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The dynamic human immune response to cancer: it might just be rocket science

"We have recognized that dynamic immune responses in cancer may mirror natural self-organizing processes ... and, as such, are amenable to hypothesis testing and data-driven predictive modeling."

KEYWORDS: angiogenesis ■ biorhythm ■ melanoma ■ mouse model ■ placenta ■ predictive modeling ■ tolerance

The immune-mediated eradication of cancer is made difficult both by immunosuppressive changes that occur within the tumor micro-environment as well as by systemic effects that lead to chronic inflammation and immunologic exhaustion. At present, the temporal dynamics and interconnectivity of these local and systemic impediments to antitumor immunity are poorly understood. Over the past several years, our laboratory has identified methods to conceptualize and study the complex immunobiologic interactions driving the balance between tolerance and immune activation over time in cancer patients. We have recognized that dynamic immune responses in cancer may mirror natural self-organizing processes, such as species dispersal into new ecological environments [1] and suburban sprawl [2] and, as such, are amenable to hypothesis testing and data-driven predictive modeling. In the following sections, we describe our approach to understanding alterations in immunity that occur as a result of the malignancy, as well as challenges posed by animal models to study this phenomenon. We show that tumor-induced immune modulation facilitates metastases and how comparative models for tolerance may help us better understand this process. Finally, we discuss a method for discovery of immune biorhythms and predictive modeling of immune responses developed with the help of aerospace engineers. With our multidisciplinary approach, we are developing methods of dynamic immune network recognition that embrace the complexities of tumor/immune interactions with the goal of real-time, individualized, curative immunotherapy for cancer patients.

Many immunotherapeutic strategies have been investigated in the treatment of advanced malignancies, including our laboratory's primary

focus: melanoma. Frequently, immunization with tumor-specific vaccines elicits measurable peripheral cytotoxic lymphocyte responses; however, associated clinical responses are seldom seen. One potential explanation for the suboptimal clinical benefit observed with cancer vaccines is that activated immune cells are rendered ineffective upon entry into the immunosuppressive tumor microenvironment, a subject of extensive exploration by our lab and others [3]. A different and complementary explanation is that immunosuppressive factors in the tumor microenvironment may impair not only local immune responses but also disturb systemic immunity. To test this hypothesis, we performed a comparative analysis of peripheral blood mononuclear cells, plasma and RNA microarray of tumor tissue of melanoma patients of all clinical stages to determine if proteins expressed by tumor cells are present in plasma and whether tumor-associated proteins have any consequence on systemic immunity [4]. We found that melanoma tumor specimens highly expressed vascular endothelial growth factor (VEGF). In plasma, high levels of VEGF were detected in patients with stage IV melanoma relative to healthy controls. In addition to VEGF, high levels of Th2 cytokines (IL-4, IL-5, IL-10 and IL-13) were measured in plasma of stage IV patients, suggesting a global state of chronic inflammation. VEGF, now increasingly recognized as an immunosuppressant [5], was shown to increase IL-4 and decrease IFN- γ production by CD4⁺ T cells in a dose-dependent fashion. Thus, Th2-associated chronic inflammation in stage IV melanoma patients is due at least in part to tumor-derived VEGF, a therapeutically targetable mediator neutralization of which (using bevacizumab) has already suggest clinical benefit in advanced melanoma [6].

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To understand the impact of VEGF and other tumor-derived mediators on tumor–host interactions *in vivo*, we comprehensively studied systemic immunity in tumor-bearing mouse models. Animal models have been used extensively to advance our understanding of the complex biological processes involved in angiogenesis and inflammation at the tumor–immune interface. Studying *in vivo* interactions between tumors and the immune system in model animals has provided insight into key immune mediators of tumor rejection and tolerance and has identified promising immunotherapeutic strategies. However, while vaccines have eradicated tumors in rodent models [7], the results of human immunotherapy clinical trials have been far less robust [8]. Our comparative studies of tumor–immune interactions in mice and humans focused on our observation of the role of VEGF in driving Th2-biased immune responses. While human T lymphocytes change polarity from Th1 to Th2 predominance, we found that mouse lymphocytes are unaffected by VEGF exposure [9]. Furthermore, the high levels of Th2 cytokines present in human cancer patients are not found in mice with advanced tumors, and lymphocytes from mice with advanced tumors function in a manner indistinguishable from lymphocytes from healthy mice. Based on these differences in tumor–immune interactions between mice and humans, we have focused on studying humans whenever possible.

“We discovered an immune biorhythm through a longitudinal analysis of time-dependent profiles of 52 different immune biomarkers ... in 11 patients with metastatic melanoma.”

Faced with the challenge of identifying appropriate models to study the evolution of immune responses in melanoma, we recognized that tumor draining lymph nodes afford a unique view into early immunologic reactions to melanoma. They are a site of contact between tumor-associated antigens and the adaptive immune system, as antigen-presenting cells migrate to these nodes to direct the immune system's response to melanoma. They also represent the first site of metastasis of melanoma and are exposed to an efflux of soluble, primary tumor microenvironment-derived soluble mediators (immunomodulatory cytokines). Using immunohistochemistry, we profiled human melanoma sentinel lymph nodes (SLNs) in order to characterize regional immunity in patients with various

stages of melanoma [10]. We were somewhat surprised that we did not find any differences in expression of multiple immunologic biomarkers between SLNs with or without metastasis. In addition, when compared with control lymph nodes, SLNs of patients with melanoma had fewer cytotoxic T cells and diminished expression of transcription factors for Th1 (T-bet) or Th2 (GATA-3) polarity. These results suggest that at a very early stage, a primary melanoma tumor is communicating with the draining lymph nodes, preparing the lymph nodes for seeding of metastatic disease by suppressing immunity. Immune dysfunction of the SLN may be a prerequisite for the metastatic cells to establish disease within the SLN. Ongoing studies are aimed at elucidating the soluble/cellular factors driving this regional immunosuppression.

“Although many of our studies have focused on immune responses that develop over weeks to months, we have recently realized that systemic immunity in patients with metastatic melanoma is a dynamic process that fluctuates over a much shorter time period.”

To better understand the complex mechanisms of regional immune regulation in the context of a growing tumor, we searched for a physiologic model of regional immunosuppression with distant immune regulation, emulating what we had increasingly realized was taking place in melanoma. The transient organ-system-based immune evasion characterized by placentation with the accompanying changes in systemic immunity during normal pregnancy is of keen interest to our group [11]. We have found that many molecular similarities exist between invading cells of the placenta and melanoma [HOLTAN S, MANUSCRIPT SUBMITTED]. Furthermore, we identified that a shift in NK cells toward an increasingly regulatory phenotype critical to tolerance induction during early pregnancy, also occurs in patients with metastatic melanoma, suggesting a parallel in immunoregulation of pregnancy and cancer [12]. Also of interest to our group is the observation that the maternal immune response to pregnancy is not static but rather a dynamic condition associated with tolerance in the early months followed by acute inflammation and maternal immune reconstitution as parturition approaches [13]. Further studies into the immunobiology of pregnancy may help us learn more about an angiogenic/endocrine/immunoregulatory network that appears to be reversible and

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1023

possible by a concerted effort of a multidisciplinary team of experts spanning basic science, clinical research and data analysis/modeling. While none of us could have anticipated that we would be looking to the stars for a cure for cancer, we are confident that, as with other notable previously insurmountable challenges facing humanity, applying a focused/multifaceted approach to the problem could lead us to success.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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