AD________________

Award Number: DAMD17-01-1-0479

TITLE: The Role of the Immune Cell Cytoskeleton in Breast Cancer Immunity: Particular Relationship Between Actin and p38 MAP Kinase

PRINCIPAL INVESTIGATOR: Alex W. Tong, Ph.D.
Tyler Curiel, M.D.
Schuang Wei, Ph.D.

CONTRACTING ORGANIZATION: Baylor Research Institute
Dallas, Texas 75204

REPORT DATE: July 2002

TYPE OF REPORT: Final

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

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.

20021231 108

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2002	Final (1 Jul 01 - 30 Jun 02)

4. TITLE AND SUBTITLE
The Role of the Immune Cell Cytoskeleton in Breast Cancer Immunity: Particular Relationship Between Actin and p38 MAP Kinase

5. FUNDING NUMBERS
DAMD17-01-1-0479

6. AUTHOR(S)
Alex W. Tong, Ph.D.
Tyler Curiel, M.D.
Schuang Wei, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Baylor Research Institute
Dallas, Texas 75204

E-Mail: Alext@Baylorhealth.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

Deformation of the cytoskeleton activates intracellular signaling pathways, including those governing differentiation. Based on the observation that monocytes reverse transmigrating through endothelial cells spontaneously undergo differentiation into dendritic cells (DCs), we hypothesized that the cytoskeleton modulation could affect DC differentiation. Monocytes were cultured with granulocyte M-CSF (GM-CSF) plus IL-4 to induce differentiation into immature DCs. The microtubule stabilizer paclitaxel significantly reduced DC CD1a and CD40 without affecting CD54, CD80 and MHC class I expression. DCs differentiating in the presence of paclitaxel secreted significantly (>55%) less IL-12 and significantly (4-fold) more IL-10 compared to control DCs following LPS-induced maturation. As a result, DCs differentiating in the presence of paclitaxel induced 9-fold less T cell interferon-γ compared to control DCs, and were inefficient at activating T cells. The microtubule destabilizer nocodazole reversed paclitaxel effects on DCs in a dose-dependent fashion, suggesting that derangements of microtubule movement and architecture were responsible for paclitaxel effects. Whereas LPS effects on differentiation are relatively irreversible, paclitaxel effects were reversible within 48 hours of paclitaxel withdrawal. Microtubules play an essential role in DC phagocytosis and migration. These data suggest that microtubules also mediate non-phagocytic DC functions including differentiation and T cell activation.

14. SUBJECT TERMS	15. NUMBER OF PAGES
cytoskeleton, dendritic cell, monocyte, breast cancer, p38 MAP kinase, actin	7
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

COVER.. P.1

SF 298.. P.2

Introduction.. P.4

BODY..P.4-5

Key Research Accomplishments..P.5

Reportable Outcomes...P.5

Conclusions..P.5

References..P.5

Appendices..P.6-7

Final Report for DAMD17-01-1-0479

MANUSCRIPTS

None to date

INTRODUCTION

The Concept Grant No. DAMD17-01-1-0479 was originally awarded to Dr. Shuang Wei at Dr. Tyler Curiel's laboratory at the Baylor Institute of Immunological Research, Baylor Research Institute, Dallas, TX in July, 2001. In the fall of 2001, Dr. Wei and Dr. Curiel, relocated to Tulane University. Based on the recommendation of the USAMRAA Grants Office, this award was retained by Baylor Research Institute with a subcontract agreement with Dr. Tyler Curiel to perform the proposed work for the balance of the funding period. This arrangement also involved the instatement of Alex W. Tong, Ph.D. at Baylor Research Institute as Principal Investigator. The following is the final report of the findings provided by Drs. Curiel and Wei.

Cell cytoskeletal elements transduce signals that relate to growth and differentiation. Little is know regarding how cytoskeletal movements influence immune events in the tumor microenvironment. A major component of the cytoskeleton is the microtubules. We used drugs that selectively interrupted normal cell microtubule movements and studied their effects on differentiation of myeloid dendritic cells such as are found in the tumor microenvironment. We hypothesized that the tumor prevented normal cell microtubule movement, and that this contributed to the dendritic cell dysfunction seen in human tumors. p38 MAP kinase is a protein cascade tied to the microtubule system that we hypothesized was specifically disrupted by defective microtubule movements.

BODY

We had two principal goals: i) to determine if microtubule movements altered myeloid dendritic cell (DC) differentiation, and ii) to determine if breast tumors contributed to microtubule dysfunction. Goal 1 was successfully completed as described. Goal 2 was not completed owing to the time involved in successfully achieving goal 1.

We used a standard system of DC differentiation by culturing blood monocytes in recombinant GM-CSF plus IL-4 for 5 or 6 days to induce DC differentiation. To block microtubule function we added paclitaxel, a microtubule stabilizer, or colchicine, a microtubule destabilizer. Paclitaxel added to cultures of monocytes differentiating into DC inhibited DC differentiation in a dose-dependent fashion. Thus, as little as 50 μM paclitaxel significantly inhibited DC differentiation after 5 days. DC differentiation was assessed morphologically as reduced DC dendrites, phenotypically by flow cytometry (FACS) as reduced CD1a, CD40, CD80, CD86 and DR and increased CD14, and by several independent functional assays.

Functional assays included a reduced activation of naïve, allogeneic $CD4^+CD45RO^-$ T lymphocytes as measured by [^{3}H]thymidine incorporation, reduced induction of T cell interferon-γ and reduced DC secretion of IL-12. Cytokines were measured by standard commercial ELISA kits (R&D Systems).

