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Evaluation of Cancer Immunity in Mice

Mary L. Disis[1,3] and **Karolina Palucka**[2,3]

[1]Tumor Vaccine Group, Center for Translational Medicine in Women's Health, University of Washington, Seattle, Washington 98109

[2]Baylor Institute for Immunology Research, Dallas, Texas 75204 and Department of Oncological Sciences, Mount Sinai School of Medicine, New York, New York 10029

Abstract

There have been significant advances in the development and application of novel therapeutic approaches and improved diagnostics for cancer in the past decade. Manipulation and/or assessment of cancer-specific immunity have been central to these advances. Murine models are a standard for the preclinical development of cancer immunotherapeutics. However, critical advances in our understanding of the role of the immune microenvironment and the assessment of cancer-specific immunity have not been fully applied to rodent models. Methods to preserve the function of immune cells after cryopreservation and standard approaches to quantitative immune assays have not been developed. Furthermore, a detailed evaluation of the immune tumor environment, which can impact a clinical response to different agents, is rarely undertaken as models are being contemplated. Rapid translation of immunoncology agents to the clinic will require standardization of immunologic assay methods and a more detailed immunologic characterization of common mouse models. Outlined here are the critical elements in assessing immunity in cancer mouse models and suggestions concerning the standardization of approaches when using these models for the study of immunoncology.

Introduction

Innovative immunotherapeutics have entered the clinic largely based on the recognition that immune cells and their mediators, when chronically engaged, may both hinder and foster tumor development. Along with stimulating specific immune responses, modulating immune-related receptors and proteins in the tumor microenvironment has become an area of intense clinical investigation. Indeed, the immune microenvironment has been shown to be an important determinant of response to standard therapies in cancer patients (Denkert et al. 2010; DeNardo et al. 2011).

Murine models have long played a major role in the evaluation of immune-based therapies for cancer. Standardized methods for cryopreservation of murine lymphocytes, measuring adaptive or innate immunity and characterizing tumor immune infiltrates, have not been fully developed across multiple mouse models as such assays have been for humans.

[3]Correspondence: ndisis@uw.edu; karolinp@baylorhealth.edu.

Developmental work is needed in several areas to improve the utility of mouse models as predictors of optimized cancer immmunotherapeutics.

Cryopreservation of Murine Lymphocytes and Immune Cells

Studies with human T and B cells have showed that there are several components in the cryopreservation process that can adversely impact the function of lymphocytes once they are thawed (Disis et al. 2006). These components include the additive in which the cells are frozen, the temperature at the time of thawing, and the length of time between thawing and use of the cells in assays. Extensive analysis of these parameters for cryopreservation of human lymphocytes has resulted in the adoption of standard operating procedures (SOPs) for the freezing and thawing of human lymphocytes to maintain basic immune functions such as antigen-specific proliferation and cytokine production and, thus, assay validity. Detailed evaluations and procedures for the standardization of cryopreservation for murine lymphocytes to retain immune function have not been published. In a recent review of procedures collecting, processing, and storing T-regulatory cells (T_{reg}), a subtype of $CD4^+$ T-lymphocytes, authors catalogued numerous methods of processing both murine and human T_{reg} (Daniele et al. 2011); however, no SOP or consensus protocol was presented.

Cryopreservation of murine lymphocytes to retain immune functionality is important for several reasons: (1) to facilitate sample sharing for the development of new immune-based technologies for the assessment of adaptive and innate cancer associated immunity, (2) to allow replicate assay performance adding to overall data quality, and (3) to allow batch analysis of similar experimental groups potentially decreasing assay variability. In general, cancer-specific immunity is of a lower functional avidity, different Th phenotype, and, in most cases, of a much lower magnitude than immunity to infectious agents. For these reasons, protocols developed in infectious disease research may not be directly applicable to the analysis of cancer-specific immune responses.

Good viability of lymphocytes (>70%) after cryopreservation and thawing has been shown to be associated with retained immunologic function. Preliminary studies with murine lymphocytes showed that viability is preserved when the lymphocytes are thawed at 37°C and the freezing media has been screened for the ability to maintain both viability and recovery of cells (E Gad, University of Washington, pers. comm.). Indeed, in a screening study of five commercially available media suitable for cryopreservation, two of the five products resulted in <70% viability after thawing in more than 20 specimens tested. Studies are ongoing to detail the elements of a protocol for cryopreservation of murine lymphocytes for immunoncology research. At minimum, investigators should record and report the median and range of viability of murine lymphocytes, when thawed, as a potential source of assay variability.

