The immunobiology of malignant gliomas

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Summary

In spite of continuing advances in surgery, radiotherapy and chemotherapy, the last few decades have brought little improvement in the prognosis of malignant brain tumours. New immunotherapeutic strategies are being studied in numerous laboratories throughout the world in an effort to gather the further clinical and biological data needed in order to render these therapies effective and selective. The authors review recent advances in understanding of the immunobiology of brain tumours, in particular malignant gliomas. The biological basis of the interaction between nervous system cells and cancer cells is still an unresolved question and a constantly evolving area of research. Particular attention is paid to interleukin-2, one of the few effective cytokines used in the adjuvant treatment of some tumours. However, there is still a long way to go before these and other immunological approaches can be considered really applicable in the treatment of malignant gliomas.

KEY WORDS: astrocytic tumours, cytokines, immunobiology, lymphocytes, therapeutic strategies.

Introduction

Even though we have seen rapid advances in surgery (1-3), the optimisation of radiotherapy (4,5), and the introduction of new chemotherapies (6-8), the prognosis of cerebral gliomas, which have an annual incidence of 4-6 new cases per 100,000 population, has remained practically unchanged in recent decades: mean survival of under 1 year in the case of glioblastomas (WHO IV). Furthermore, increasing emphasis has been placed on the need to ensure that cancer treatments associated with severe neurological side effects respect, as far as possible, the patient's quality of life. For many years, treatment of these neoplasias focused on surgical removal of the tumour. However, given their infiltrating character, surgery is unable to eradicate them completely. There have recently been various attempts to develop treatment protocols that introduce adjuvant immunological treatments (9-12) alongside traditional chemotherapies (ACNU, carboplatin, etoposide) (13,14). Human gliomas are known to be immunosuppressive and recent reports have suggested novel strategies to overcome this immunosuppression, including immunogene therapy (15,16). Moreover, in the field of gene therapy, the possibility of using oncolytic viruses, such as HSV-1, for glioma therapy (specifically of high-grade astrocytomas) is being explored (17). In some cases, the results obtained with various interleukins have been quite promising and further studies could lead to the clinical use of cytokines in some forms of cancer, including gliomas (18). The use of cytokines and growth factors in immunotherapy is justified by their capacity to boost one or more components of the cellular immune system (19). However, the immune activation status of individuals with cancer, particularly those affected by malignant gliomas, is a question that is still debated. In particular, severe immune system depression and reduced T-cell activation have been described in these patients (20). The biological basis of the interaction between nervous system cells and tumour cells has still not been fully clarified. The human immune system is extremely complex and highly specific (21). In each individual, the relationship between immune system cells and cancer cells is, by nature, unique and this makes immunotherapy studies complicated and difficult to conduct. Even though animal responses to chemotherapies, radiation treatments and surgical interventions are generally predictive of the effectiveness of these treatments in humans, the same cannot be said of immunotherapy. Many immunotherapeutic agents are inactive in other species and animal models of immunotherapy fail to "mimic" the biology of human tumours (22). New progress in the immunotherapy of cancer will require further basic studies and the gathering of a large quantity of clinical data from patients undergoing treatment with cytokines or adoptive immunotherapy.

Role of the immune system cells

In addition to cytotoxic T lymphocytes (CD8+), the literature describes numerous subsets of lymphocytes that exhibit cytotoxic antitumour activity, such as natural killer (NK) cells, phytohaemagglutinin activated killer (PAK) cells, lymphokine activated killer (LAK) cells, and interferon activated killer (IAK) cells. The existence of these subsets came to light thanks to the discovery of
cytokine genes and the subsequent availability of recombinant cytokines, which made it possible to study their effects in in vitro cultures. It appears that all these cells recognise the absence of MHC determinants of self and thus eliminate the altered, non MHC-expressing cells, for this very reason, escaped the immune surveillance of the CD8+ cytotoxic T cells. The relationship between the expression of MHC antigens on cancer cells and the recognition of these antigens by the immune system cells is currently the focus of intense research (23). To date, little information is available regarding HLA antigen as well as antigen processing machinery (APM) component expression in human malignant brain tumours. Recently, we utilized an mAb panel capable of detecting monomorphic, locus- and allo-specific determinants of HLA class I antigens, β2-microglobulin (β2m), APM components (LMP2, LMP7, TAP1, TAP2, calnexin, calreticulin and tapasin) and HLA class II antigens in surgically removed malignant astrocytic tumours by immunohistochemical (IHC) staining to investigate HLA antigen and APM component expression in human malignant brain tumours. A total of 88 malignant astrocytic tumours, differently classified according to the World Health Organisation (WHO), were investigated. The results indicated total HLA class I antigen loss in 22 out of 47 (46.8%) glioblastoma samples and in 3 out of 18 (16.6%) grade II astrocytoma samples. HLA class II antigen expression was detected in 20% of the lesions analysed. APM component expression was investigated in 44 surgically removed malignant astrocytic tumours and the staining of these MHC and tapasin expression was observed in 20% of the glioblastoma lesions analysed and were associated, although not significantly, with HLA class I antigen down-regulation. The presence of HLA antigen defects in human malignant brain tumours may provide an explanation for the relatively poor clinical response rates observed in the majority of the T cell-based immunotherapy clinical trials conducted to date (24).

