CHAPTER V

CONCLUSION

Hb Bart's hydrops fetalis syndrome is perilous not only to the fetus but also the pregnant mother. This disease, however, can be prevented by identifying its carriers and inform them to its harmfulness. The aim of this study was to produce the monoclonal antibodies against Hb Bart's and embryonic ζ globin chain that contained in Hb Bart's hydrops fetalis hemolysate. These mAbs will be useful for development of the immunodiagnostic for screening of α-thalassemia 1 carrier in the future. To achieve this purpose, Hb Bart's hydrops hemolysate and purified Hb Portland were used as the immunogens for immunization of BALB/c mice.

Hemolysate of Hb Bart's hydrops fetalis were immunized to BALB/c mice without adjuvant to avoid fibroblast overgrows. After appearing of the specific antibodies, splenocytes of the immunized mice were fused with myeloma cells to generate the hybridoma cells. The produced monoclonal antibodies were characterized for their specificity by ELISA and Western blotting using different types of hemolysates, purified hemoglobin A, A_2, E and F and various globin chains including ζ, $^{A}\gamma$, $^{G}\gamma$, α, β and δ globins. By this immunization method, we could not produce monoclonal antibody specific for Hb Bart's. The produced monoclonal antibodies HB1, HB2, HB3, HB4 are IgM isotype and react to all type of hemoglobins.

Purified Hb Portland was also immunized to BALB/c mouse by using Freund's adjuvant to enhance the antibodies response. After appearance of

the specific antibodies, mouse was subjected to cell fusion and the generated mAbs were characterized for their specificity with the same antigen and methods as the mice that immunized with Hb Bart's hydrops fetalis hemolysate. By this immunization method, a monoclonal antibody against embryonic ζ globin chain was generated (mAb Thal-PL1). This mAb is IgG1 isotype and specifically reacted to ζ-globin chain without cross reaction with the other globin chains.

The generated anti-hemoglobin mAbs, in this study, may be useful in the development of immunological test for occult blood diagnostic in stool and urine. Anti-ζ globin chain mAb will be useful in the development of immunodiagnostic test kits for mass screening of α-thalassemia 1 (SEA type) carriers which are highly incidence in Thailand. This test kit may combine with anti-Hb Bart's antibodies to screen the other types of α-thalassemia carriers and, finally, reduce the incident of Hb Bart's hydrops fetalis cases in the future.