THE ROLE OF DENDRITIC CELL SURVIVAL ON

T CELL ACTIVATION AND TOLERANCE

CLAUDIA LIZETT VARGAS

Department of Biological Sciences

APPROVED:

_____
Kristine M. Garza, Ph.D., Chair

_____
Todd Primm, Ph.D.,

_____
Wen-Yee Lee, Ph.D.

_____
Charles H. Ambler, PhD.
Dean of the Graduate School

DEDICATION

This thesis is dedicated to my wonderful parents whom have given me all their support, patience, and advice. I would like to thank my dad for always guiding me through life, teaching me the value of a great education and hard work, and simply being a great role model for me. I would also like to thank my mom for being my best friend, always providing that shoulder when I need it, and teaching me to love the simple things in life.

THE ROLE OF DENDRITIC CELL SURVIVAL ON T CELL ACTIVATION AND TOLERANCE

by

CLAUDIA LIZETT VARGAS

THESIS

Presented to the Faculty of the College of Science of

The University of Texas at El Paso

in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE

Department of Biological Sciences

THE UNIVERSITY OF TEXAS AT EL PASO

July 2004

UMI Number: EP10808

ProQuest Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

ACKNOWLEDGEMENTS

I would like to extend my sincere gratitude to Dr. Garza for believing in me, giving me the opportunity to work with her, and for all her support, guidance, and friendship she extended to me throughout the course of this project. I would also like to acknowledge and thank the members of my committee, Dr. Todd Primm and Dr. Wen-Yee Lee, for their contributions to this thesis.

I am also extremely grateful to Theresa G. Powell and January Carrillo for their help and contributions to this project when I was overwhelmed with work. Thanks for the lending hand! My very special thanks to the members of the Garza lab for making my research experience fun, entertaining, informative, and enlightening.

This research was supported by NIH-SCORE funds (GM008012-33), NIH-RCMI (2G12RR008124), and Bridges to the Doctorate Program.

This thesis was submitted to the committee on July 21, 2004

ABSTRACT

Ligation of the T cell receptor (TCR) by self-antigen/MHC complexes results in T cell activation or tolerance, leading to autoimmunity or protection, respectively. The factors however, that contribute to either outcome are not well defined. We hypothesize that the balance between the induction of autoimmunity and tolerance may be a function of the lifespan of the antigen presenting cell (APC) presenting self-antigen, where prolonged survival promotes the induction of autoimmunity and shortened survival promotes the induction of tolerance. To assess the affect of APC lifespan on T cell activation, dendritic cells (DC) of increased lifespan and shortened lifespan, relative to normal DC, were generated and assessed for their ability to activate antigen-specific T cells. DC generated from bone marrow (BM) of normal mice and treated with ceramide (C2) as well as BM-DC generated from CD40-deficient mice ($CD40^{-/-}$) were utilized as DC with shortened lifespans. BM-DCs of normal mice were treated with a general caspase inhibitor (APO-Block) to generate DC with prolonged life spans. As shown by flow cytometry for the relative expression level of the costimulatory molecules CD80 and CD86, the adhesion molecule CD54, and class II MHC, all groups of DC ($CD40^{-/-}$ DC, C2-DC, APO-DC, and normal-DC) were phenotypically similar prior to presentation of antigen. Their ability to activate antigen-specific T cells was measured in co-cultures with TCR transgenic T cells as a function of the upregulation of T cell activation markers (CD25 and CD69), the induction of proliferation, and the production of interferon gamma (IFNγ). Data demonstrates that T cell activation in response to antigen presented by $CD40^{-/-}$ DC and C2-

DC was less robust in comparison to control and were particularly marked by an inhibition of expression of the high affinity receptor for IL-2, CD25. The activation level of the DC, as measured by the level of CD80, CD86 and CD54, was similar between the experimental and control DC populations at the termination of the co-culture. However, when assessed for survival in the co-cultures, the $CD40^{-/-}$ DC and C2-DC presented with a greater apoptotic rate. BM-DC treated with APO-Block induced T cell responses similar to normal BM-DC, however their survival in co-culture was slightly increased. In addition, survival of T cells responding to antigen presented by APO-DC was suppressed relative to the T cells responding to peptide presented by normal DC as determined by an increase in apoptotic $CD8^{+}$ T cells in the co-culture. The data demonstrates that when APC survival is decreased during presentation, they are poor inducers of T cell responses, which correlates with low expression of CD25, suggesting that short immunological synapse duration leads to sub-optimal activation due to an inability to respond to IL-2. The possible induction of tolerance by the short-lived DC remains to be assessed. When APC survival is enhanced, T cell responses appear normal however this may be due to the induction of T cell apoptosis through activation induced cell death, thus appearing as a normal response relative to the control where in actuality, the T cells are responding more vigorously. The possible association with autoimmune disease induction also remains to be assessed. Thus, APC survival appears to directly affect the outcome of TCR interactions with self-peptide/MHC ligands and may influence the balance between autoimmunity and tolerance.

