CHAPTER I

INTRODUCTION

Rationale for the Study and Statement of Problem

The immune system is body's ability to eliminate invading pathogens, including bacteria, viruses and substances that appear foreign and harmful to the body. The immune response protects the body from potentially harmful abnormal cells and substances by recognizing and responding to antigens which are usually proteins. The immune system can be divided into two branches; innate and adaptive immunity. Innate immunity functions as the first line of defense which is thought as the unspecific response. It consists of soluble factors, such as complement proteins, and diverse cellular components including granulocytes (basophils, eosinophils and neutrophils), mast cells, macrophages, dendritic cells and natural killer cells. The acquired or adaptive immunity is slower to develop, but manifests as increased antigenic specificity and memory. It consists of antibodies (Ab), B cells, and $CD4^+$, $CD8^+$ and gamma-delta T cells. There are two types of adaptive immune responses: humoral and cell mediated immunity (Chaplin, 2003, pp. S442-S459). In contrast to innate immunity, adaptive immunity exhibits specific recognition of the pathogens, can amplify and sustain its responses, and has the unique ability to remember the pathogen and quickly produce heightened immune response on subsequence encounters with the same agent (Ahmed and Gray, 1996, pp. 54-60). However, innate and adaptive immune response function cooperatively with numerous cells and molecules involving as a network.

T cells are a key cellular constituent of the immune system that possess the capacity to directly destroy cells infected with a pathogen and that also have a central role in stimulating and coordinately other components of the immune response, such as B cells. T cells originate from haematopoietic stem cells in the bone marrow and later move to the thymus where they develop into mature T cells (Res and Spits, 1999, pp. 39-46). The central function of the adaptive immune system is when the TCR:CD3 complex recognizes a foreign peptide presented by a major histocompatibility (MHC) molecule and binds to it, initiating a specific immune response. T cell signaling is

transduced not by the TCR itself but by invariant proteins called CD3 and ζζ within the TCR complex. The immunoreceptor tyrosine-based activation motifs (ITAMs) located in the cytoplasmic domains of CD3 and ζ chains are phosphorylated, resulting in activation of downstream signaling molecules and initiation of signaling complexes (Figure 1).

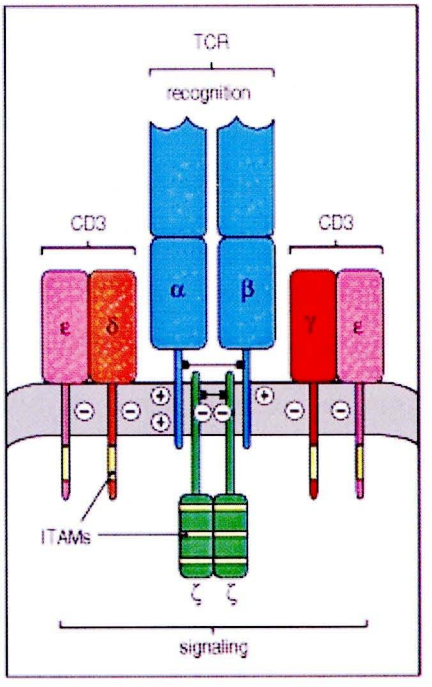

Figure 1 The T-Cell Receptor Complex is Made up of Antigen-Recognition Proteins and Invariant Signaling Proteins.

Source: Janeway, et al., 2001, p. 229

The major issue in T cell activation is to understand how the ligand-binding component initiates, or triggers, a signal from TCRs upon appropriate interaction with MHC-peptide complex. Numerous proteins and protein complexes constitute the T cell signaling pathway. Adaptor protein Nck has been reported that it acts as a link between extracellular and intracellular signaling molecules and cytoskeleton (Lehmann, Riethmuller and Johnson, 1990, p. 1048; Park, 1997, pp. 231-236; Ullrich and Schlessinger, 1990, pp. 203-212). In T cells, Nck links the TCR:CD3 complex

with proteins that mediate signaling and cytoskeleton rearrangement. Several studies have shown that Nck involves intracellular T cell signaling in both an ITAM-requiring mechanism (Alberola-Ila, et al., 1997, pp. 125-154; Bubeck, et al., 1998, pp. 607-616; Galisteo, et al., 1996, pp. 20997-21000; Lin and Weiss, 2001, pp. 243-244) and a non-ITAM-requiring mechanism (Gil, et al., 2002, pp. 901-912; Gil, et al., 2005, pp. 517-522). In contrast, Szymczak et al. have shown that interaction between the CD3ε proline-rich sequence (PRS) and Nck is not required for T cell development and function (Szymczak, et al., 2005, pp. 270-275). Moreover, the Nck-CD3ε is also capable of downregulating T cell activation by inhibiting of CD3ε ITAM phosphorylation and/or reducing TCR cell surface expression (Takeuchi, et al., 2008, pp. 704-716).

