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Award Number: W81XWH-04-1-0372

TITLE: CTL-Tumor Cell Interaction: The Generation of Molecular Probes Capable of Monitoring the HLA-A*0201-HER-2/neu Peptide Complex

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REPORT DATE: March 2007

TYPE OF REPORT: Annual Summary

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

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-03-2007	Annual Summary	27 Feb 2004 – 26 Feb 2007

4. TITLE AND SUBTITLE

CTL-Tumor Cell Interaction: The Generation of Molecular Probes Capable of Monitoring the HLA-A*0201-HER-2/neu Peptide Complex

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-04-1-0372

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Health Research, Incorporated
Roswell Park Cancer Institute
Buffalo, NY 14263

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. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The overall goal of this project is to probe the CTL – tumor cell interaction by generating scFv probes that are able to recognize the HLA-A*0201-HER-2/neu369-377 peptide complex. In the second 12-month funding period (Feb 2005 – Feb 2006), scFv 2.3.5-58-53 was isolated following site-directed mutagenesis in the VL CDR3 and VL CDR1 of HLA-A*0201:HER-2/neu369-377 complex-specific scFv 2.3.5. In the 12-month period covered by this report (Feb 2006 – Feb 2007), I aimed to further improve the recognition of HLA-A*0201-HER-2/neu369-377 complexes by scFv 2.3.5-58-53. To this end, an scFv-Fc construct was utilized in an attempt to improve the avidity of the scFv for HLA-A*0201:HER-2/neu369-377 complexes expressed on tumor cells. Although scFv 2.3.5-58-53-Fc demonstrated modestly enhanced reactivity with HER-2/neu369-377 peptide-pulsed T2 cells when compared with scFv 2.3.5-58-53, it did not exhibit enhanced reactivity with HLA-A2+HER-2/neu+ tumor cells PCA-30 and MDA-MB-231. We are actively investigating several possibilities that may account for this discrepancy.

15. SUBJECT TERMS
Tumor immunology; Immunotherapy; HLA class I antigen; Antigen presentation

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	10	19b. TELEPHONE NUMBER *(include area code)*

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INTRODUCTION

This document is the final report of DOD Predoctoral Fellowship Award BC030039, entitled “CTL – Tumor Cell Interaction: The generation of molecular probes capable of monitoring the HLA-A*0201-HER-2/neu peptide complex”. It is intended to communicate the research progress related to this award mechanism in the 12-month period spanning from February 2006 through February 2007.

A major limitation to the utilization of the HLA-A*0201:HER-2/$neu_{369-377}$ complex-specific scFv 2.3.5, which was isolated during the first 12-month funding period, was its low affinity to the native complexes on breast carcinoma cells. During the second 12-month funding period, we utilized molecular biology approaches to introduce site-specific amino acid changes in the complementarity-determining regions (CDRs) of scFv 2.3.5, with the aim of isolating a clone with higher affinity for the HLA-A*0201:HER-2/$neu_{369-377}$ complex. scFv 2.3.5-58-53 exhibited markedly higher affinity for the HLA-A*0201:HER-2/$neu_{369-377}$ complex than the parental scFv 2.3.5.

In the previous 12-month funding period, we sought to further enhance the affinity of the scFv 2.3.5-58-53 to detect HLA-A*0201:HER-2/$neu_{369-377}$ complexes, whether these complexes were those found on peptide-pulsed T2 cells or native complexes located on the surface of breast carcinoma cells. To accomplish this goal, we utilized an available DNA construct, which inserted human Fc at the C-terminal end of the scFv in order to increase its avidity. Although we noted a modest improvement in the ability of scFv 2.3.5-58-53-Fc to detect HER-2/$neu_{369-377}$ peptide-pulsed T2 cells, compared with the parental scFv 2.3.5-58-53, no improvement was noted in its ability to react with PCI-30 or MDA-MB-231 cells, each of which is HLA-$A2^{+}$HER-2/neu^{+} and expected to express some native HLA-A2:HER-2/$neu_{369-377}$ complexes on their surface. It is not clear if these negative findings are due to the concentration of scFv-Fc reagent utilized, and/or the low density of native HLA-A2:HER-2/$neu_{369-377}$ complexes on these cells.

