ABSTRACT

As a result of transformation, a normal cell undergoes changes in a restricted set of cellular genes. During the course of this development, the properties of a malignant cell are altered considerably and changes such as anchorage independence, loss of contact inhibition, increased uptake of essential nutrients, low serum requirements in culture, invasiveness and ability to metastasize are frequently observed. As a result of accumulating mutations, the protein profile of a malignant cell is altered considerably with respect to its normal tissue counterpart. Some of these altered proteins or neoantigens are capable of evoking an immune response against the tumor in the host and these are known as tumor specific antigens.

A large amount of data regarding the biochemical and immunological characterization of tumor specific antigens has been generated as a result of analysis of protein preparations from various tumors. Tumor specific antigens are primarily defined by their ability to evoke an immune response in a syngeneic host. The ability of a tumor antigen to evoke an immune response in the host varies depending upon the tissue of origin and the initial carcinogenic event. Based on the causal agent, type of cell and the molecular characterization of the antigen expressed tumor antigens have been classified as being unique to i) a tumor, ii) to a class of tumor, iii) to the transforming agent, and iv) common to many types of tumors. Numerous examples of these various classes have been identified and characterized from different tumors and the immune responses evoked by these antigens also vary considerably ranging from the weakly immunogenic tumor antigens of spontaneous tumors to the highly immunogenic viral antigens.

The type of immune responses evoked by these various classes of antigens ranges from humoral to cellular or both. In the recent past, CD8+ T cell responses have been shown to play a major role in the rejection of tumors. Many tumor antigens which specifically evoke cytotoxic T cell responses have been shown to be effective in protecting the host from tumors. CD4+ T cells, natural killer cells and cytokines like IL-2, IL-12 and IFN-γ have also been shown to play important roles in the rejection of tumors, and antigens evoking one or
more of these responses have been identified and characterized from various tumors. Humoral responses though insufficient for the elimination of tumors on their own have been shown to augment the cellular responses in the rejection of tumors.

The system chosen to investigate the role of tumor specific antigens in the rejection of tumors is the AK-5, a rat macrophage-like cell line, passaged intraperitoneally as ascites in inbred Wistar rats. The AK-5 is a highly immunogenic cell line. When injected intraperitoneally, AK-5 is highly malignant, killing 100% of the animals within 8 days. However, subcutaneous injections of AK-5 into syngeneic Wistar rats result in solid tumors which grow relatively slowly. By day 14 post-transplantation, the solid tumor starts regressing spontaneously, accompanied by necrosis in about 80% of the animals and disappears completely by day 25 in these animals. Animals that reject the tumor do not accept further challenges of AK-5, either intraperitoneally or subcutaneously. This property of AK-5 is extremely interesting, since spontaneous tumors are not known to be immunogenic.

Earlier work in our laboratory had shown that the mechanism of rejection of AK-5 involves activation of multiple factors and effectors of the immune system. Animals that reject the tumor have high titres of circulating anti-tumor antibody in their serum, which reach peak values around day 10 post-transplantation. The anti-AK-5 antibody obtained from tumor regressed animals can lyse the tumor cells in the presence of rabbit complement in a complement mediated lysis assay (Khar, 1993). The anti-AK-5 serum was specific in that it did not lyse other tumor cells like ZAH, YAS or Meth-A. Membrane or cell extracts of AK-5 can prevent the complement mediated lysis of AK-5 cells by the anti-AK-5 antibody. It was also shown that NK cells from tumor bearing animals mediate killing of AK-5 cells in vitro through ADCC, anti-AK-5 being the specific mediator in this reaction.