To date, the function of Nck in T cell receptor signaling have not been entirely established. Nck is involved in the activation of downstream members of the DNA damage cascade and cell-cycle arrest (Kremer, Adang, and Macara, 2007, pp. 837-850), but the relationship of Nck1 with apoptosis remains to be defined. Nck has been shown to be essential in T cell proliferation because Nck-deficient murine T cells fail to proliferate upon TCR-mediated stimulation (Roy, et al., 2010, pp. 15529-15534). In contrast, the proliferative response of CD3ε PRS motif-deficient murine T cells to strong antigens was normal (Szymczak, et al., 2005, pp. 270-275). Thus, additional studies must be performed to address the role of Nck on T cell proliferation. There have been few reports on the association of T cell activation markers, such as CD69 and Interleukin 2 (IL-2), and Nck1. CD69 expression represents one of the earliest available markers for T cell activation. CD69 acts as a potent signal-transmitting receptor on lymphocytes. It is involved in cytokine gene regulation and cell migration upon lymphocyte activation (Shiow, et al., 2006, pp. 540-544). IL-2 is a critical autocrine growth factor upregulated upon T cell activation that is required for the clonal expansion of T cells (Gillis and Watson, 1980, pp. 1709-1719). Nck-CD3ε interaction-inducing (OKT3) Ab can induce both IL-2 release and CD69 expression in Jurkat cells (Gil, et al., 2002, pp. 901-912). However, one functional study has shown that there was no difference observed in CD69 expression after staphylococcal enterotoxin B (SEB) stimulation in T cells from mice expressing wild-type or CD3ε PRS mutation (Szymczak, et al., 2005, pp. 270-275). Therefore, the present study is

aimed to assess the role of Nck in T cell apoptosis, T cell proliferation, as well as T cell receptor activation and function.

Objectives of the Study

The purpose of this study is to investigate the function of adaptor protein Nck1 in T cell receptor activation and function including: 1) the proliferation and apoptosis of Nck1 knockdown Jurkat T cells using RNA silencing method, 2) CD69 expression of Nck1 knockdown Jurkat T cells, and 3) the association of Nck1 in Jurkat T cell IL-2 cytokine production.

Hypothesis of the Study

Nck adaptor protein has been reported to play an important role in linking the TCR:CD3 complex with proteins that mediate T cell activation. However, the role of Nck-CD3ε interaction is still controversial, as it has been suggested that the interaction is not required for T cell development and function, or is required for signal amplification and ITAM phosphorylation following weak TCR ligation. Thus, we hypothesize that Nck1 may not be essential in TCR activation.

1. The proliferation and apoptosis of Nck1 knockdown Jurkat T cells is not different from negative-control Jurkat T cells.

2. CD69 expression of Nck1 knockdown Jurkat T cells is not different from negative-control Jurkat T cells.

3. IL-2 production from Nck1 knockdown Jurkat T cells is not different from negative-control Jurkat T cells.

Scopes of the Study

Jurkat T cells (clone E6-1), a human leukemic T-cell line, originally obtained from the American Type Culture Collection (Rockville, MD, USA) will be used to study the role of Nck1 in T cell activation and function. These include investigation of the proliferation and apoptosis of Nck1 knockdown Jurkat T cells using RNA silencing method, CD69 expression of Nck1 knockdown Jurkat T cells using immunofluorescence flow cytometry analysis, and study the association of Nck1 in IL-2 cytokine production using ELISA.

Expected Benefits

This study will answer the question on the necessary role of Nck1 in the activation and function of human T cells. The information obtained will help to understand, at least in part, the involvement of Nck1 in the control of T cells responses. The subsequent applications may contribute to find new approach for diagnosis or treatment methods in immunopathologic conditions.