Abstract

Abrus agglutinin, isolated from the seed kernel of *Abrus precatorius,* is a heterodimeric (two A chains and two B chains) glycoprotein of Ribosome Inhibitory Protein (RIP) II family. The protein has molecular weight 134 kDa and carbohydrate specificity towards [Gal β (1-3) GalNAc]. Previous studies showed that both native agglutinin (NA) and heat denatured agglutinin (HDA) induced high NO, IL1 and TNF productions from peritoneal macrophages and enhanced murine splenocytes and thymocytes proliferation *in vitro*. The cytokines released by stimulated splenocytes was biased to Th-1 types of immune response. The cytotoxicity effects of NK cells were also enhanced when stimulated by NA and HDA. Based on this background information, the present study aimed to demonstrate the cell specific immunomodulaory effects of NA and HDA on the differentiation and activation of splenocytes. There was an increase in the expression of both CD3 and CD19 which proved that both B and T sub-population of cells were proliferated following the stimulation by NA and HDA. There were greater expressions of lymphocyte activation markers CD25 and CD71 by splenocytes on activation by NA and HDA. Both NA and HDA induced proliferation of the individual B and T cells isolated from splenocytes by magnetic activated cell sorter, whereas, neither A nor B chain isolated from *Abrus* agglutinin could stimulate splenocytes proliferation and NO production from macrophages. The *Abrus* agglutinin was reported to have anticancer effects in S-180 bearing mice model, but its mode of action is not clearly known. In the present study, the anti-tumor activity of *Abrus* agglutinin (NA, HDA) was evaluated in murine Dalton's lymphoma ascites tumorogenic model. This study showed that treatment with both NA and HDA were able to decrease the tumor cell number *in vivo* and significantly increased median survival time. *Abrus* agglutinin (NA, HDA) also inhibited the growth of Dalton's lymphoma ascites cells *in vitro* at the concentrations of 1 μg /ml and above, whereas, *in vitro* stimulation of peritoneal macrophages and spleen derived NK cells by *Abrus* agglutinin (NA, HDA) at lower concentration (~1ng/ml) demonstrated cytotoxicity against Dalton's lymphoma ascites cells. The cell cycle analysis of Dalton's lymphoma ascites cells showed the increased number of cells in Pro-G_o/G_1 phase for *in vitro* and *in vivo* treatments. *Abrus* agglutinin (NA, HDA) at non-toxic concentrations was able to elicit anti-

tumor effect in Dalton's lymphoma bearing mice by stimulating the innate immune system and Th1 type immunomodulation. Attempts were also undertaken to evaluate the potentials of NA and HDA in chemoimmunotherapy regimen in Dalton's lymphoma bearing mouse model. Combined treatment by NA or HDA along with low dose of cyclophosphamide (4mg/kg body weight) showed greater reduction in the number of ascitic tumor cells and increased the percentage in apoptosis phase, compared to the treatment with NA, HDA or cyclophosphamide (4mg/kg body weight) alone. Intra-peritoneal injection of cyclophosphamide at 4mg/kg alone was well tolerated but produced a weak anti-tumor effect in Dalton's lymphoma cancer model. However, a significant inhibition of tumor growth was observed when *Abrus* agglutinin was administered in combination with low dose of cyclophosphamide (4mg/kg body weight). These results indicate that both NA and HDA are immunostimulatory in nature and are potential candidates as biological response modifier to be used in cancer treatment. The agglutinin may also be useful in chemoimmunotherapy regimens.

Key words

Abrus agglutinin, anti-tumor, biological response modifier (BRM), cyclophosphamide, Dalton's lymphoma, immunomodulaory, lectin, macrophages, Natural Killer cells (NK cells), splenocytes, thymocytes