

# **GM-CSF and IFN- $\gamma$ Deficiency Link Autoimmune Diseases and Cancer – A Cancer Immune Surveillance Controversy**

Ph.D. Thesis

Prepared at the Dana-Farber Cancer Institute/Harvard Medical School  
in the Laboratory of Professor Glenn Dranoff

Submitted in the Department of Biology, Chemistry, and Pharmacy  
of the Free University Berlin

of  
Thomas Enzler, M.D.  
from St. Gallen, Switzerland

May, 2004

**Thesis Committee:**

**Thomas Blankenstein, Ph.D.**

Professor

Institute of Immunology

Free University Berlin

and Max-Delbrueck-Centrum for Molecular Medicine

Berlin, Germany

**Glenn Dranoff, M.D.**

Associate Professor

Department of Medical Oncology, Dana-Farber Cancer Institute

Department of Medicine, Brigham and Woman's Hospital

Harvard Medical School

Boston, MA, U.S.A.

This Ph.D. thesis was prepared between November 2000 and April 2004.

Date of thesis defence: 14.06.2004

## ACKNOWLEDGEMENTS

I would like to thank my mentor Professor Glenn Dranoff, Medical Oncology, Dana-Farber Cancer Institute, Boston, for his excellent support and the valuable discussions. I thank Professor Thomas Blankenstein, Max-Delbrueck Center for Molecular Medicine, Berlin, and Institute of Immunology, Free University Berlin, for his engagement to supervise this Ph.D. thesis.

I would also like to acknowledge the help of Dr. Silke Gillessen, Professor Fred Alt, Professor Martin Mihm, Dr. John Manis, Dr. David Ferguson, Dr. John Daley, Dr. Donna Neuberg, Dr. Jan Schmollinger, Professor Stanley Korsmeyer, Professor Jeffrey Kutok, and Professor Brian Wilson of the Harvard Medical School, Boston.

I would also like to thank Professor Jim Allison, Howard Hughes Medical Institute, Cancer Research Laboratory, University of California, Berkeley, for providing the hybridoma producing anti-CTLA-4 antibody, and Professor Myung Lee, Samsung Medical Center, Seoul, for his advice.

A special thank goes to Dr. Ewen Gallagher for critically reading the manuscript, and to Professor Michael Karin, both at the Laboratory of Signaltransduction and Gene Regulation, Department of Pharmacology, UCSD Medical School, University of California, La Jolla.

I thank Professor Willi Vetter and PD. Matthias Barton, Dep. of Internal Medicine, University Hospital of Zurich, for their support.

Last but not least, I would like to thank my parents, Hildegard and Alfred, for their support and interest in my work through all the time.

The author was supported by the Hanne Liebermann Foundation, University of Zurich.

**TABLE OF CONTENTS**

<b>1.</b>	<b>INTRODUCTION</b>	<b>6</b>
1.1	Cytokines in Autoimmune Diseases and Cancer	6
1.2	IFN- $\gamma$	7
1.3	GM-CSF and IL-3	8
1.4	Cytotoxic T Lymphocyte Associated Antigen-4	10
1.5	Mechanisms Leading to Autoimmune Diseases	11
1.6	The Cancer Immune Surveillance Theory	13
1.7	Experimental System and Hypothesis	15
<b>2.</b>	<b>MATERIALS AND METHODS</b>	<b>16</b>
2.1	Mice	16
2.2	Pathological Procedures	17
2.3	ELISAs	18
2.4	Generating Apoptotic Thymocytes	19
2.5	<i>In Vivo</i> Phagocytosis of Apoptotic Thymocytes	20
2.6	B Cell Purification	20
2.7	Genomic DNA Isolation from Tissue	21
2.8	Isolation of Total RNA	21
2.9	Protein Isolation and Protein Concentration Measurement	21
2.10	FACS <sup>®</sup> Analysis	22
2.11	Anti-CTLA-4 Antibody Purification and Injection Into Mice	23
2.12	RNase Protection Assays	24
2.13	Immunoblots	24
2.14	Statistical Analysis	25
2.15	Apoptosis Assays and Correction of the Fas Resistance	25
2.16	Determination of Cell Viability by Trypan Blue Exclusion and by Annexin V/Propidium Iodide Staining	26
2.17	Cell Cycle Analysis	26
2.18	Blood Sugar Measurements and Glucose Tolerance Tests	27
2.19	Southern Blot Analysis	27

2.20	Electromobility Shift Assays	28
2.21	Spectral Karyotype Analysis	28
2.22	Purification of DNA by Precipitation	29
2.23	First Strand cDNA Synthesis from RNA and Real Time PCR	29
<b>3.</b>	<b>RESULTS</b>	<b>31</b>
3.1	GM-CSF- and GM-CSF/IL-3-Deficient Mice Develop Autoimmune Diseases	31
3.2	GM-CSF/IFN- $\gamma$ - and GM-CSF/IL-3/IFN- $\gamma$ -Deficient Mice Often Develop Lethal Infections	41
3.3	GM-CSF/IFN- $\gamma$ - and GM-CSF/IL-3/IFN- $\gamma$ -Deficient Mice Develop Cancer	43
3.4	Lymphomas Show Upregulation of the Pro-Inflammatory Protein COX-2	47
3.5	Antibiotics and Anti-Inflammatory Agents (COX Inhibitors) Suppress Tumor Formation in GM-CSF/IFN- $\gamma$ - and GM-CSF/IL-3/IFN- $\gamma$ -Deficient Mice	48
3.6	Lymphomagenesis in GM-CSF/IFN- $\gamma$ - and GM-CSF/IL-3/IFN- $\gamma$ -Deficient Mice	50
<b>4.</b>	<b>DISCUSSION</b>	<b>53</b>
4.1	Autoimmune Diseases in GM-CSF/IFN- $\gamma$ - and GM-CSF/IL-3/IFN- $\gamma$ -Deficient Mice	53
4.2	GM-CSF/IFN- $\gamma$ - and GM-CSF/IL-3/IFN- $\gamma$ -Deficient Mice Develop Cancer, But Not Under Anti-infectious Or Anti-inflammatory Therapy – Inconsistency to the Cancer Immune Surveillance Theory	57
<b>5.</b>	<b>SUMMARY</b>	<b>62</b>
	<b>ZUSAMMENFASSUNG</b>	<b>63</b>
<b>6.</b>	<b>ABBREVIATIONS</b>	<b>65</b>
<b>7.</b>	<b>REFERENCES</b>	<b>68</b>
<b>8.</b>	<b>CURRICULUM VITAE</b>	<b>90</b>
<b>9.</b>	<b>PUBLICATIONS RELATED TO THIS THESIS</b>	<b>91</b>