The aim of the present study was to identify and characterize the antigenic molecule(s) responsible for such a strong humoral response in the tumor bearing animals. To achieve this end, we decided to employ a molecular cloning strategy using the anti-tumor antibody as probe to screen an expression cDNA library of the AK-5 tumor.
Screening of the AK-5 cDNA library constructed in the expression vector λ gt 11, with the anti-AK-5 antiserum was done and a putative antigen clone 1(1) carrying an insert of approximately 1.0 kb was obtained. This recombinant λ clone was used to lysogenize the E.coli vector Y 1089, and the fusion protein expressed as a β-gal fusion protein. The size of the protein expressed by the clone was approximately 15 kDa. Western blot analysis of the fusion protein revealed that it is recognized strongly by the anti-AK-5 serum indicating that the 1(1) clone indeed encodes an antigen defined by the autologous anti-AK-5 antibody. In order to further test the immunogenicity of the putative antigen, the insert carried by the 1(1) clone was subcloned into an overexpression vector pGEX. The antigen was overexpressed as a GST fusion protein, and sufficiently large amounts necessary for immunization experiments were purified using a glutathione Sepharose affinity column. In addition to anti-AK-5 antibody, two monoclonal antibodies against AK-5, raised independently (Khar et al., 1992), were found to recognize the antigen encoded by 1(1) specifically in a western blot analysis. The GST-1(1) fusion protein was used to immunize syngeneic animals and was found to evoke a strong humoral response in all the animals tested. Further these immunized animals were challenged with AK-5 cells intraperitoneally and monitored for tumor growth and death of the host. There were significantly more survivors in the GST-1(1) immunized group than in the GST immunized or the unimmunized control groups indicating that active immunization by the antigen encoded by 1(1) does afford some protection against the tumor in syngeneic animals. All these data indicate that the protein encoded by 1(1) is a tumor specific antigen of AK-5.

Northern blot analysis revealed that the message for 1(1) is about 2.5 kb in size, is unaltered in AK-5 as compared to normal macrophages and is expressed in brain, testes, spleen and thymus in addition to AK-5 and macrophages. Southern blot analysis of AK-5 tumor DNA, normal macrophage DNA and rat genomic DNA isolated from liver indicated that the organization of the gene for 1(1) is unaltered in AK-5. Sequencing of the complete 1(1) cDNA was done and homology search in the database revealed it to be a novel gene. Since by northern analysis it was found to be a partial clone, another cDNA library of AK-5 was constructed in the vector λ zap and screened using the 1(1) cDNA as a probe. A larger clone
A1 was obtained and was sequenced completely. Sequence analysis of the cDNA and homology search in the database revealed that it is homologous to a human cDNA clone c-leng08 expressed in the brain. The function of this gene is as yet unknown.

Our studies indicate that the 1(1) cDNA encodes a novel protein which is unaltered in the AK-5 as compared to its normal tissue counterpart. Tumor antigens were earlier thought to be proteins altered as a result of transformation and hence express novel tumor specific epitopes which are recognized as foreign by the host immune system. However an increasing amount of data in the recent past shows that this assumption may be incorrect. A large number of tumor antigens have been characterized which are found to be unaltered when compared to their normal tissue counterparts. Recent advances in the understanding of the mechanisms of antigen presentation have shown that for the immune system to mount a response, the antigenic peptide has to be recognized by either the CD8+ or CD4+ T cells in the context of the MHC molecules. Thus any peptide presented in the context of the MHC class I molecule is a potential antigen. It is becoming accepted that a large set of antigenic determinants of the self have not induced self-tolerance and that these peptide determinants furnish target structures for autoimmune attack and could provide potential targets for immune responses directed against cancer. T cells can evade negative selection during the process of thymic education in the developing immune system possibly due to low affinity for the peptide being presented and these can become potential effectors if sufficiently activated. It is known that in a tumor bearing host there is an increased expression of cytokines and other costimulatory molecules and general activation of the immune system. Normal peptide epitopes under these circumstances may activate these T cells sufficiently for them to mount a response. It has been demonstrated that tumor cells transfected with genes for costimulatory molecules, cytokines, and MHC class II molecules have increased immunogenicity due to the increase in the efficiency of antigen presentation.

Natural killer cells and anti-tumor antibody have been shown to be the main effectors in the regression of AK-5 through ADCC. In addition cytokines like IL-12, IL-2 and TNFα have also been shown to be involved. AK-5 is a macrophage like cell line and possesses all the
phenotypic characteristics of antigen presenting cells. Most importantly it expresses MHC class II molecules and has the ability to phagocytose bacteria. The attractive possibility that the AK-5 is capable of presenting self peptides through MHC class II is discussed.