Monitoring Immune Responses After Glioma Vaccine Immunotherapy

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• Immunomonitoring • Immune response
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Glial tumors are the most common type of primary brain tumor, and malignant gliomas are the most common glial tumors. Furthermore, malignant gliomas are refractive to current therapeutic approaches including surgery, radiotherapy, and chemotherapy. The current median survival of patient with glioblastoma multiforme with today's standard therapy including radiation and temozolomide is 14 months.[1,2] Given their complex biology, and invasive spread along white matter tracts, an alternative treatment strategy is needed. Surgical resection cannot completely remove the numerous infiltrating neoplastic cells, and radiation subjects non-neoplastic tissues to toxic and collateral damage. Modulation of the immune system, or immunotherapy, provides the ideal candidate of therapeutic attack as it allows for specific targeting of cancer cells.[3–15] In recent years, several candidates have been identified as reasonable approaches to combating malignant gliomas.

The recent expansion of knowledge regarding tumor immunobiology and the clinical application of this knowledge has led to a need for immune monitoring technology to identify successful and unsuccessful therapies. Immunomonitoring could provide more information about the immunologic state and overall effectiveness of the intervention than the current practices of serial radiologic imaging. Given the multimodal therapies that exist, it is not surprising that there are multiple means of monitoring immune system function. This article gives a broad overview of standard approaches used to examine current immunotherapies and their clinical potential in monitoring immunotherapy for malignant gliomas.

T-CELL MEDIATED RESPONSES AND T-CELL FUNCTION ASSAYS

Based on the current understanding of the immune response to cancerous cells, gliomas create a tumor microenvironment that suppresses immune function and allows the tumor to evade the host, cell-mediated, killing pathways. In the past, cellular immune responses were detected by measuring cytotoxicity, proliferation, or the release of cellular mediators such as cytokines. These laboratory tests typically involved in vitro preincubation of antigens with cultured cells. These analyses, however, were limited in their ability to estimate the quantity of cells that existed in a given population. The advent of the enzyme-linked immunosorbent spot (ELISPOT), Tetramer, cytokine flow cytometry, and chromium-release assays provide the capability of measuring clonal expansion of populations of antigen-responsive T cells.

A study by Galon and colleagues[16] demonstrated that T-cell presence in colorectal cancer tissue is predictive of overall prognosis. Furthermore, data has demonstrated that T-regulatory cell activity increases in patients with known malignant gliomas.[17] These cells are thought to

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later suppress cytotoxic T-cell activity and reduce the systemic response to tumor burden. Current clinical trials are monitoring systemic T-cell responses after vaccinations.

ENZYME-LINKED IMMUNOSORBENT ASSAY

Proteins that are secreted into the extracellular space, by tumor cells or neighboring host immune cells, can be monitored to identify multiple variables. These agents can identify if the host is amounting an attack on the tumor cells. It can also identify if the tumor is actively evading immune surveillance. Therefore, understanding the composition of circulating proteins in the intercellular space of tumor and self tissue provides valuable therapeutic and prognostic implications. The enzyme-linked immunosorbent assay (ELISA) was first developed in 1983 by Czerinkinsky and colleagues for the purpose of identifying and quantifying antibody secreting cells. It was later also used for quantifying antigens. The assay involves the administration of a known antibody to a solution of unquantified antibody or antigen. The known antibody is tagged with a known signal that is used for analysis and identification. It is common practice to add a second antibody to bind to the first administered antibody, to amplify the signal that is produced.

ELISAs are highly sensitive, reliable, and accurate tests.[18] This method of analysis will prove invaluable in different methods of monitoring the immune response for various different therapies due to its accuracy and wide applicability in protein analysis.

ELISPOT

The ELISPOT assay is a modified version of the ELISA immunoassay. ELISPOT assays were originally developed to quantify B cells secreting antigen-specific antibodies. They were later used to quantify the agents that were secreted by these cells, such as cytokines.[19] The ELISPOT assay provides both qualitative (type of immune protein) and quantitative (number of responding cells) information. The ELISPOT assay is exceptionally sensitive, capable of detecting cells that secrete 100 molecules of a given protein.[19] This exceptional sensitivity is because the protein of interest is rapidly captured around the secreting cell, before it is either diluted in the supernatant, captured by receptors of adjacent cells, or degraded. There is growing interest in increased use of ELISPOT as a measure for cytotoxic T lymphocytes (CTL) responses, in large part because it is reliable and highly sensitive. Results from various clinical trials, including peptide and whole tumor cell vaccination and cytokine treatment, are now available and show the suitability of the ELISPOT assay for monitoring T-cell responses. The ELISPOT technique allows for quantification of tumor-specific T lymphocytes from peripheral blood by detecting antigen-induced cytokine secretion.