Standardization and Standard Operating Procedures for Immune Assays

Over the last decade, there has been an explosion in the development of quantitative immune assays to both characterize and enumerate tumor-specific T- and B-cell responses that are suitable for use with both human and murine cells. Immune competent murine models of common cancers could allow a greater understanding and/or delineation of useful immune

biomarker candidates for validation in human clinical trials, but there has been little effort to develop the standardization needed to intensely mine rodent models for immune biomarkers that could be surrogates of clinical response after immune therapy.

The two major quantitative assays used in translational immunoncology in both humans and rodents are enzyme-linked immunosorbent spot assay or ELISPOT and flow cytometry. ELISPOT is a more labor-intensive assay than flow cytometry and requires the stimulation of lymphocytes with antigen- and protein-based assessment of cytokine secretion. There are many sources of variability in ELISPOT. First is the length of time the T cells are allowed to produce cytokine after antigen stimulation and whether two or more stimulations are needed to obtain signal. The longer the incubation the greater the potential for cytokine production from unstimulated cells that will increase the background of the assay and lower sensitivity. As stated above, cancer-specific responses are often of low magnitude and the number of lymphocytes plated in each well may impact signal. The greater the number of lymphocytes, the more likely low signal will be detected. However, sensitivity may also be decreased by an increase in the background due to nonspecific secretion by resting cells. Finally, appropriate cryopreservation methods that preserve lymphocyte viability will impact assay validity (as discussed above). Studies of immunomodulation in mouse models should include basic assay development to ensure high antigen-specific signal with low background responses.

Flow cytometric methods are less labor intensive but are highly dependent on quality reagents. Monoclonal antibodies used to stain surface receptors or intracellular cytokines can vary greatly in their avidity for target and their utility in a variety of assays. If a specific antibody works well for immunohistochemical staining, that same antibody may not be as effective in flow cytometry. Flow cytometric analyses should use positive controls to titer antibody concentrations to optimize signal. Groups such as the National Cancer Institute–supported Mouse Models of Human Cancer Consortium are creating reference lists that provide usage data on common immune-related antibodies along with performance comments and suggested starting concentrations (see further description below).

A final consideration in assessing the validity of immune response data in any murine model is the genetic background of the model itself. As inbred strains, it is well known that T cells in mice can be skewed to a predominant Type I versus Type II response. Specific strains are commonly used in studies of infectious disease to evaluate lack of response to infectious agents, but a catalogue of these differences has not been made for cancer models. For example, FVB, a common strain for transgenic mice construction, have a Th2 bias in cellular immunity as compared to a strain such as BALB/c (Kim et al. 2012). The phenotype bias of a particular strain may greatly impact immunologic monitoring as well as ability to respond to immune-based cancer therapies. There are no existing protocols to fully assess Th1/Th2 skewing before the use of a particular strain. A thorough evaluation of the literature should be the first step when contemplating a particular model. The use of two or more strains in testing cancer immunotherapeutics is an alternative. Finally, some information may be gleaned by stimulating T cells with nonspecific mitogens and assessing whether the immune response elicited is predominantly Th1 or Th2 (Christie et al. 1999).

Characterization of the Tumor Immune Environment

There are many rodent models available for the translational evaluation of immune-based cancer therapeutics. Rarely are tumors developing in those models fully characterized at baseline for infiltrating immune elements or other factors that might influence data quality. Given the functional relevance of immune cellular networking in tumors, it is imperative to incorporate immunometrics such as “the immunoscore” (a score including a composite of immune cellular and protein biomarkers assessed in tumor tissue) (Fridman et al. 2011) into traditional experimental schemes. These predictors, if present in rodent models, could greatly assist with defining a hierarchical prioritization among future newly developed immune therapeutic approaches.

