CHAPTER VI
DISCUSSION

Blimp-1 is a Zinc finger-containing protein encoded by *prdm1*. The organization of human Blimp-1 gene is similar to that of the mouse. In mouse, it contains 8 exons and the five zinc finger domains critical for DNA binding are encoded in exon 6 and 7. Several Blimp-1 mRNA isoforms have been found in mouse plasmacytoma cells. 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.

As Blimp-1 was later found in many cell types other than plasma cells, it was questioned how Blimp-1 functions in different types of cells or are different accessory molecules needed in association with Blimp-1 for specific cell types or stages. According to the finding of a minor isoform, Δ7 (8), it was speculated that full length was a functional isoform and expressed when needed, while Δ7 isoform, a non functional isoform, expresses in a resting stage. Surprisingly, study in bone marrow mononuclear cells of patients with leukemia and lymphoma showed that all patients expressed 2 isoforms, the full length and Δ7. Most of the patients presented more deleted Δ7 than full length isoform of Blimp-1, except 4 patients who expressed only Δ7 isoform. As the majority of patients expressed both isoforms, it was therefore concluded that a general expression profile should express both isoforms, and not one type separately, as speculated (33).

In further study, several human B (Nalm-6: pre B cell line, Raji, Ramos: mature B cell lines) and non B cell lineages (U937: monocytic cell line, A549: lung fibroblast, KU562-F: basophilic cell line, LP-1, U266, RPMI8266: plasma cell lines), were then investigated for profile of expression. Initially, it was speculated that the Δ7 isoform should be higher than full length in cells that did not need Blimp-1 such as a pre B cell or mature B cell lines. All types of cell lines showed an unexpected difference in full length, more than Δ7 isoform, and the highest ratio was in plasma cell lines (Pachana. Termpaper, 2008).

As Blimp-1 full length was found in the pre (Nalm 6) and B cell stage (Raji and Ramos), it was considered that expression of full length Blimp-1 over Δ7 isoforms might be insufficient in fulfilling its function and a very high ratio is needed to drive B cells into plasma cells. These were shown in 3 plasma cell lines (LP-1, U-266, RPMI-8266).

As the study of Blimp-1 isoform expression was compared in different cell types, monitoring the kinetic expression of Blimp-1 isoforms during the terminal B cell differentiation in a single cell type would be a better way to confirm the data.

Raji cells, a mature B cell line, were used in this study. LP-1, a plasma cell line, secreting IgM antibody was used as a positive control for the experiments. Raji cells were stimulated with 2.5 μg/ml of pokeweed mitogen in combination with 20 unit/ml of IL-2. The cells and supernatants were harvested on day 0, 3, 6 and 9 for the following studies; 1) expression of CD138, a plasma cell marker using flow cytometry 2) antibody secretion by using sandwich ELISA, and 3) kinetic expression of a)

Blimp-1 mRNA isoforms by RT-PCR and b) intracellular Blimp-1 protein by flow cytometry.

Optimal conditions for different techniques used in this study were performed. For the detection of antibody secretion in cell supernatants by ELISA, the 0.2 ug/ml of goat anti IgG+IgM coated plate, and peroxidase anti human IgG+IgM dilution of 1:20,000 could detect standard IgM as low as 6.25 pg/ml sensitivity. The mouse anti CD138 dilution of 1: 10 and FITC rabbit anti mouse IgG1 dilution of 1:100 were used for staining plasma cell phenotype. For the detection of Blimp-1 expression, by flow cytometer, the goat anti Blimp-1 dilution of 1:50 and FITC conjugated rabbit anti goat IgG at 1:200 gave the optimal staining. Comparing the permeability reagents used for intracellular Blimp-1 protein, both 0.1% Triton X-100 and cold methanol showed a similar pattern when methanol was used.

