1. Abstract

Plasmodium yoelli sporozoite surface protein 2 (*py*SSP2) is considered as an important antigen for protection studies in malaria vaccine development. For the liver stage protection, anti-*py*SSP2 cytotoxic T lymphocyte (CTL) activity against SSP2 was investigated in BALB/c mice. Radiation induced leukaemia (RL♂) cells were transfected with retrovirus encoding SSP2 gene and stably expressing cell line was selected by puromycin. In addition, dividing bone marrow cells from BALB/c mice were cultured for 7 days with Growing marrow cell stimulating factor (GMCSF) and Interleukin 4 (IL-4) and transfected with retrovirus encoding SSP2 gene *in vitro*. These dividing bone marrow cells were infected with retrovirus expressing SSP2 on 5^{th}, 6^{th} and 7^{th} days of culture, by prolonged centrifugation for 1 hour at 32^0C. Cultured bone marrow cells demonstrated 70-80% of dendritic cells (DCs) with high CD11c, CD80, CD86 and MHC class I (I-K^d) expression.

Degree of SSP2 expression in transfected DCs and RL♂ cells was assessed by immuno-precipitation of SSP2 protein with mouse *py*SSP2 antibody. Results showed that SSP2 protein was clearly expressed in both types of transfected cells (DCs and RL♂ tumor cells) until the day 6 since transfection. Transfection efficacy of DCs was also assessed using retrovirus shuttled with green fluorescence vector (EGFP). Sixty four percent of CD11c expressing transfected DCs showed green fluorescence. Both SSP2 and EGFP transfected DCs had prolonged expression of the relevant engineered genes until day 6 since the transfection.

To analyze the antigen presentation, mice were immunized with either genetically engineered RL♂ tumor cells or bone marrow derived DCs expressing pySSP2 peptides. For the identification of SSP2 MHC class I peptide motif, BALB/c mice were immunized with RL♂ tumor cells expressing SSP2 for three times at weekly intervals. Seven days after last immunization, spleen cells containing CTLs were induced with SSP2 peptides *in vitro* and

IFN-γ secretion was assessed by ELISPOT and ELISA assays. Immunization of SSP2 encoding RL♂ tumor cells resulted in identification of two new MHC class I K^d restricted CTLs binding motifs (A and C) in SSP2 protein. A and C peptide specific CTLs from spleen cells secreted significant amount of IFN-γ over the recognition of relevant peptides on tumor target cells. To assess the capacity of antigen presentation in genetically engineered dendritic cells, BALB/c mice were immunized with retrovirus infected DCs similar to the RL♂ tumor cell immunization. DC immunization also resulted in recognition of the two specific MHC class I (I-K^d) restricted binding motifs, A and C, in *py*SSP2 protein. Both A and C SSP2 peptides induced antigen specific IFN-γ secreting cytolytic CTLs upon antigen recognition on target cells. Immunization of tumor cells or DCs encoding SSP2 gene resulted in identification of two K^d restricted CTL epitopes and induction of IFN-γ secreting cytolytic $CD8^+$ T cells. Taken together, these data indicate that bone marrow DCs which were genetically modified by prolonged centrifugation strongly enhanced the antigen presentation and the induction of antigen specific CTLs in mice.