REFLECTIONS ON HUMAN TUMOR IMMUNOLOGY

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BACKGROUND

The establishment of inbred mouse strains laid the ground for studies of immunological host response against tumor cells. Graft rejection experiments showed that immunity can be induced against syngeneic tumors. The following general rules emerged: 1) Chemical carcinogen induced tumors have individual antigenicity; 2) Tumors induced by the same virus cross react; 3) Spontaneous tumors have low or no antigenicity; 4) Cell mediated effector mechanisms are mainly responsible for graft rejection; 5) The take of tumor grafts can be counteracted by active or passive immunization, but the growth of established grafts is difficult to influence.

Through the years expectations for the exploitation of immunity for therapeutical measures have been rising and waning several times. As main aspects which influenced the attitudes the weak or low antigenicity of spontaneous tumors and the discovery of natural and activated killer cells can be mentioned. Development of techniques for lymphocyte subset characterization, their cultivation and the knowledge about the details of the antigen recognition by T lymphocytes allow now to attempt definition of the antigenic epitopes on tumor cells. With this goal prospects for cancer immunotherapy occupy a large number of researchers applying technical and conceptual innovations of basic immunology and molecular biology.

T lymphocyte mediated recognition of antigenic cells

A paradigmatic change occurred in cellular immunology when it was discovered that cytotoxic T lymphocytes recognize antigenic carrying targets in connection with the surface localized molecules encoded by the major histocompatibility complex, MHC. This discovery initiated the analysis of those molecules on the T cells and on the targets, which are responsible for the immunological rec-ognition steps and delivery of the functional signals. The demonstration of antigenic epitope carrying peptides in a special groove of the MHC molecules explained their importance in the immune reaction against autologous cells.

Before this discovery the search was directed to surface localized proteins. Now it is known that peptides processed from intracellular proteins can be transferred to the cell surfaces and determine the antigenicity. Thereby the possibility for provision of immunodeterminants became considerable wider. A large variety of proteins may be selectively expressed in the tumor cells particularly in those which carry viral genomes. Each of these proteins may provide several peptides with antigenic epitopes. Generally, peptides fit selectively to the various MHC class I alleles and therefore the antigenic specificities show variations according to the HLA genotype of the individual.

On the part of the T lymphocyte, the anatomy of the surface structure which interacts with the antigenic target and transmits signals for various functions has been largely clarified. The antigen recognizing molecules which define specificity are associated with other molecules which represent T cell differentiation markers, and interact with the MHC molecules on the target. In addition, the lymphocytes and the target cells carry interacting receptors, cell adhesion molecules which strengthen their contact. These are independent of the antigen recognition structures.

In addition to the search for antigens expressed on tumor cells, studies of their immunogenicity and immunosensitivity have to include thus the expression of MHC molecules and the cell adhesion molecules. Appropriate amounts of MHC molecules on the tumor cells which can present the antigen epitope carrying peptides are required for an efficient anti-tumor response.

IMMUNE RESPONSE DIRECTED TO TUMORS IN HUMANS

The incidence of malignancies in immunosuppressive conditions, congenital or iatrogenic, and the natural course of the diseases should be indicative of whether or not immune responses occur against human tumors. Sufficient
time has passed since the introduction of organ transplantation, made possible by administration of strong immunosuppressive measures, to provide information on whether tumor incidence is increased in such patients. The results indicate that this indeed is the case. The tumors which appear with higher frequencies, however, are those to which viruses contribute in some way (for reviews see ref. 9). Thus anti-viral immunity may play an important role in the avoidance of these tumors under normal conditions and this can be weakened or put out of function in these patients.

**Immune response against Epstein-Barr virus (EBV) carrier B lymphocytes**

DNA tumor viruses provide the best examples of efficient immune surveillance, e.g. polyoma virus is ubiquitous in mice and can induce a variety of tumors if infection occurs early in life. Maternal antibodies can protect against tumor development. In older mice, no tumors are induced unless their T-cell system is seriously impaired. We humans carry EBV, a herpes virus, acquired through horizontal transmission. It is an efficient transforming virus for B cells in vitro. The cell-virus and the host-cell relationships are highly informative about the capacity and strength of the mechanisms which operate in immune surveillance.