Stimulation of Raji B cells was fulfilled, as the cells showed differentiation to plasma cells, which was proved by the expression of CD138 and antibody secretion. The kinetic expression ratios of full length and Δ7 mRNA isoforms were monitored by RT-PCR. The primers were designed to bind between the exon 6 and exon 8 so that both full length and Δ7 mRNA could be detected. The unstimulated B cells showed little increase in the ratio of full length and Δ7 mRNA on day 3, with no further change, while stimulated Raji cells increased rapidly from an average 1.8 on day 0 to the highest ratio of an average 14.5 on day 9. Although, this ratio was not as high as found in plasma cell lines but they were about 5-7 times more than the resting stage of different cell types. When Blimp-1 protein was investigated, both unstimulated and stimulated Raji B cells expressed intracellular Blimp-1 at the basal level and slightly higher expression after stimulation. This result correlated with the expression of

Blimp-1 at the mRNA level, which indicated how the 2 isoforms related to the function. It was considered that merely a higher expression of full length rather than exon 7 deleted isoforms might not be sufficient, but a certain high level of full length isoform is needed to drive the function of Blimp-1.

The staining of intracellular Blimp-1 protein showed very low expression by flow cytometry and, therefore, it was very difficult to follow the kinetic expression. This was due to either a limitation quality of anti Blimp-1 antibody, which was a polyclonal antibody, or a very low amount of protein like transcription factors or both factors. In this study, Raji B cells showed an unexpected expression of Blimp-1 protein even in resting or unstimulating stage. However, it was not concluded whether or not it was the functional full length protein. Unfortunately, so far there is no available anti-Blimp-1 antibody that can distinguish the full length and Δ7 proteins. This would be a very useful tool for following up where the 2 isoforms are located in cells as well as the level of expression at time intervals.

Regulation of cell function by isoforms has been reported in transcription factors such as XBP-1(11). 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 spliced XBP-1 protein, a potent transactivator, then translocates into the nucleus, where it binds to its target sequence for regulating UPR gene expression. The XBP-1 spliced isoform has 26 base pairs deleted, in which a frame shift leads to a stop codon in translation and this leads to a non functional shortage protein of a 261 amino acid, 33 kDa rather than a 371 amino acid, 54 kDa, XBP-1spliced isoform. The Blimp-1 isoforms regulate their function differently, in that the Δ7 has 129 base pairs deleted,

in which the protein can be translated without frame shift. It could be the same as a full length sequence, but the protein is only 43 amino acids shorter. There is no Blimp-1 antibody available to distinguish between the full length and Δ7 isoform proteins for further investigation.

Besides the Δ7 Blimp-1, the Blimp-1 isoform has demonstrated relevance as a PRDI-BF1β (synonym of Blimp-1) isoform in patients with multiple myeloma (30). The PRDI-BF1 deleted protein (called PRDI-BF1β) lacks 101 amino acids that comprise most of the regulatory domain. 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.

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.

Since the Δ7 isoform is unable to bind DNA, it is likely to interfere with the full length Blimp-1 by poisoning it rather than competing for the regulatory sequence of their target genes. The Δ7 protein may form a dimer with the full length protein and render it unable to bind DNA, as shown in another zinc-finger protein, Ikaros (12, 13). It has been shown that the transcriptional repression activity of Blimp-1 depends upon

its association with protein encoded by Grouch-related genes, histone deacetylases, and the G9a histone H3 methyl transferase (27, 28).

Ectopic Blimp-1Δ7 expression in WEHI 231 cells, a Blimp-1 negative murine lymphoma line, showed that Δ7 and Blimp-1 proteins interact with themselves as well as form a heterodimer with each other. The Blimp-1Δ7 could be demonstrated in both cytosol and sub-nucleus localization. Accordingly, it has been suggested that the auto-regulatory mechanism of Blimp-1 activates by which Δ7 Blimp-1 interferes with endogenous Blimp-1 expression (32).

Blimp-1 is not only required in the plasma cell differentiation, but it is also needed to maintain long-lived plasma cells (34). Treatment of leukemia and autoimmune diseases by targeting B cells has not always succeeded, as long-lived plasma cells are not targeted. Interference of plasma cells by targeting at Blimp-1 isoforms would be considered for therapeutic proposes.

This study helps understanding of how transcription factor Blimp-1 isoforms regulate the function of B cells, especially the termination of differentiation into plasma cells. It provides insight into transcriptional controls operating in cells responsible for humoral immunity. The therapeutic implication of having molecular information enables manipulation of a humoral response for different purposes including vaccination, autoimmune pathology and myeloma malignancies. If the level of Blimp-1 isoforms regulates the function of Blimp-1, these isoforms can be a target in the treatment of autoimmune disease or myeloma.