Abstract

The seed kernels of the plant *Abrus precatorius* contain a lectin, which is an agglutinin. *Abrus* agglutinin is a 128kDa heterotetrameric glycoprotein. The lectin is of Gal (β 1$\rightarrow$ 3) Gal/NAc specificity and is reported to be a T cell mitogen. The immunostimulatory and adjuvant properties of *Abrus* agglutinin in native (NA) and heat denatured (HDA) conditions were studied both *in vitro* and *in vivo*. The adjuvant properties of both native and heat denatured agglutinin was studied in mice model system with different antigens (Ovalbumin/OVA, Diphtheria toxoid/DT, Lysozyme/LYZ) and by different routes of administration (i.p., oral and skin) in an aqueous formulation. The humoral response induced by NA and HDA were comparable to Freund's adjuvant. The type of immune response differed with the antigen type. In case of OVA and DT an inclination towards the Th1 response was observed whereas for LYZ a distinct Th2 type of response was seen. Both NA and HDA also induced a high avidity antigen specific antibody response. An *in vivo* induction of cell mediate immunity was also observed both by NA and HDA in the form of delayed type of hypersensitivity (DTH) response. NA and HDA also reduced the effect of immunosuppressive drug cyclosporin A on the mice immune system. *In vitro* stimulation of murine macrophages by NA and HDA was also studied. An alteration in different macrophage functions like nitric oxide (NO), superoxide anion, hydrogen peroxide, interleukin 1(IL-1) production was observed. A change in the phagocytic and bactericidal activity of the macrophages was also observed. An increased production of NO, H_2O_2 and IL-1 and higher phagocytic and bactericidal activity resulted due to the stimulatory effects of NA and HDA. Stimulation of other murine immune cells like splenocytes, thymocytes and NK cell by NA and HDA were also observed. Proliferation of splenocytes and thymocytes and increased cytolytic activity of NK cell was seen. The cytokine (IL-2, IFN-γ) released by NA/HDA stimulated splenocytes *in vitro* indicated a Th1 type of response. The positive bioactivity of HDA led to the next part of the study, in which the bioactivity of tryptic digest of *Abrus* agglutinin (TDA) was studied *in vitro*. The effect of TDA on murine macrophages, splenocytes, thymocytes and NK cells were studied. A positive stimulation of the murine cells by TDA was observed. But in comparison to NA and HDA, TDA induced higher cell proliferation and a very low NO

production. The loss of haemagglutination activity of *Abrus* agglutinin due to heat denaturation was observed. HDA was further characterized to determine the changes in the sugar-binding pocket that resulted due to heat denaturation. The effect of lactose on the cell binding properties of NA and HDA was studied. Lactose was seen to interfere with the cell binding properties of NA but not HDA. Fluorimetric studies showed fluorescence quenching in case of lactose interaction with NA. But in case of HDA the presence of lactose showed no fluorescence quenching. HDA did not show any binding affinity to the lactamyl sepharose affinity matrix as determined from the frontal affinity chromatography whereas NA showed a dissociation constant of 33.38μM. These results indicate that both NA and HDA are immunostimulatory in nature and are potential candidates for developing alternate adjuvants. Heat denaturation results in the loss of carbohydrate binding pocket of the lectin and the bioactivity of tryptic digest agglutinin suggests the presence of immunostimulatory peptides in the protein. These results also indicate a probable alternate cell activation pathway by which lectins work.

Key words

Abrus agglutinin, alternate adjuvant, immunostimulation, biological response modifier, lectin, macrophages, splenocytes, thymocytes, NK cells, humoral immune response, cell mediated immune response, ELISA, avidity,