INTRODUCTION

The biological basis of the interaction between immune system cells and tumor cells is at present, still unknown. Host immunity and tumor biology are important cofactors in brain tumor immunotherapy. Many studies have been focused on evaluation of tumor cell antigenicity and cell-mediated immune responses. It is well known that a host versus tumor reaction occurs at the site of tumor growth and that the cell-mediated immune system may have a central role in possible control of the tumor growth. The discovery that interleukin-2 (IL-2) confers oncolytic properties on lymphocytes has determined the development of new strategies and innovative therapeutic protocols in human tumor (1-7).

Over the past years, populations of immunological effector cells induced by IL-2 named “lymphokine activated killer (LAK) cells” have been discovered. These cells are able to recognise and destroy tumor targets specifically and have been used for adoptive immunotherapy.

Little is known about the mechanism by which LAK cells recognise their targets. Since LAK cells can distinguish between normal and
tumor cells, they represent a valuable model system for studying the mechanism of tumor cell recognition.

MATERIALS, METHODS AND RESULTS

Peripheral blood mononucleate cells (PBMCs) were treated with low doses of IL-2 (Eurocetus) for one week. LAK cells were tested for their capacity to kill transformed cells and the following were used as targets: Chang cells, HeLa, K562, normal fibroblast and fresh tumoral cells (bronchogenic carcinoma, mesothelioma, renal carcinoma). After IL-2 stimulation, an active proliferation of lymphocytes with characteristic features, such as “hand mirror shape” HMS, was observed.

The activity of IL-2 stimulated lymphocytes against transformed cells was evaluated examining May-Grunwald Giemsa (MGG) stain samples from mixed LAK-target cultures. Chang cells are sensitive to LAK lysis while K562 are sensitive to NK lysis. The reactivity against normal fibroblasts and tumoral cells is quite different. In the normal cells there is no lysis of the target, while the interaction between LAK-tumoral target instead showed destruction of the cells. The different reactivity against normal and transformed cells probably reflects differences between normal and transformed cells in the surface molecules and in the presence of structures that regulate specific lysis signals (8-13).

Tumors of the central nervous system (CNS) belong to a peculiar group of neoplasia. Because activated leukocytes are able to cross the blood-brain barrier (BBB), a suitable stimulation of the immune system can increase the host’s defence against brain tumor. Our studies carried out in recent years have investigated the function and role of the defensive cells within human brain tumor. Some gliomas are very invasive and a severe depression of the immunological system has been described. In particular, subjects with glioblastoma show more severe depression than patients with astrocytoma. Our results indicated that in malignant glioma there is stimulation of the immune system as witnessed by the presence of the activated cells inside tumor tissue and soluble activating factor in serum (IL-2, sIL-2R) (14). However, immunodepression in brain tumor patients occurs and reduced response to mitogens, functional deficiencies in circulating T-helper cells and blocking serum factors that repress the cytotoxic action of T-cells have been described. We hypothesized that the failure of the immunological defence against tumors could be due to a block during the defensive cell effect, rather than to the lack of lymphocyte stimulation. Soluble serum factors with a specific reactivity for activated lymphocytes might be involved in these processes. In fact, in many malignancies, the amount of soluble intercellular adhesion molecules is increased and in vitro experiments demonstrated that their presence blocks the reactivity of the cytotoxic cells against their targets. Then, we evaluated the expression of lymphocyte function-associated antigen-1 (LFA-1) in glioblastoma patients by flow cytometrical analysis. LFA-1 belongs to the family of leukocyte integrins and is detected on the membrane of many cellular lines. Intercellular adhesion molecule-1 (ICAM-1) is a member of the immunoglobulin family and represents the ligand for LFA-1. ICAM-1 and its counter receptor LFA-1 act as accessory molecules in the activation of T lymphocytes. Cytokines and ICAM-1 play an important role in the recruitment of activated lymphocytes to the sites of inflammation within the central nervous system. Our data showed that no statistically significant difference of LFA-1 expression was found in lymphocytes of glioblastoma (GBL) patients and soluble ICAM-1 serum levels compared with healthy control lymphocytes and serum. The absence of significant differences between LFA-1 expression in GBL patients and controls suggests that the
LFA-1 / ICAM-1 system does not play an important role in downregulating immunoreactivity in glioblastoma (15).

To continue these studies, we focused our attention on malignant glioma. To evaluate whether IL-2 may induce in vitro proliferation of tumor-infiltrating lymphocytes (TILs) capable of destroying tumor cells, tumoral fragments obtained from different areas of the astrocytic tumor were cultured with and without rIL-2. Short-time cultures were submitted to morphological (also ultrastructural) (Fig.s 1a, 1b) and immunocytochemical analysis. We used primary malignant tumor cultures observed at short time because of the cells tend to change their characteristics during pro-longed culture time. In this condition the in vivo situation could correlate with the in vitro situation. We observed the ability of the T cell growth factor IL-2 to support the proliferation of defensive cells inside the tumor. In the presence of IL-2, growth of tumor cells was also observed (Fig. 2a, see over). On the contrary in the absence of IL-2 only few colonies derived from tumor fragments were obtained after two weeks (Fig. 2b, see over). The immunocytochemical study revealed that IL-2 induces the expression of IL-2 receptor on the surface of human glioblastoma cells (16,17).

In our experimental conditions, IL-2 affected the proliferation not only of infiltrating lymphocyte cells, but also of tumor cells. In fact IL-2 is not a specific T cell activating factor, but a factor involved in general activation processes shared by a variety of cells types. IL-2 belongs to a large group of hemopoietins that signal through the common γ chain receptor. For this reason, the local treatment of human GBL cells using IL-2 might produce tumor cell proliferation due to the presence of TAC receptor on microglia and glial cells.

CONCLUDING REMARKS

These findings suggested that immunotherapy with IL-2 may be either damaging or useful depending on the type and localization of tumor, mode of administration and dosage.

Human gliomas are known to be immuno-

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Fig. 1 - Electron micrographs of glioblastoma cells cultured in the presence (a) and in the absence (b) of IL-2 and observed after 3 days of culture.

1a: infiltrating lymphocytes (arrow, X4000); 1b: apoptotic tumoral cell (X4000)
suppressive. Recent reports have suggested novel strategies to overcome this immunosuppression, including immunogene therapy (18). Some gene therapy clinical protocols are in progress (19). In addition, the treatment of intracranial gliomas with dendritic cells pulsed with tumor antigens is a promising experimental model. Preclinical work on the use of dendritic cell-based vaccines for malignant brain tumors are encouraging (20,21). Currently, our studies are aimed at evaluating HLA class I antigen and antigen-processing machinery components in human glioma. The abnormalities in the MHC class I antigen are found in some human malignant cells where they represent a major obstacle to the successful implementation of T cell-based immunotherapy (22). In conclusion, in spite of copious efforts in many institutes, studies into immunotherapy for brain tumor are still in their early stages of development.

REFERENCES


Fig. 2 - Cells obtained from human glioblastoma fragment-cultured for 13 days in the presence of IL-2 (a) and in the absence of IL-2 (b).
2a: tumour cells with many infiltrating lymphocytes (arrow) (X1200); 2b: tumour cells colonies without infiltrating lymphocytes (X1200).
7. Fletcher M, Goldstein AL. Recent advances in the understanding of the biochemistry and clinical pharmacology of interleukin-2. Lymphokine Research 1987; 6:45-57