TABLE OF CONTENTS

Page

LIST OF FIGURES

CHAPTER 1

INTRODUCTION TO IMMUNOLOGY

"Immunology is the study of the physiological mechanisms that humans and other animals use to defend their bodies from invasion by other organisms" [1]. The immune system can be divided into two types of defense mechanisms, the innate and adaptive. Innate immunity, which is the body's first line of defense since it comes into play within minutes of a microbial invasion, is the recognition by nonspecific effectors such as granulocytes and macrophages. Although helpful in the removal of the infectious agent, this does not generate a lasting immunity since effector cells provided by the innate response have a limited life-span. During the adaptive immune response, which is mediated by antigen-specific lymphocytes, some cells persist after the antigen has been eliminated. These are known as memory cells and make up what is known as immunological memory, which provides protection against reinfection with the same pathogen [2]. Upon a second encounter with a pathogen, the memory cells ensure a more rapid and effective response against the culprit.

Adaptive immunity is dependent on the acquisition, processing, and presentation of antigen. This is mediated by antigen presenting cells (APCs): B lymphocytes, macrophages, and dendritic cells. An adaptive immune response starts with the engulfment of a pathogen or extracellular material by immature dendritic cells (DCs) [3]. DCs originate from bone marrow precursors that migrate from the bone marrow to peripheral stations in lymphoid and non-lymphoid tissues, where they carry

out a sentinel-like function [4]. These long-lived cells have a slow turn-over rate and, therefore, are constantly moving throughout the periphery in search of potential threat. They not only have the capacity to uptake extracellular fluid continually by the receptor-independent mechanism of macropinocytosis, where large amounts of fluid are taken up in single vesicles, but are primarily responsible for the phagocytosis and endocytosis of potential pathogens by a number of different mechanisms [2].

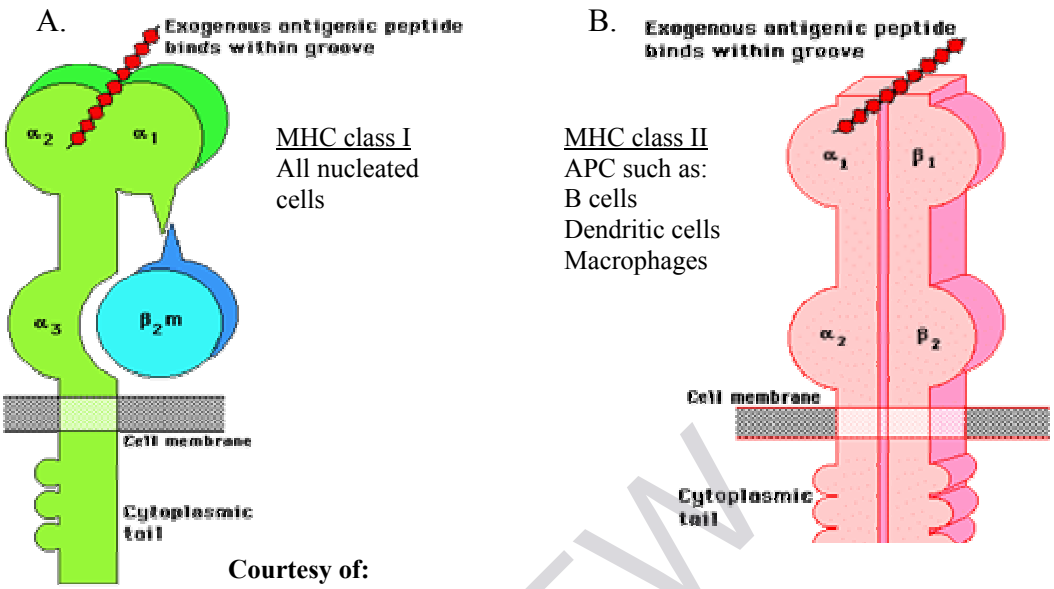
During their immature phase, dendritic cells express many surface receptors that allow them to recognize certain features that are common to many pathogens, such as their cell wall. Once the "potential pathogen" is targeted, its extracellular proteins become attached to the surface receptors on the DC, which signal particle uptake by phagocytosis. In this mechanism, the ingested material is contained in phagosomes that fuse with lysosomes and allows for the destruction and degradation by acid proteases triggered by low pH resulting in small molecules or peptides [5]. The peptide is then mounted onto a molecule called the major histocompatibility complex class II (MHC II), that displays it once it reaches the cell surface (Figure 1B). Class II MHC is thus responsible for presentation of peptide antigens derived from exogenous sources.

