ABSTRACT

A neoplastic cell develops from its normal progenitor as a consequence of changes in some members of a restricted set of cellular genes. During the course of this development, cell surface profile of a malignant cell is altered considerably. This fact is reflected in its properties, which are largely dependent on the constituents of the cell surface such as anchorage independence, loss of contact inhibition, increased uptake of essential nutrients and modified patterns of lectin agglutinibility. Another characteristic feature of a tumor cell is expression of neoantigens which are involved in modulating the response of the host's immune system to a newly invading tumor cell.

Tumor-specific antigens are primarily defined by their ability to evoke an immune response in a normal syngeneic host. The capacity of a tumor-specific antigen to act as an 'immunogen' is variable. Tumor-specific antigens continue to be interesting molecules to immunologists, primarily due to the role they play in modulating the immune response evoked, as well as the possible direct relationship they might have with molecular mechanisms of malignant transformation itself. The classical definition of tumor-specific antigens implied their presence only on tumors, but not on normal tissues. However, among the various tumor-specific antigens discovered so far, normal cellular proteins which can act as an antigen and evoke an immune response in a syngeneic host have
attracted considerable amount of attention. The immunogenicity of such antigens can be explained by either the increased concentration or by alterations in the pattern of the post translational modifications such as glycosylation leading to the changes in the normal molecule on the cell surface.

The system chosen to investigate the alterations in cell surface profile upon transformation and the precise role of tumor specific antigens is a macrophage-like cell line, the AK-5. The macrophage identity of the cell line has been established using cell surface markers such as FC receptors, C3d receptors, leucocyte common antigen and la determinant and hydrolytic enzymes like lysozyme, collagenase, non-specific esterase. Also, the phagocytic property of the cells has been demonstrated using fluorescence labelled bacteria and electron microscopy. AK-5 is being maintained by serial passaging in the peritoneal cavity of Wistar rats and grows as solid tumor when injected subcutaneously or intradermally. AK-5, when injected intraperitoneally, kills all the animals, whereas with subcutaneous administration, a proportion of the animals reject the tumor suggesting the highly immunogenic nature of the tumor cells.

It was convenient to exploit such a system to address an unambiguous question regarding the exact role of tumor specific antigens in eliciting and modulating immune responses against the tumor. This thesis reports results regarding the purification and molecular characterization of an antigen from AK-5 cells which has a strong homology with serum albumin. The tumor specific nature of an albumin-like antigen is analysed in light of the aberrant expression
of this protein in a macrophage-like cell line. Results regarding the tumor-specific nature of the antigen, its subcellular localization and immunologically distinct identity compared to rat serum albumin are presented.

The important results obtained during the course of these studies can be summarized as follows: A tumor-specific antigen of molecular weight 67,000 has been purified to homogeneity from the cell-surface extracts of a rat macrophage cell line AK-5. The purified antigen can neutralize the anti-AK-5 antiserum raised in normal syngeneic hosts thereby protects the AK-5 cells from lysis in an in vitro lysis protection assay. The antigen purified from crude plasma membranes can be used to raise cytotoxic antibodies against the tumor. Also, immunoprecipitation experiments performed using anti-AK-5 antibody indicate the presence of a 67-kDa protein confirming its tumor specific nature.

The antigen behaves like rat serum albumin when analysed by SDS-PAGE, isoelectric focussing, single dimensional peptide mapping and PAS-staining. Isoelectric point of the antigen (5.7) is comparable to that of rat serum albumin. Single dimensional peptide maps of rat serum albumin and the antigen are identical. Upon PAS staining the antigen appears to be unglycosylated. N-terminal sequence of the antigen and rat serum albumin are similar. Seventeen residues out of the twenty two residues sequenced are identical while three amino acid residues at the N-terminus are altered. The protein crossreacts with anti-rat albumin antibodies raised in rabbit. Analysis of
the polyA* message from AK-5 indicates the presence of an albumin-specific transcript (1.7 kb) which is similar to the one in normal liver in terms of size. The organisation of the gene coding for an albumin-like protein in AK-5 appears to be comparable to that of rat liver and a rat hepatoma (Zajdela ascitic hepatoma). The extent of expression of this gene in AK-5, though comparable to the hepatoma, is lesser than that observed in liver. Ectopic expression of the liver specific gene in a macrophage cell is addressed. Based on the observations a causal relationship between the aberrant presence of liver specific transfectors and ectopically expressed hepatic functions is suggested. The immunologically distinct nature of the antigen as compared to albumin has been demonstrated. Possibilities which can account for the unusual behaviour of a normal secretory protein acting as a tumor antigen are outlined.

Lastly, the possible relationship that an albumin-like antigen might have with the origin of a macrophage cell line, AK-5, is discussed.