CHAPTER II

LITERATURE REVIEW

Blimp-1 is a transcriptional repressor containing five zinc finger motifs that confer DNA binding ability (13). Mouse Blimp-1 and its human orthologue, PRDI-BF1 (14), have long been implicated in the differentiation of B cells into plasma cells. Mouse Blimp-1 contains 856 amino acids and is predicted to be a 98 kD protein. Human Blimp-1 has 789 amino acids and is predicted to have a molecular weight of 87 kD. The mouse and human Blimp-1 proteins are highly homologous and are interchangeable in function assays. Blimp-1 has been the common name used for both the human and mouse protein (8).

Within the B-cell lineage, Blimp-1 is specifically expressed in all antibody secreting cells including plasma cells and plasmablasts (15). Ectopic expression of Blimp-1 is sufficient to drive mature B cells towards the antibody producing cell phase (16), whereas gene-knockout studies have demonstrated that Blimp-1 is also required for maintaining the plasma cell phase (17). Originally, three known targets of Blimp-1-dependent repression explained important aspects of the plasma cell phenotype, and that is how a single transcriptional repressor drive plasma cell developed (18). Thus Blimp-1 is called a master regulator of terminal B cell differentiation. Blimp-1 directly represses at least 3 target genes in the B cell lineage as follows:

1. Repression of *c-Myc* transcription correlates with the cessation of cell division, which is characteristic of B cell terminal differentiation (19).
2. Repression of promoter III of CIITA, a transcription co-activator and thus leading to the downregulation of class II MHC genes (20, 21).
3. Repression of the promoters of Pax-5, and potentially of Bcl6, thereby providing the mechanism by which its induction leads to antibody secreting cell differentiation and loss of the B cell phenotype (22).

Pax-5 gene is required to maintain B cell identity and repress the expression of XBP-1. XBP-1 in itself plays an essential role in plasma cell differentiation, and directly induces transcription of many genes necessary for ER function and protein secretion (10). The diagram below shows the cross regulation between the 3 transcription factors, Blimp-1, Pax-5 and XBP-1 in the B cell terminal differentiation (23).

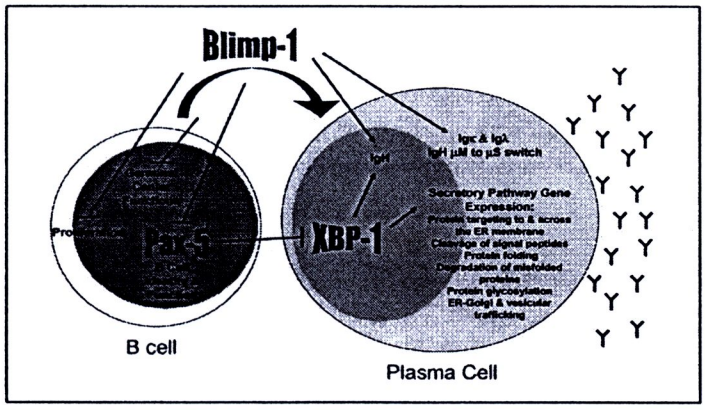

Figure 2. Model for the role of Blimp-1 in repressing B cell fate and inducing plasma cell fate. Blimp-1, through direct and indirect action, represses genes involved in

proliferation, germinal center functions, and B cell identity while inducing genes involved in the Ig secretory pathway (23).

Repression of c-*myc*, *CIITA*, and *PAX5* alone is not sufficient to explain the entire program of plasma cell development activated by Blimp-1. Microarray experiments indicate that Blimp-1 regulates a large set of genes that constitute a significant part of the plasma cell expression signature. Blimp-1 represses 228 genes and induces 32 genes in multiple system using human B cell lines. This analysis revealed both direct and indirect Blimp-1 targets (24, 25). Three main programs of gene expression were altered by Blimp-1 are 1. a proliferative program including c-Myc, E2F1, and other genes required for entry into cell cycle and cell division was repressed (26). 2. a program involved in Ig secretion, including J chain, XBP-1 as well as IgH and IgL chain genes. 3. an extensive program of gene expression characteristic of activated or GC B cells was repressed, including genes encoding the critical transcription factors Pax-5 and Bcl-6 (24).

Organization of the Blimp-1 genes in mouse and human

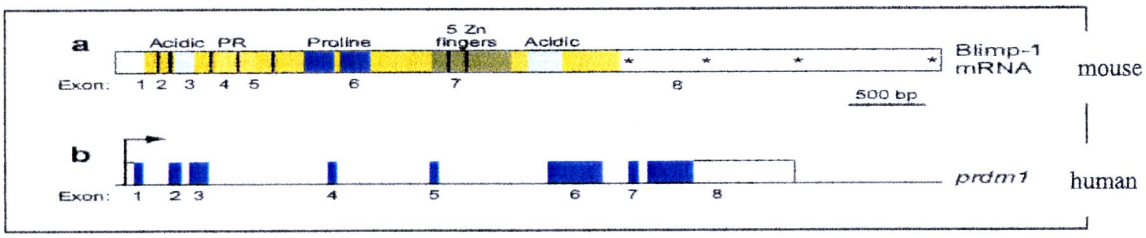

Figure 3. Murine Blimp-1 mRNA and the human Blimp-1, *prdm1* gene (8)

Blimp-1 is a zinc finger-containing protein, which binds to DNA sequence and is characteristic of many transcription factors. Murine Blimp-1 gene contains 8 exons, which consist of functional domains: acidic, PR, proline-rich and the 5 zinc finger domains. The PR domain is encoded in the exon 4 and 5, while the zinc finger

domains are encoded in exon 6, 7 and 8. Zinger finger 1, one of the 2 critical zinc fingers for Blimp-1's ability to bind DNA, is encoded in exon 6 and 7. Three major Blimp-1 mRNA isoforms result from the use of different polyadenylation sites were identified by using Northern blot analysis. These isoforms encode the same protein. In addition, RT-PCR analysis of Blimp-1 mRNA reveals the minor isoform lacking exon7 (Δ7 isoform) (8).