In children EBV infection usually occurs unnoticed. About half of the individuals infected during and after adolescence suffer the syndrome of infectious mononucleosis. The life long presence of antibodies in the serum witness the acquisition of the virus in the past which is thereafter harbored in the B lymphocyte population and in the epithelial cells of the oropharynx (for reviews see refs. 10,13).

The harmless existence of EBV in the human species is ensured by highly efficient multicomponent control mechanisms. EBV transformed B lymphocytes growing to lymphoblastoid cell lines, LCL, can be established from the blood of seropositive individuals if from the explanted populations the T cells are eliminated or their function is inhibited. The role of T cells in the in vitro transformation reflects the fact that growth variety of EBV-carrying B cell in vivo is an extremely rare phenomenon; it can be regarded as an accident. The T cell mediated inhibition of B cell transformation, shown also in experiments with in vitro exposure to the virus suggests that if not entirely, then at least in great part, mobilization of immune responses against the virus and against the virus genome carrier cells are responsible for the in vivo control. When the immunological control is relaxed, due to congenital defect or immunosuppressive treatments, the risk for EBV-carrying lymphoproliferations increases.

Antibodies directed against some of the viral structural and the viral encoded cellular proteins contribute to the generally harmless host-virus symbiosis. It is known however that immune surveillance is exercised mainly by cell-mediated mechanisms, alone and in concert with antibodies. Specific T-cells which recognise EBV-carrying B-cells were demonstrated in virus carrying i.e. seropositive individuals. Cytotoxic T-cells directed against 7 (EBNA2-6 and LMP1-2) of the 8 EBV encoded proteins expressed in the viral genome carrying LCL were shown to function following the rules of MHC restriction.

The patients with EBV carrying Burkitt lymphomas (BL) are immunocompetent. The malignant cells escape immune recognition by several measures. They do not express the above mentioned EBV-encoded immunogenic proteins present in the LCLs and they have lower levels of the cellular adhesion molecules and MHC class I antigens. The capacity to escape reactive T-cells is reflected also in the considerably lower capacity of BL-lines to stimulate allogeneic lymphocytes in comparison to LCLs.

Thus the EBV-genome carrying B-cells with proliferative capacity can grow to malignancies either when the immune response is severely impaired (e.g. ‘transplant’ lymphomas) or when several of the cellular properties change which render them non-recognizable to T cells.

**Immune response against solid tumors. The importance of MHC Class I antigen expression**

The natural course of the diseases and the results of various immunotherapeutical protocols suggest that melanoma and renal carcinoma cells may elicit an immune response. Indeed, in vitro experiments show the existence of humoral and cellular immunity in these patients. Even at the clonal level, auto-tumor-specific T cells have been detected in their lymphocyte populations.

It has been shown by immunohistology that non-malignant cells, benign and malignant tumors on one hand, primary tumors and their metastases on the other hand often differ in expression of MHC class I molecules. These experiments showed that tumor cells have a relatively low level of class I molecules, compared to the adjacent non-malignant cells.

Many but not all, experimental models showed that deficiency in certain class I and/or class II antigen expression is associated with increased tumorigenicity and metastasizing tendency of tumor cells (reviews in refs. 18,19).

In melanoma and renal carcinoma patients some studies indicated an association between high levels of MHC class I antigens on the tumor cells and favorable prognosis. On the other hand high expression of MHC class II antigens on melanoma cells was associated with bad prognosis.

We showed the involvement of MHC class I antigens on the tumor cells in the in vitro recognition by T lymphocytes. Combined IFN-gamma and TNF-alpha treatment of tumor cells in vitro induces elevated levels of MHC class