to detect what appears to be a subtle

enhancement of tumor growth in mice depleted of $\gamma\delta$ -T cells. Too high a tumor cell inoculum appears to mask the effects we expect to see. Our initial studies – though not conclusive – suggest that 4T1 cells implanted in mice depleted of $\gamma\delta$ -T cells using the GL3 anti- $\gamma\delta$ TCR antibody do indeed appear to grow faster, but that this is a very subtle effect requiring larger numbers of mice to achieve statistical significance.

Our initial studies using a commercially available anti- $\gamma\delta$ TCR antibody. We have noted that the cost of the commercially available reagent has proven to be prohibitive—especially in studies where larger numbers of mice are required. Accordingly, we have now acquired the hybridoma which makes the GL3 antibody and are now producing the purified antibody for use in the larger studies now underway. These will be reported in the 2nd annual report.

2. Studies to assess immunotherapeutic potential of adoptively transferred $\gamma\delta$ -T cells in the setting of established disease. Here we are determining the extent to which adoptively transferred $\gamma\delta$ -T cells can moderate growth or metastasis of established breast cancer cells.

These studies are possible as we are able to obtain $\gamma\delta$ -T cells by direct isolation (immunomagnetic separation) from spleen cell preparations. Alternatively, we can also isolate mouse $\gamma\delta$ -T cells from spleen cell preparations first expanded in a process similar to that used to expand human $\gamma\delta$ -T cells.

However, we have discovered that when large numbers of $\gamma\delta$ -T cells are required for multiple treatments of multiple mice, reliably generating the number of mouse $\gamma\delta$ -T cells can be limiting. Although we can usually obtain sufficient numbers of $\gamma\delta$ -T cells from cultured spleen cell preparations, we have realized that the variations in the cell yield on expansion of $\gamma\delta$ -T cells from healthy, wild type BALB/c mice can compromise our studies.

To solve this problem, we have elected to take the approach of generating $\gamma\delta$ -T cells from BALB/c mice which are deficient in $\alpha\beta$ -T cells. This is a commonly used approach that assures that the T cells expanded from the spleen cell preparation are all $\gamma\delta$ -T cells and not contaminated with $\alpha\beta$ -T cells. This approach then assures us that we will have a pure population of $\gamma\delta$ -T cells for adoptive transfer into tumor-bearing mice. This $\alpha\beta$ -T cell deficient mouse on the BALB/c background is available and we are in the process of obtaining a breeding pair from The Jackson Laboratory.

KEY RESEARCH ACCOMPLISHMENTS

- Very early data support our prediction that tumorigenic 4T1 breast cancer cells grow more rapidly in mice depleted of $\gamma\delta$ -T cells. However, as noted above, we are currently optimizing our model by downwardly titrating the number of 4T1 cells used to establish tumors. Also, we will no longer use the commercially available GL3 antibody to deplete mice of $\gamma\delta$ -T cells on account of cost. We are now purifying our own GL3 using a hybridoma obtained from a colleague.
- Immunotherapy studies in the syngeneic mouse model at this point are inconclusive as we have not been able to reliably generate the larger numbers of $\gamma\delta$ -T cells needed for the larger studies. This problem is being directly addressed by the purchase of $\alpha\beta$ -T cell deficient mouse on the BALB/c background which will then serve as our source of $\gamma\delta$ -T cells for our studies.

REPORTABLE OUTCOMES none to date

CONCLUSION none to date

REFERENCES none

APPENDIX none