A direct involvement of microtubules in these effects was confirmed in two independent experiments. First, we used phalloidin-conjugated Texas red to demonstrate abnormal clustering of microtubules in paclitaxel-treated DCs by confocal microscopy. Second, the microtubule destabilized colchicine reversed paclitaxel effects in a dose-dependent fashion. Toxic effects or effects on specific DC subsets were ruled out by washing out the paclitaxel from culture after 5 days. In this case, the DC phenotype reverted to normal in 2 days or less. Adding paclitaxel to control DCs that were previously differentiated in 5 days of GM-CSF+IL-4 had no effect on DC function or phenotype.

We obtained DCs directly from the tumors of patients with ovarian carcinoma by FACS sort of cells in malignant ascites. We used these instead of cells from breast cancer patients as we were unable to obtain sufficient cells from breast cancers at present for this work. The DCs we obtained had low expression of CD1a, and DR and increased expression of CD14, similar to observations with paclitaxel-treated DCs. Insufficient cells for study of other markers and for functional assays were available. These data are consistent with our concept of defective microtubule movement in these tumor-associated DCs, but further work is required to establish a causal link.

Interestingly, neither colchicine nor paclitaxel had any measurable effect on p38 MAP kinase activation in DCs. Nonetheless, addition of SB203580, a specific p38 MAP kinase inhibitor, replicated the DC differentiation defects of paclitaxel. Thus, it appears that p38 MAP kinase activation is required for DC differentiation as we predicted, but its activation is not through the cell microtubule system as we predicted.

KEY RESEARCH ACCOMPLISHMENTS

- **demonstration of microtubule movement in DC differentiation**
- **demonstration that in the absence of normal microtubule movement, DC function (IL-12 secretion, T cell interferon-γ induction, naïve T cell activation) is impaired**
- **demonstration that p38 MAP kinase activation is required for normal DC differentiation, but this activation is independent of microtubule movements**

REPORTABLE OUTCOMES

Manuscripts	none
Abstracts	1 to the 2002 ERA of Hope Meeting
Presentations	1 to the 2002 ERA of Hope Meeting
Patents and licenses	none
Other listed outcomes	none

CONCLUSIONS

Current work suggests that microtubule movements are critical to normal DC differentiation and function. DCs differentiating in the presence of the microtubule stabilizer paclitaxel had reduced capacity to activate T cells and induce interferon-γ. Tumors are known to inhibit microtubule movement in adjacent cells, and DCs in tumors have properties similar to the DCs we derived in vitro when microtubule movement was impaired. Nonetheless, further work will be required to establish a link between tumor-mediated microtubule dysfunction, and DC dysfunction. Such work will be the object of an R01 application on our part. Further, our work suggests that paclitaxel might induce tumor tolerance by inducing DCs that activate T cells poorly. This finding also bears further investigation.

REFERENCES

None

PERSONNEL RECEVING PAY

Alex W. Tong, PhD
Schuang Wei, PhD

APPENDICES

1. Abstract submitted to the 2002 ERA of Hope Meeting.

Appendix 1

MICROTUBULES PARTICIPATE IN CYTOKINE-INDUCED MYELOID DENDRITIC CELL DIFFERENTIATION, MATURATION AND FUNCTION

Shuang Wei, Ben Daniel, Tyler Curiel

Tulane Medical School, Section of Hematology and Medical Oncology,
1430 Tulane Avenue SL78, New Orleans, LA 70112

Email: swei@tulane.edu

Deformation of the cytoskeleton activates intracellular signaling pathways, including those governing differentiation. Based on the observation that monocytes reverse transmigrating through endothelial cells spontaneously undergo differentiation into dendritic cells (DCs), we hypothesized that the cytoskeleton could play an important role in DC differentiation. We used a well-described model of DC differentiation by culturing monocytes with granulocyte M-CSF (GM-CSF) plus IL-4. Monocytes differentiating into immature DCs in the presence of the microtubule stabilizer paclitaxel had significantly reduced expression of CD1a and CD40; CD54, CD80 and MHC class I expression were unaffected. DCs differentiating in the presence of paclitaxel secreted significantly (>55%) less IL-12 and significantly (4-fold) more IL-10 compared to control DCs following LPS maturation. As a result DCs differentiating in the presence of paclitaxel induced 9-fold less T cell interferon-γ compared to control DCs, and they were poor at activating T cell proliferation. Induced T cell IL-4 was equivalent to control DCs. Following LPS-mediated DC maturation HLA-DR and CD86 were upregulated only half as much as control, suggesting a defect in maturation. This observation was confirmed functionally in that LPS-matured DCs differentiated in the presence of paclitaxel were significantly impaired in their capacity to activate T cell proliferation. Paclitaxel has some LPS-like effects itself, which might explain some of these results. However, IL-12 and IL-10 secretion, and regulation of HLA-DR, CD40 and CD86 were distinct when induced by LPS, demonstrating that the underlying mechanisms of defective differentiation/ maturation in the presence of LPS compared to paclitaxel were distinct. Further, the microtubule destabilizer nocodazole reversed paclitaxel effects on DCs in a dose-dependent fashion, suggesting that the derangements in microtubule movement and architecture were responsible for paclitaxel effects. Further, whereas LPS effects on differentiation are relatively irreversible, paclitaxel effects were reversible within 48 hours of paclitaxel withdrawal. Microtubules play an essential role in DC phagocytosis and migration. These data suggest that microtubules also mediate non-phagocytic DC functions including differentiation and T cell activation. Defining the underlying mechanisms of perturbation of differentiation following microtubule disruption may help elucidate important pathways in DC differentiation. For example, NF-kB activation, and cytokine signaling affect, and are affected by cytoskeletal movements, including movements of microtubules. Furthermore, paclitaxel is used to treat certain cancers. These data suggest that chemotherapy may have unexpected effects on DCs such as induction of immunosuppressive IL-10.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0479 supported this work."