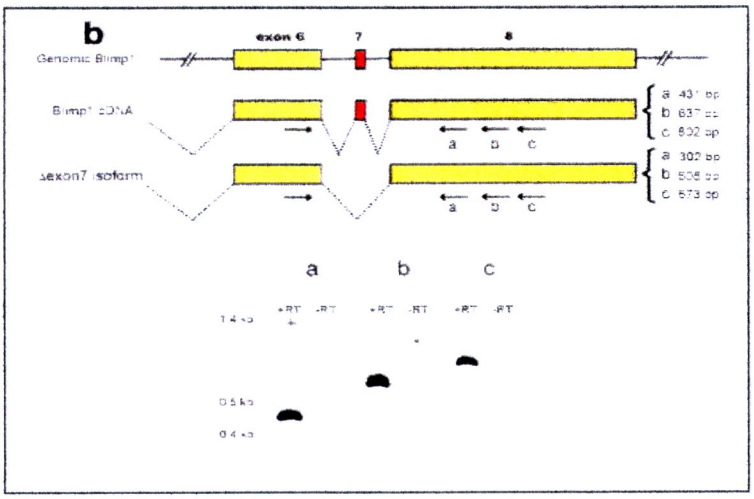

Figure 4. RT-PCR analysis of mouse Blimp-1 mRNA. The diagram show the possible alternative splicing involving exon 7 and the rationale for PCR products design. The expected sizes of PCR products from cloned cDNA and potential Δ7 isoform are indicated (8).

Exon 7 encodes part of zinc finger 1, all of zinc finger 2 and part of zinc finger 3. The exon 7 deleted protein (Δ7 protein) is incapable of binding DNA and probably does not function as a transcriptional repressor. It is expressed at less than 10% of the total Blimp-1 mRNA. Zinc fingers 1 and 2 are also important for Blimp-1 to associate

with histone methyltransferase G9a (27, 28), and therefore the Δ7 protein is expected to be incapable of associating with G9a as well. This minor isoform might therefore interfere with the function of full length Blimp-1. The functional significance of the Δ7 isoform is still opened to investigate. Both human and mouse Blimp-1 gene organization show a very similar exon-intron organization, except the 5'UT in human is encoded in only one exon, while that in mouse is encoded in 2 exons. Thus, mouse exon7 is comparable to exon 6 in human. In this study, the Δ7 isoform, instead of exon 6, is used for human as originally applied to mouse.

Regulation of cell function by isoforms has been reported in transcription factors such as XBP-1 (10), Ikaros family (20). XBP-1 has 2 isoforms; spliced and unspliced. The spliced isoform of XBP-1 is active, whereas the unspliced one is not. Plasma cell differentiation is dependent on UPR induced IRE1α splicing of XBP-1. The generation of spliced XBP-1 protein requires stimuli that evoke the UPR through the accumulation of Ig heavy chain in ER. The spliced XBP-1 protein, a potent transactivator, then translocates into the nucleus, where it binds to its target sequence for regulating UPR gene expression (29).

The Blimp-1 isoform has demonstrated relevance as a PRDI-BF1β isoform in patients with multiple myeloma (30). This isoform of human Blimp-1 is generated by transcription initiation at an alternative promoter located at 5' of exon 4 of human *PRDM1* (synonym of Blimp-1). The PRDI-BF1 deleted protein (called PRDI-BF1β) lacks 101 amino acids that comprise most of the regulatory domain. Since this molecule contains the DNA-binding domain, but bears a disrupted regulatory domain, PRDI-BF1β might behave as an inhibitor of functional PRDI-BF1. Interestingly, the transcription level for this isoform was markedly low in normal human plasma cell,

but far higher in malignant cells, thus suggesting interference with the normal cell differentiation program (28).

Compared to PRDI-BF1β isoform, the Δ7 Blimp-1 and full length isoforms have been found to express in many cell types including non B cell lineage and normal cells. Thus, the Δ7 Blimp-1 does not result from an aberrant process, but is a normal isoform that has expressed physiologically. Interestingly, steady state mRNA encoding Δ7 blimp-1 showed an expression lower than the encoding full length protein. Thus, merely a larger amount of full length rather than Δ7 Blimp-1 isoform might not be sufficient, but a certain high level of full length is needed to drive the function of Blimp-1. It would be interesting to hypothesize that the Δ7 Blimp-1 isoform interferes with the function of the full length Blimp-1. Since the Δ7 Blimp-1 isoform is unable to bind DNA, it would likely interfere with the full length Blimp-1 and render it unable to bind DNA.

Understanding the regulation and functions of key transcription factors such as Blimp-1, helps in elucidating molecular mechanisms that function during B cell development to provide specific antibodies in response to appropriate stimuli. The role of Blimp-1 at these early developmental stages of B cells is not yet known and neither is the function of the Δ7 isoform. The precise role of Δ7 isoform in plasmacytic differentiation and its effect on the activity of Blimp-1 has not been performed. Thus, the role of Blimp-1 isoforms, within regulation of their function, is an interesting area in which to investigate more precisely, in order to offer novel therapeutics for improving vaccine efficacy or treatment of autoantibody-mediated or malignant diseases, such as multiple myeloma.