CHAPTER VII

SUMMARY

In this thesis, the kinetic expression of Δ7 Blimp-1 and full length isoforms during the differentiation of B cells to plasma cells was determined in Raji cells, a human mature B cell line. 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 mg/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. The results were as follows:

1. For the detection of antibody secretion in cell supernatants by ELISA, the 0.2 mg/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.
2. For the verification of plasma cell phenotype, by flow cytometer, the mouse anti CD138 dilution of 1:10 and FITC rabbit anti mouse dilution of 1:100 were used for staining both stimulated and resting Raji cells.
3. 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 optimal staining. When comparing the permeability reagents used for intracellular Blimp-1 protein, both 0.1% Triton X and cold methanol showed a similar pattern when methanol was used.

4. 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.
5. The kinetic expression ratios of full length and D7 mRNA isoforms were monitored by RT-PCR. The unstimulated B cells showed little increase in the ratio of full length and D7 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 that found in plasma cell lines, they were about 5-7 times greater than at the resting stage of different cell types.
6. It was therefore considered that expression of full length Blimp-1 over Δ7 isoforms was insufficient in fulfilling its function and a very high ratio is needed to drive B cells into plasma cells.
7. 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.
8. Since the D7 isoform is unable to bind DNA, it is likely to interfere with the full length Blimp-1 by forming a dimer with itself or the full length protein and render it unable to bind DNA or other accessory molecules such as Grouch-

related genes, histone deacetylases, or the G9a histone H3 methyl transferase and the function of Blimp-1 is blocked.

9. Understanding the mechanism how D7 isoform related to the Blimp-1 function, it could be used for therapeutic application for autoimmune diseases and multiple myeloma by interfering the antibody producing cell development.