RESULTS

I. Reactivity of purified scFv-Fc with peptide-pulsed T2 cells

During the course of the second 12-month funding period for this fellowship, we utilized molecular biology approaches to increase the affinity of scFv 2.3.5, which is specific for the HLA-A*0201:HER-2/neu$_{369-377}$ complex. We performed three rounds of mutagenesis: (i) Alanine-scanning mutagenesis of V_L CDR3; (ii) Alanine-scanning mutagenesis of V_L CDR1; and (iii) Site-directed random mutagenesis of V_L CDR1. We obtained a scFv clone, named 2.3.5-58-53, which has been mutagenized in both its V_L CDR3 (M89T, Q90A, L94V) and V_L CDR1 (L27cA, H27dA, S27eP) regions. scFv 2.3.5-58-53 exhibited markedly higher affinity for the HLA-A*0201:HER-2/neu$_{369-377}$ complex than the parental scFv 2.3.5.

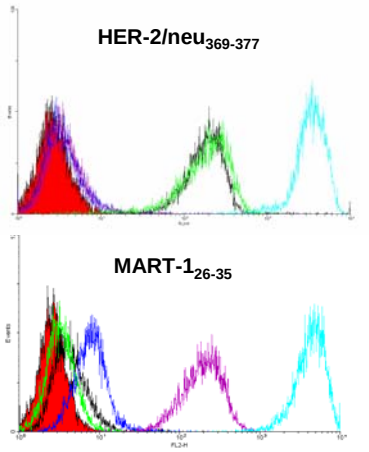

Fig. 1. Reactivity of purified scFv-Fc with peptide-pulsed T2 cells. scFv 2.3.5-58-53-Fc (25 µg/ml) was compared to parental scFv 2.3.5-58-53 for its reactivity to T2 cells pulsed with HER-2/neu$_{369-377}$ peptide (50 µg/ml) (top panel). The HLA-A*0201:MART1$_{26-35}$ complex-specific scFv 8.3-21, scFv 8.3-21-Fc, and the HLA-A2,A28-specific mAb KS-1 were utilized as negative and positive controls, respectively. T2 cells pulsed with MART1$_{26-35}$ peptide (50 µg/ml) (bottom panel) were utilized as a specificity control.

In the most recent 12-month funding period, we sought to further improve the affinity of scFv 2.3.5-58-53, in order to increase its sensitivity in detecting low to moderate amounts of native HLA-A*0201:HER-2/neu$_{369-377}$ complexes found on breast carcinoma cells. To this end, we collaborated with investigators at EMD-Lexigen (Billerica, MA), who had a DNA construct that allowed insertion of human Fc at the C-terminal end of our protein of interest. We expected this modification to improve the avidity of scFv 2.3.5-58-53, thereby enhancing its ability to detect HLA-A*0201:HER-2/neu369-377 complexes. We noted that there was a modest improvement in the reactivity of scFv 2.3.5-58-53-Fc with HER-2/neu$_{369-377}$ peptide-pulsed T2 cells (**Fig. 1**). Significantly, this modification did not affect the background reactivity of the same scFv-Fc reagent with irrelevant MART1$_{26-35}$ peptide-pulsed T2 cells.

II. Reactivity of scFv 2.3.5-58-53-Fc with malignant tumor cells

We also tested the reactivity of scFv 2.3.5-58-53-Fc with a panel of malignant tumor cells, including PCI-30 (HLA-$A2^{+}HER\text{-}2^{+}$), MDA-MB-157 (HLA-$A2^{-}$ $HER\text{-}2^{+}$), MDA-MB-231 (HLA-$A2^{+}HER\text{-}2^{+}$), SK-OV-3 (HLA-$A2^{-}HER\text{-}2^{+}$), and PCI-13 (HLA-$A2^{+}HER\text{-}2^{-}$) (**Fig. 2**). Very little to no reactivity was noted for all of these cells, including HLA-$A2^{+}HER\text{-}2^{+}$ PCI-30 and MDA-MB-231 cells. It is not clear if these negative findings are due to the concentration of scFv-Fc reagent utilized, and/or the low density of native HLA-A2:HER-2/$neu_{369\text{-}377}$ complexes on these cells.

