STUDY ON TUMOR REGRESSION BY PRESENTING LIPOSOME-ENCAPSULATED TUMOR-ASSOCIATED ANTIGENS (TAA) TO THE IMMUNE SYSTEM

ABSTRACT

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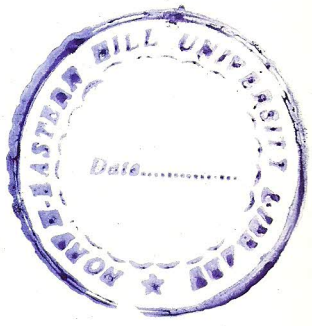

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Abstract

Immunotherapy has been a fast-evading area of cancer research because some clinical responses have been demonstrated against malignant tumors. This therapy has an advantage of specific lysis of malignant cells without destroying the normal cells. One approach is specific active immunotherapy with antigenic tumor cells (rendered non-tumorigenic by, e.g., irradiation) or with extracted tumor-associated antigens (TAA), the objective being to stimulate the host's immune response against the tumor. Antigens on tumor cells are usually also found on normal cells. The expression of such molecules on tumor cells, however, differs from the expression on normal cells. For instance, they are present at high levels on tumor cells and in trace amounts on normal cells, they are usually distributed over the cell membrane, or they are expressed at an inappropriate phase of ontogenesis, as in case of fetal antigens on tumor cells in adults. Little evidence is available on the existence of real tumor-specific antigens in man. Therefore, antigens on tumor cells are usually called tumor-associated antigens (TAA).

The main obstacle in specific active immunotherapy is the generally weak immunogenicity of TAA. Therefore a major challenge in tumor immunology is to develop methods that augment the immune response to TAA. Different methods have been explored in order to achieve a potent immune response against tumor and are as follows:

1. Instead of whole tumor cells, preparations containing TAA of tumor cells (TAA extract) have been explored for active immunization. Potential advantages of the use of TAA extracts over whole tumor cells include the immunization of contamination with potentially oncogenic nuclear materials from tumor cells as well as the opportunity of chemical characterization, sterilization, and refinement of the antigen preparation.
2. Tumor cells have been modified to enhance their immunogenicity. This has been accomplished in various ways, for example, by enzymatic unmasking of TAA with enzymes such as neurominidase, by additional immunogenic cell surface determinants, or by rigidification of cell membranes with lipids. All these make the antigen more immunogenic.
3. Another approach is to employ immunological adjuvants. Recently less toxic immunostimulants such as cytokine and analogs of bacterial products are under investigation. Beside these, extensive efforts are currently being made to augment anti-tumor immunity by using genetically modified autologous cancer cells as

vaccines and also the cloning of genes encoding TAAs which have significantly improved the prospect for cancer immunotherapy.

4. Several investigators have focused on the delivery of TAA-derived proteins/peptides or TAA genes to professional antigen presenting cells, to elicit immune responses capable of eradicating tumor cells.

The recent isolation and biochemical characterization of certain TAA enabled the study of immune reactions against highly purified TAA. Encouraging results were reported in cancer patients for specific active immunotherapy with crude as well as highly purified TAA preparations. Considering the antigenic heterogeneity of human tumors it is expected that tumor vaccine should contain multiple TAA. Ideally, vaccines should be prepared from a mixture of pure antigens.

The TAA responsible for mediating tumor rejection are integral membrane molecules or molecules associated with the outer cell surface. Their immunogenicity is closely related to their presentation form.

Liposomes have been found to elicit the immune response to a variety of antigens and therefore proposed as biodegradable vehicles for the presentation of antigens to the immune system. Liposomes are lipid vesicles made up of concentric bimolecular leaflets of phospholipids separated by aqueous spaces (0.1 – 1.0 μm). They are versatile presentation vehicles, as antigen can be included in the aqueous or lipid phase of liposome, and their size, surface charge, and other properties can be controlled.

The work embodied in this thesis is an attempt to develop a safe and effective liposomal-TAA formulation that could mount a strong rejection response against the host's tumor. Liposome has successively been used as a potential carrier in drug delivery and drug targeting with a number of cancer therapies. However, its application in tumor immunotherapy as carrier in yet to be proved.

Diethylnitrosamine (DEN), a potent hepatocarcinogen was used for tumor induction in mice. The carcinogen administered by i.v. route for a period of about three

months at weakly intervals. Cancer induction studied by monitoring the marker enzymes activities, i.e. γ-glutamyl transpeptidase (GGT) and acetylcholine esterase (AChE). Histology and electrophoretic studies of surface membrane glycoproteins in liver were also carried out in support of cellular transformation. Tumor-associated antigen (TAA) was extracted from the liver cells of DEN-treated animals using 1-butanol, which extract exclusively membrane surface glycoproteins. Mice having complete DEN treatment were immunized with TAA-extract after encapsulating it into liposomes, which served as carrier for the presentation of TAA to the immune system. Antibody response elicited by liposomal-TAA formulation monitored using ELISA, whereas the cellular response followed by cell proliferation assay using BrdU labeling kit. The assay is a cellular immunoassay, which uses a mouse monoclonal antibody directed against BrdU based on ELISA principle. Effect of immunization on tumor regression was studied. For the regression studies the same parameters employed in induction studies were used.

In the present investigation it was found that a strong immune response against host's tumor could be achieved by administering TAA encapsulated in liposomes. Liposome encapsulated TAA was found to be more potent immunogen compared to TAA alone. The elicited immune response produced satisfactory results. The main points emerging from the work embodied in the thesis are:

⇒ Chronic exposure of DEN to Swiss albino mice induced cellular transformations in the liver, as substantiated by the pronounced alterations in the activities of the marker enzymes such as γ-glutamyl transpeptidase and acetylcholine esterase.

⇒ DEN treatment resulted in a distinct change in the morphology of the hepatocytes such as the variations in the cell shape and size, appearance of more densely stained nuclei and multinucleated cells as elucidated in the histological studies.

⇒ Cell surface membrane glycoproteins in liver exhibited differential expression upon DEN exposure. A glycoprotein of approximately 68 kDa was over expressed while some between 20 kDa to 29 kDa molecular weight found under expressed as compared to their normal counterparts as revealed by SDS-PAGE electrophoretic study.

⇒ Liposomes prepared by the dry film method exhibited highly reproducible entrapment efficiency. Further, the immunogenicity of entrapped TAA remained unaltered.

⇒ DEN-exposed mice upon immunization with liposomal-TAA formulation elicited humoral immune response against TAA as substantiated by the presence of significantly high circulating antibody concentration in immune serum.

⇒ Cell proliferation assay *in vitro* of lymphocytes, obtained from immunized animals, clearly indicate the induction of cell mediated immune response in DEN-exposed mice upon immunization.

⇒ Reversal of the marker enzyme activities, in the liver of DEN-exposed and immunized mice, towards normal level is likely due to the effect of immunization on tumor regression.

⇒ Attainment of normal morphology and cell shape of hepatocytes upon immunization in DEN-exposed mice supports tumor regression.

From the above observations, it is evident that this particular approach could be useful to elicit immune response against tumor-associated antigens. It is further suggested that vaccine based on liposomal antigen formulation in particular with non-fractional cell extracts as a source of tumor antigen may be equally effective to induce immune responses to that of the cell based modified tumor vaccine.