A second pathway of antigen processing and presentation involve antigen fragments that bind to MHC class I (MHC I) and are typically derived from viruses, intracellular pathogens, tumor proteins, and self-proteins [6]. These peptide fragments are generated by degradation of the protein in a cylindrical multicatalytic protease complex called the proteasome that consists of 28 subunits with a hollow core. Once the proteins are broken down into peptides in the cytosol, they are translocated into the endoplasmic

reticulum lumen by TAP (transporters associated with antigen processing) and are ready to be mounted onto newly synthesized MHC I (Figure 1A). Class I MHC is thus responsible for presentation of peptide antigens derived from endogenous sources [6].

Different type of stimuli such as the uptake of antigen, tissue damage, or even exposure to UV light, trigger the migration of DCs from the periphery to secondary lymphoid tissue. Along the way, they lose their ability to capture antigen and gain the capacity to upregulate their surface expression of costimulatory and adhesion molecules, such as CD80, CD86, CD40 and CD54. These molecules promote DC interaction with T lymphocytes for the initiation of an adaptive immune response [4].

T lymphocytes, otherwise known as T cells, originate in the bone marrow, but development occurs in the thymus. T cell precursors arriving in the thymus from the bone marrow spend up to a week developing before they enter the proliferation stage. During their developing phase, T cells undergo a selection process before they are released to the periphery. During the selection process, a person's own antigen is taken in by antigen presenting cells, is processed and presented as "self-peptide" on a "self-MHC" to developing thymocytes. T cells whose receptors interact weakly with the self-MHC:peptide complex, receive signals that enable them to survive; a process called positive selection. Those that do not recognize self-MHC are not "saved" considering



Courtesy of:
www.tulane.edu/.../WWW/ 335/335Immunopath.html **[7]**
Cann A.J: Principles of Molecular Virology. Academic Press, 2nd Edition, 1997 **Chapter 6. [8]**

Figure 1: Construct of MHC class I and class II. MHC class I is a heterodimer made up of a membrane spanning α chain bound covalently to β_2-microgobulin. It folds into three domains, in which α_1 and α_2 domains create a long cleft for peptide binding. MHC class II is composed of two parallel transmembrane chains, α and β, each consisting of two domains α_1, α_2, β_1, and β_2. The α_1 and β_1 domains create a peptide binding groove that is open at both ends.

that they cannot interact with the body's own MHC molecules and therefore would not have the ability to mount an immune response against antigenic peptide that is being presented. Negative selection occurs when a T cell's receptors bind too strongly with self MHC:self peptide complex and must be removed due to their ability to initiate damaging autoimmune reactions. This selection process, both positive and negative, is known as central tolerance because only the cells that interact weakly with self-MHC and potentially have the capacity of mounting an appropriate immune response against foreign antigens are released into the periphery and all those that have the potential to create havoc (responses against self antigens) are deleted [2,9].