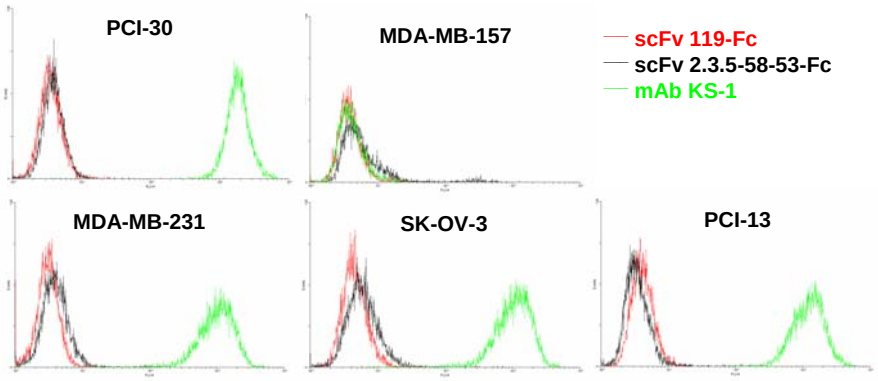

Fig. 2. Reactivity of scFv 2.3.5-58-53-Fc with malignant tumor cells. scFv 2.3.5-58-53-Fc (25 µg/ml) was incubated with a panel of tumor cells: PCI-30 (HLA-$A2^{+}HER\text{-}2^{+}$), MDA-MB-157 (HLA-$A2^{-}HER\text{-}2^{+}$), MDA-MB-231 (HLA-$A2^{+}HER\text{-}2^{+}$), SK-OV-3 (HLA-$A2^{-}HER\text{-}2^{+}$), and PCI-13 (HLA-$A2^{+}HER\text{-}2^{-}$). scFv 119-Fc (25 µg/ml) was utilized as a negative control, and HLA-A2,A28-specific KS-1 (5 µg/ml) was utilized as a positive control.

KEY RESEARCH ACCOMPLISHMENTS

- Synthesized and purified scFv 2.3.5-58-53-Fc protein, which is based on the HLA-A*0201:HER-2/neu$_{369-377}$ complex-specific scFv 2.3.5-58-53.

- Demonstrated that scFv 2.3.5-58-53-Fc has modestly enhanced reactivity with HER-2/neu$_{369-377}$ peptide-pulsed T2 cells, compared with scFv 2.3.5-58-53.

- Demonstrated that scFv 2.3.5-58-53-Fc has very low reactivity with HLA-A2^{+}HER-2/neu^{+} tumor cells PCI-30 and MDA-MB-231, which is consistent with findings utilizing scFv 2.3.5-58-53. It remains unclear whether the limited reactivity with tumor cells, despite positive findings with peptide-pulsed T2 cells, reflects the concentration of scFv-Fc reagent utilized, and/or the low density of native HLA-A2:HER-2/neu$_{369-377}$ complexes on the tumor cells.

REPORTABLE OUTCOMES

1. Ogino T, Miyokawa N, Ko E, Ferrone S. HLA class I antigen abnormalities in cancer: Molecular mechanism, clinical significance and negative impact for T cell-based immunotherapy. *Manuscript in preparation*.

CONCLUSIONS

In the course of the last 12 months, I have carried out a series of experiments to further enhance the reactivity of the HLA-A*0201:HER-2/neu$_{369-377}$ complex-specific scFv 2.3.5, and its mutagenized derivative scFv 2.3.5-58-53. In collaboration with EMD-Lexigen, an scFv-Fc reagent was generated, based on scFv 2.3.5-58-53 and hence termed scFv 2.3.5-58-53-Fc. The latter reagent showed moderately improved reactivity with HER-2/neu$_{369-377}$ peptide-pulsed T2 cells, when compared to scFv 2.3.5-58-53; however, it did not exhibit improved reactivity with HLA-A2^{+}HER-2/neu^{+} tumor cells. We are actively investigating the reasons for the limited reactivity of our scFv-Fc reagent to these tumor cells.

In conclusion, the research activities supported by this DOD Predoctoral Fellowship Award have served as an ideal training vehicle for multiple laboratory techniques, including (i) the generation scFv-Fc fusion proteins via molecular biology approaches; (ii) the purification of scFv-Fc fusion proteins; and (iii) the analysis of the reactivity of scFv-Fc fusion proteins with HLA-A2:HER-2/neu peptide complexes, expressed either on peptide-pulsed T2 cells or HLA-A2^{+}HER-2/neu^{+} tumor cells.

REFERENCE

1. Lo KM, Sudo Y, Chen J, Li Y, Lan Y, Kong SM, Chen L, An Q, Gillies SD. High level expression and secretion of Fc-X fusion proteins in mammalian cells. Protein Eng 1998; 11:495-500.