CHAPTER I

INTRODUCTION

1. Principle and rationale

Production of antibodies by B cells is essential for the successful removal of pathogens. Conversely, there are a number of autoimmune diseases, in which pathogenesis is mediated by autoantibody. Soluble antibodies or immune complexes are pathological in many autoimmune diseases, including myasthenia gravis, Graves' disease, systemic lupus erythematosus and rheumatoid arthritis. In addition, overproduction of one type of antibody in hematopoietic malignancy, such as myeloma, causes diseases. Studying the regulation and requirement for B cell development will help to regulate the production of antibodies in the correct form, i.e. to suppress or stimulate the humoral immune system.

Differentiation of B cells is divided in to 2 phases. Early differentiation from stem cells to mature B cells takes place in the bone marrow generative lymphoid organ, while the terminal differentiation of B cell is raised when encountering specific antigen in the secondary lymphoid organ. In the early stage of B cell development, the key is to generate B cell receptors by rearrangement of Ig genes. After the B cell receptor generation and selection process in bone marrow, the immature B cells migrate to peripheral lymphoid tissues and continue to develop to mature B cells. Upon encountering specific antigen, the mature B cell differentiates into antibody producing plasma cells. Both early and terminal phases of B cell differentiation are regulated by several transcription factors along the way. The transcription factors that

are involved in B cell differentiation are, for example, PAX-5, c-myc, Bcl-6, IRF-4, XBP-1 and Blimp-1 (1,2). These transcription factors express at different stages of B cell development and have cross regulation between each other as show in Figure 1.

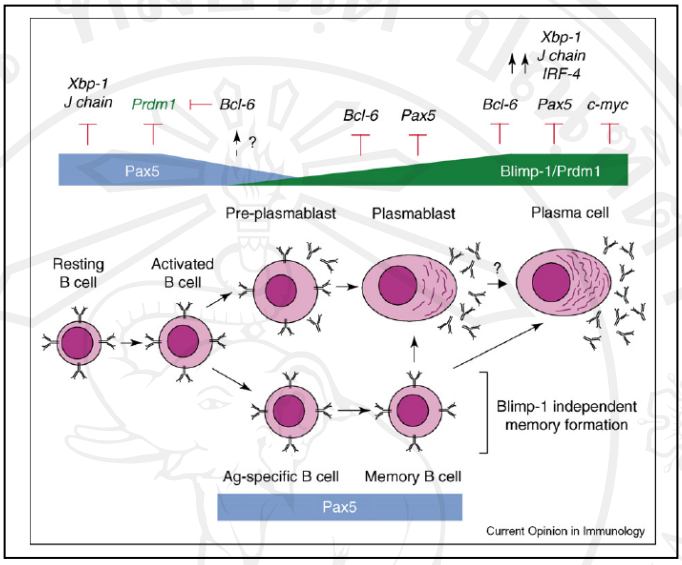


Figure 1. Blimp-1 controls plasma cell differentiation. The stages of cellular differentiation from a resting B cell to an antibody-secreting plasma cell are indicated, as are the relative levels of Pax5, Blimp-1 and key target genes (2).

During the differentiation of B cells into plasma cells, the two main lineage transcription factors; PAX-5 and Blimp-1, counterbalance each other (3). *Pax5* is a transcription factor that is critical for commitment to and maintenance of the B-lineage and for B cell function. Pax5 activates genes involved in B cell identity, and suppresses genes involved in antibody secretion such as J chain, IgH, and XBP-1 (4). Upon the activation of resting B cells, B cells are differentiated into preplasma blast, plasmablast and finally plasma cells (5). During the differentiation into plasma cells, Blimp-1 expression increases, and genes expressed in B cell phase and involved in

cell cycles are inhibited (6). Blimp-1 has a broad role in promoting plasma cell differentiation by repressing the characteristic gene expression signature of mature B cells. Thus, Blimp-1 is considered a master regulator of terminal B-cell differentiation (1, 7).