The surviving T cells that are released into to periphery fall into two major classes that have different effector functions, $CD8^+$ and $CD4^+$. The two distinct classes of T cells differ in the type of MHC they have the ability to recognize [10]. $CD8^+$ T cells, known as cytotoxic T cells, bind to MHC class I molecules expressed on all cell types that present endogenous antigen. These are "killer" cells since they have the capacity to kill any infected cell. On the other hand, $CD4^+$ T cells, otherwise known as helper T cells (T_H cells), bind to MHC class II molecule that are only expressed on professional APCs presenting exogenous antigen. $CD4^+$ cells are specialized to activate other immune cells and fall within two categories, T_H1 or T_H2. The former category is known as pro-inflammatory T cells. Marked by the production of interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα), their main function is to activate macrophages, to promote the killing of the intravesicular pathogens that they harbor and to recruit other pro-inflammatory leukocytes. T_H1 cells also induce B cells to make antibodies that are

effective at "marking" extracellular pathogens for uptake by APC. The latter helper T cells (T_H2) initiate a humoral immune response by activating antigen-specific B cells to produce antibodies against the antigen for neutralizing, opsonizing (alteration of a pathogen's surface so it can be engulfed by an APC) or destruction by means of complement. They are marked by the production of interleukin (IL)- 4, IL-5, and IL-10 and are generally thought to partake in the removal of parasites [2,10].

Once matured, T lymphocytes are responsible for the cell-mediated immune responses of adaptive immunity. In order to recognize the peptide that is being presented by the DC via the MHC, the T cell needs to establish contact with it by forming a synapse with the DC whereby the MHC and a T cell molecule known as T cell receptor (TCR) forms the central molecular interaction (Figure 2) [10]. The TCR consists of two different polypeptide chains, TCRα and TCRβ, linked together by a disulfide bond. A minority of T cells convey an alternative, but structurally similar, receptor made of γ:δ T cell receptors. The array of the T cell repertoire comes from the somatic rearrangement of different TCR gene segments. In either case, both chains have an amino-terminal variable region to determine T cell specificity that is encoded by the variable (V), diversity (D), and joining (J) segments, and a constant region encoding a short hinge region consisting of cysteine that forms disulfide bonds between the two chains, a hydrophobic transmembrane domain, and a short cytoplasmic tail [2,11]. Because of the short cytoplasmic tail, the TCR signal indicating contact with another cell does not reach the interior of the cell. For this reason, signaling is aided by an amino acid sequence called immunoreceptor tyrosine-based activation motifs (ITAMS), which become

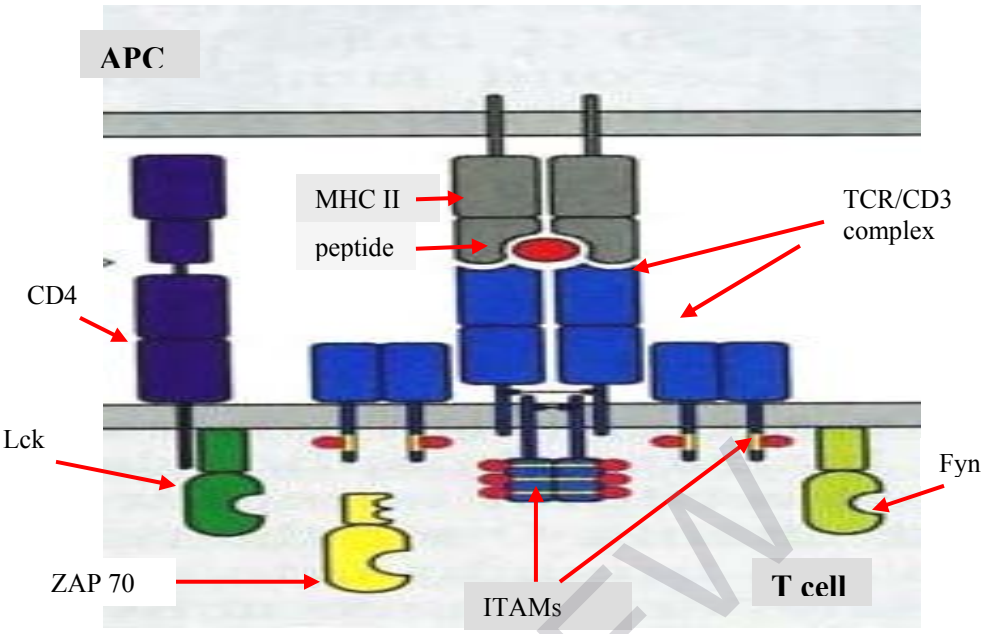


Figure 2: APC (DC) and T cell synapse. When an APC interacts with a T cell via the MHC:TCR complex, a number of other signaling molecules are activated to allow for full presentation and activation.