The immunobiological effect of interleukin-2

Even though the new surgical techniques allow a more radical elimination of the brain tumour mass, infiltrating tumour cells can remain and are the most frequent cause of relapse of the tumour. These disseminated cells constitute the real “target” of immunotherapy using cytokines such as IL-2. The first evidence that IL-2 confers oncolytic properties on lymphocytes dates back to 1981 (25). The ability of lymphocytes to kill, in vitro, a wide variety of tumour cells led to the supposition that the inoculation of these lymphocytes into cancer patients may produce positive results. In 1985, Rosenberg et al. (26) were the first to report an antitumoral response in vivo following the administration of autologous cytotoxic lymphocytes grown in vitro in the presence of IL-2. The use of IL-2 is an innovative therapeutic strategy based on the exploitation of substances or cells normally present in the body, and it can be carried out either through the direct administration of IL-2, or through the administration of a combination of LAK cells and IL-2 (adoptive immunotherapy). Experimental studies involving the transfer of the IL-2 gene into human cancer cells have been started (27,28). Many positive results have been achieved in the local and systemic treatment of melanoma, renal carcinoma, and other solid neoplasias, which have been shown to be responsive to conventional chemotherapies. However, numerous problems have arisen in relation to the therapeutic use of LAK cells and these problems have concerned different aspects: treatment modalities, dose-dependent effects and subjective conditions of the patient. It has, in fact, been seen that the adoptive transfer of LAK cells into patients affected by tumour induces a regression of the cancer in only 20% of cases. This disappointing result may be due to a form of resistance on the part of the neoplastic cells, to incapacity of the LAK cells to reach the neoplasia in effective concentrations, or to genetic factors.

In these studies, it is crucial to establish the real correlation between the cytotoxic activity of LAK cells and doses of IL-2 (29). One major difficulty, in vivo, is created by the high doses of IL-2 that are administered to patients, given that this cytokine, in long-term therapies, has been found to be highly toxic (30). To reduce this toxicity, lower concentrations of IL-2 were used, and the first results obtained prompted a series of in vitro studies that analysed the observed interaction between the stimulated lymphocytes and specific target cells. Cells displaying LAK activity have been obtained in vitro using very low concentrations of IL-2, and it has been demonstrated that the capacity to stimulate cell proliferation or to induce cell death depends on the concentration used (31-33). Some researchers have also demonstrated that the addition of mitogen to human peripheral blood lymphocytes stimulated with different concentrations of IL-2 increases LAK activity at least tenfold compared with the use of IL-2 alone (34). These findings are encouraging from the perspective of a possible in vivo application, since if there does indeed exist a synergism between IL-2 and these mitogens in stimulating the proliferative function (rather than toxicity), it is possible to think in terms of developing new, effective immunotherapeutic methods with fewer side effects.

From as early as the start of the 1980s, opinions on the origin of LAK cells differed. Many authors, including Rosenberg (26), affirmed that these cells were part of the NK cell population. More recently, cells displaying LAK activity have been obtained from CD4+ lymphocytes (starting with in vitro cultures of peripheral blood stimulated with low doses of IL-2), and from CD8+ lymphocytes, using low doses of IL-2 in combination with GM-CSF, whose receptor is also present on cytotoxic T cells. NK cells express constitutively only the p70 chain of the IL-2 receptor (with high affinity) and thus require, in order to be activated, much higher doses of IL-2 than other cell subsets (such as the CD4+ and CD8+ subsets). Inconsistencies regarding the LAK cell phenotype may be due to differences in the different experimental conditions: different doses of IL-2, the presence of other lymphokines, the tissue used. It is also likely that LAK activity is related to a function, rather than to a clearly defined cell subset. However, there continues to be little new information regarding the basic biology of LAK cells and the questions regarding the differentiable “pathway” that gives rise to these cells remain unanswered (35).
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Immunobiological findings in malignant glioma

Numerous trials (36-38) have evaluated patients with cerebral tumours treated with various cytokines (IL-2, IL-4, IL-7) and/or through the autologous transfer of LAK cells. The rationale for the use of T-cell growth factor in these patients is based on the observation of changes in immunobiological reactivity, i.e., reduced plasma IL-2 levels, a low T cell response to mitogens, and the presence of T cells that are unable to produce IL-2. On the other hand, in other diseases (mesotheliomas, lung tumours), administration of IL-2 induces an increase of defensive cells within the tumour mass (39). Nevertheless, the interaction between IL-2 and glial cells is a fascinating but complex problem, due to the presence of the Tac receptor (IL-2R) on glial and microglial cell membranes (40). Glial cells, in normal conditions, do not express the receptor, but IL-2 acts as a factor stimulating the derepression and synthesis of the receptor. In co-cultures of glioblastoma fragments obtained immediately after the neurosurgical intervention and cultivated with low doses of IL-2, we observed, in our laboratories, an active proliferation not only of defensive cells but also of tumour cells (32). These findings suggest that IL-2 is not specific for the activation of T cells, but is a factor contributing to a general process of activation that involves different cell types. IL-2 belongs to a large group of haemoproteins that communicate via the y chain receptor, which they both have. This receptor is a mediator of intracellular communication that interacts with various kinase proteins, such as Janus kinase, and with molecules containing SH2 domains (STAT). An alternative pathway of activation, which has been demonstrated, is that of the receptor. Recent data obtained from our experiments together with our previous observations (41) on the activation of cells with immune functions and the dose-dependent induction of apoptotic phenomena (42), suggest that the benefits (or otherwise) that patients may derive from IL-2 immunotherapy depend on the tumour isotype, on the location of the tumour, and thus on the method of dose administration used. The results obtained from these ongoing experiments, while not allowing IL-2 to be considered a valid alternative to traditional treatments, are nevertheless encouraging and an incentive for the study and use of new ‘in vitro-educated’ cells, such as dendritic cells, which play an important role in the induction of immune responses (43).

References