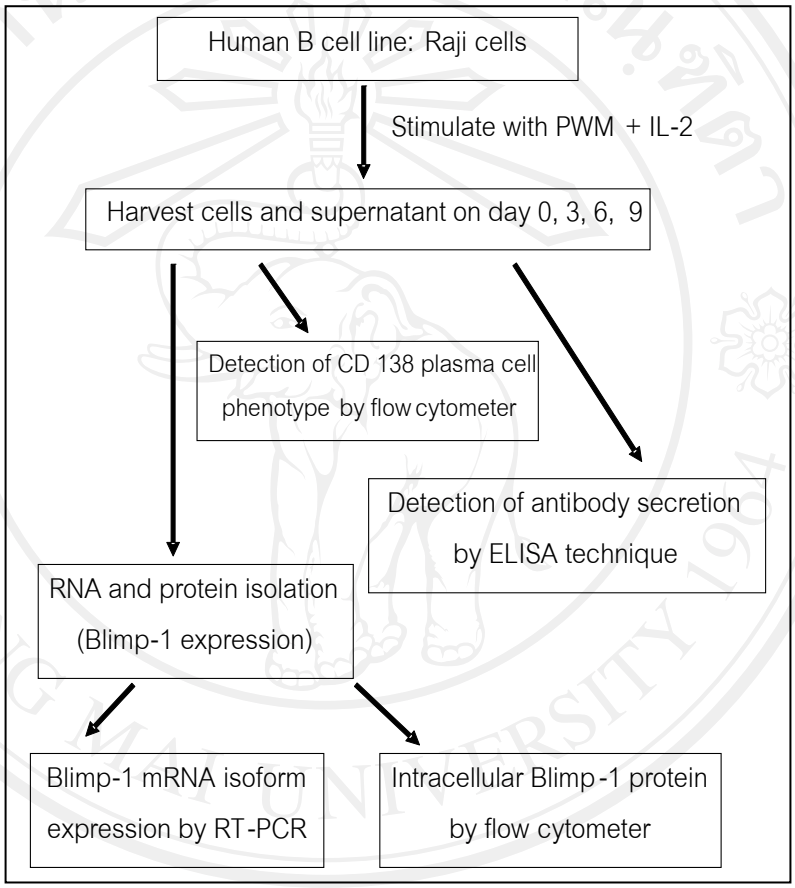
In early reports, Blimp-1 was only found in the plasma cell stage of B cell differentiation. With more sensitive methods, Blimp-1 was later demonstrated in B mature and pre-B cell stage (Laddawan Thesis, 2005). The finding of Blimp-1 at the developmental stage of B cells preceding plasma cells is of interest, since Blimp-1 directed cells to exit cell cycles, but these cells are highly proliferative. The role of Blimp-1 at these early developmental stages of B cells has not been reported.

Several Blimp-1 mRNA isoforms have been found in mouse plasmacytoma cells. Northern blot revealed 3 isoforms of Blimp-1 mRNA generated by differential usage of polyadenylation sites. However, RT-PCR revealed a minor isoform that resulted from differential splicing of exon7 (Δ7 isoform). Thus, the protein encoded by this Δ7 isoform is unable to bind DNA (8). As Blimp-1 is found in the pre and B cell stage (9, 10), it is of interest to consider that expression of Blimp-1 might not be sufficient to fulfill its function. However, the isoforms might actually regulate the function of Blimp-1, as found in several transcription factors such as XBP-1 (11), Ikaros family (12, 13).

In this study, the existence of the Δ7 isoform in human cells will be confirmed first, and then the levels of full-length and Δ7 isoforms of Blimp-1 compared during the differentiation of B cells into antibody secreting plasma cells.

To determine the kinetic expression of the full length and Δ7 Blimp-1 isoforms at the mRNA and protein level and correlation with their function in differentiation of B cells to plasma cells

Research design, scope and methods



1. Cell line used: A human B cell line, Raji cell was used in this study. Raji cell line is a mature B cell line derived from a Burkitt's lymphoma patient. It expresses surface IgM.
2. Stimulation of B cells: Raji cell line was stimulated for 9 days with pokeweed mitogen (PWM) in combination with IL-2.

3. Verification of B cell activation:

    a. Surface expression of a plasma cell marker: CD138 was stained and analyzed at different time intervals by flow cytometer.

    b. Antibody secretion in the supernatants was measured by ELISA technique.

4. Blimp-1 gene expression:

    a. Total RNA was isolated from cell culture by using RNA isolation kit.

    b. RNA was reverse transcribed in to cDNA by using first stand cDNA synthesis kit.

    c. Kinetic expression of the full length and Δ7 isoform of Blimp-1 was determined by PCR. GAPDH, a house keeping gene was used as a control.

5. Intracellular Blimp-1 protein expression: Blimp-1 protein was stained with goat anti Blimp-1 antibody and analysed by flow cytometer.