Tumor infiltrating $CD8^+$ T cells, memory T cells, and a lack of infiltrating immune regulatory cells have been shown to be associated with clinical outcomes across several human cancer types. In melanoma and colon cancer such infiltrates provide prognostic information that adds power to already existing staging systems (Bindea et al. 2010). As stated in the introduction, immune infiltrates in tumors may contribute significantly to clinical response to common cancer therapies. An “immune score” has not been developed for rodent tumors nor have immune infiltrates been assessed extensively in common rodent models used for the study of cancer immune therapies. Our lack of understanding of the baseline immune microenvironment in therapeutic models may be a source of variability in both the preclinical and correlative science assessment of new agents and a major reason for a lack of correlation of rodent models with disease outcomes in human clinical trials.

An evaluation of a basic panel of markers should be the standard for the minimal characterization of the murine tumor immune environment. Such a panel would include analysis of infiltrating cells similar to the human immune score (T cells infiltrating tumor), regulatory elements; both cell- and receptor-based (T_{reg} and myeloid-derived suppressor cells [MDSCs]), and markers that would further phenotype the immune microenvironment (e.g., PD-1, PDL-1, Tbet, GATA3, CTLA-4) (W Fridman, R Schreiber, E Engleman, et al., pers. comm.). Work is ongoing to determine appropriate antibodies and methods for the development of a murine immune score. SOPs must be created for immune assessment with the expectation that baseline data will be provided as an essential component of overall data interpretation.

One key component in determining the extent of immune infiltrates in a tumor is the use of monoclonal and polyclonal antibodies in the context of flow cytometry or immunohistochemical staining. Commercial antibodies vary greatly in quality for specific procedures. Members of the Mouse Models of Human Cancer Consortium have instituted a glossary of validated reagents and protocols that are useful for functional phenotyping of the immune system and immune environment in murine cancer models: http://emice.nci.nih.gov/characterizing-models/mice/antibody/?searchterm=antibodies. The glossary includes basic information on reagent performance, concentrations needed for signal detection, and clone numbers for easy identification.

Conclusions

Detailed immune characterization of common rodent models and development and standardization of immune-based assays, methods, and operating procedures are needed to better use mouse models for the rapid clinical translation of immune-based cancer therapy. Full characterization of the tumor immune microenvironment and an ongoing quality assurance plan for immune monitoring should be put in place to maintain data reproducibility during the period of model use. These data are also key for understanding response, or lack of response, when evaluating immune-based cancer therapeutics.

References

Bindea G, Mlecnik B, Fridman WH, Pagès F, Galon J. Natural immunity to cancer in humans. Curr Opin Immunol. 2010; 22:215–222. [PubMed: 20207124]

Christe M, Rutti B, Brossard M. Influence of the genetic background and parasite load of mice on the immune response developed against nymphs of Ixodes ricinus. Parasitol Res. 1999; 85:557–561. [PubMed: 10382605]

Daniele N, Scerpa MC, Landi F, Caniglia M, Miele MJ, Locatelli F, Isacchi G, Zinno F. T_{reg} cells: Collection, processing, storage and clinical use. Pathol Res Pract. 2011; 207:209–215. [PubMed: 21397410]

DeNardo DG, Brennan DJ, Rexhepaj E, Ruffell B, Shiao SL, Madden SF, Gallagher WM, Wadhwani N, Keil SD, Junaid SA. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer Discov. 2011; 1:54–67. [PubMed: 22039576]

Denkert C, Loibl S, Noske A, Roller M, Müller BM, Komor M, Budczies J, Darb-Esfahani S, Kronenwett R, Hanusch C, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol. 2010; 28:105–113. [PubMed: 19917869]

Disis ML, dela Rosa C, Goodell V, Kuan LY, Chang JC, Kuus-Reichel K, Clay TM, Kim Lyerly H, Bhatia S, Ghanekar SA. Maximizing the retention of antigen specific lymphocyte function after cryopreservation. J Immunol Methods. 2006; 308:13–18. [PubMed: 16337957]

Fridman WH, Galon J, Pagès F, Tartour E, Sautès-Fridman C, Kroemer G. Prognostic and predictive impact of intra- and peritumoral immune infiltrates. Cancer Res. 2011; 71:5601–5605. [PubMed: 21846822]

Kim EM, Bae YM, Choi MH, Hong ST. Cyst formation, increased anti-inflammatory cytokines and expression of chemokines support for *Clonorchis sinensis* infection in FVB mice. Parasitol Int. 2012; 61:124–129. [PubMed: 21820080]