TETRAMER ANALYSIS

A tetramer assay is used to detect the presence of antigen-specific T cells. In order for a T cell to detect the peptide to which it is specific, it must both recognize the peptide and the major histocompatibility complex (MHC) at the surface of a cell as it contacts it. Because the binding affinity of a T-cell receptor (TCR) to MHC complexed with a peptide is so low, creating a sensitive assay was historically difficult. This problem was solved by creating a tetramer of MHC molecules in which all four molecules present an identical peptide. In this manner, the avidity of the binding of the T cell of interest was increased. The resulting mechanism of the assay involves adding the MHC-peptide tetramer to an unknown quantity of T cells. The tetramer is then bound to the T cells that recognize the peptide sequence of interest. The tetramer is then stained typically by fluorescence labeling and bound T cells are measured using flow-cytometry.

Currently tetramer analysis has been used successfully in several phase I and II clinical trials for advanced staged melanoma vaccine trials and malignant gliomas.[14,15] When combined with functional analyses such as staining for specific cytokines, tetramer analysis can provide valuable information about T cells and their activation state.

CHROMIUM RELEASE ASSAY

The chromium release assay was developed by Brunner and colleagues[20] and quantifies CTL activity by measuring target cell lysis. The process involves bathing target cells with chromium. These labeled cells are then mixed with various amounts of effector T cells. The killing of these labeled cells causes a release of chromium into the supernatant after cell lysis. The accumulated chromium is quantified and compared with spontaneous release rates, to quantify functional CTL activity. Results are typically reported in lytic units.

This assay is dependent on the binding of CTL to target cells via TCR-binding and other accessory adhesion molecules. The crosslinking and binding of the TCR-receptor complex initiates the T-cell lytic process. The lytic process can occur over

several minutes to several hours, and variations in any of the above-mentioned processes can alter results. Consequently, the chromium release assay is not a good assay to examine slow-acting mediators of cytotoxicity. Furthermore, the development of tetramer staining of MHC class I complexes has provided a more rapid and sensitive method of quantifying antigen-specific CD8 T cells.

DISCUSSION

Given the multimodal nature of immunologic therapy regimens today. Monitoring the immune response to immunotherapy covers a number of technological modalities and a broad understanding of current practices in immunotherapy. As these therapies continue to take a foothold in current clinical practice, a basic understanding of the principles, techniques, and weaknesses of these assays should be understood. Of the above-mentioned assays, the ELISPOT has been used the most frequently for monitoring of vaccination trials. Its frequent use is a direct result of its highly sensitive capabilities in detecting and quantifying antigen-reactive T cells.[21]

Several pitfalls of immune monitoring have been noted and often provide some difficulty in analyzing gathered data. A great potential source of variability lies in the heterogenous nature of the tumors being evaluated. It is commonly understood that features that make tumors highly aggressive make them also highly variable in their genetic and pathologic phenotype. In malignant gliomas alone, the location of the tissue and the medical comorbidities of the patients can also affect findings. Many of these patients have undergone, and are still being treated with other previous therapies that could affect immune function. The frequent use of dexamethasone in combating brain edema and the use of Temodar, and more recently Avastin, have unknown effects on current immune monitoring practices.

Correlation with Clinical Outcome

The ultimate question that remains, does immunologic monitoring provide any prognostic significance? Although little data exists in the field of immunotherapy of gliomas, data gathered from systemic cancers suggests that immunologic response may be important to prognosis.[22]

One reason for the lack of correlation between immunologic response and clinical outcome may be the complexity of the responses required for an anti-tumor response. For example, cytokine production, usually interferon gamma, by T cells in vitro, in response to antigen-specific or polyclonal stimuli, is measured in an attempt to demonstrate type 1 helper T cells cellular responses.[13]

Moreover, it has been clearly shown that the presence of a high number of circulating effector-type T cells is not enough to induce clinical efficacy.[23–27]

Reliability of Assays

Many of these assays are currently used at the benchside for accurate and reliable measurements of the presence and quantity of antibody or antigen. The highly controlled nature and systematic controls offered with lab work may not be practically or financially feasible for clinical use. Significant work in producing clinically accessible assays is still left to be fully explored and developed.

Standardization of Immunological Monitoring

Harmonization of methods for monitoring the induction of antigen-specific T-cell responses in clinical vaccination trials has been identified as a key area of development within the field. The purpose of monitoring immunologic outcomes in response to immunotherapeutic treatments is fourfold:

1. To determine the effectiveness of a vaccine to elicit the correct type of immune response. This will further aid in understanding the nature and dynamics of the response elicited by a particular modality and provide proof-of-principle for ensuring trials.
2. Determination of elements of the immune response that correlate with clinical response. This is vital to understanding the dynamics and magnitude of immune responses that are required to elicit clinical responses.
3. Armed with such knowledge, immunologic responses may be uses as surrogate markers for clinical responses, which may ultimately be useful, if validated, as end-points in the later clinical trials.
4. There is an increasing recognition of the importance of obtaining immunologic data that are comparable between different clinical trials.

This area has been identified as highly important in developing to help move the field forward. Therefore, several consortiums have been created to address this issue, including the Immunoassay Proficiency Panel (organized by the Cancer Vaccine Consortium) and the Cancer Immunotherapy Monitoring Panel.

The future of immune monitoring

It is become increasingly clear that monitoring T-cell function in the patient with malignant cancer will be one of the mainstays of clinical oncologic practice. The ultimate goal for immunomonitoring in immunotherapy is to provide enough information to influence decision-making for later therapy or therapies.

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