Opinion

Tricking the tumour microenvironment into becoming our best rational drug design factory: reversal of immune suppression

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Abstract

The immune cellular components of the tumour microenvironment are a diverse group of cells that paradoxically are now appreciated to have a coordinated opposing duality of either promoting or retarding tumour growth. Manipulating this seemingly dynamic interaction for therapeutic benefit is a hotbed of much research. Recent findings in tumour immunology (both preclinical and clinical) build on more than a century of insights and provide a way forward to improving patient outcomes, long term survival and the predictability of "cures". This opinion piece attempts to summarise some of these historical and contemporary insights with a view to describing eminently testable therapeutic solutions.

Keywords: Tumour microenvironments, immune suppression, regulatory T cells, immune modulation, reversal, plasticity, spatio-temporal

INTRODUCTION

In 1926, Morris Fishbein, MD, the editor of the *Journal of The American Medical Association* (*JAMA*), wrote, "*Cancers must be treated early, once a cancer has spread to surrounding tissue all attempts to halt the disease are usually hopeless*"[1].

In 2008, the United States Presidential Report on Cancer stated, "*The toll of cancer has become simply an awful part of life; incidence is rising for several cancers; the most intransigent of malignancies remain impervious to treatment; (and) an absolute cure remains elusive*". The report further added, "*Despite declaring a national war on cancer in 1971 and investing many billions of dollars since then to understand and defeat cancer, our success against the disease in its many forms has been uneven and unacceptably slow*"[2]. Previously in 1996, Director of the Pittsburgh Cancer Centre Michael Lotze said, "*Mono and multi-agent chemotherapy just do not work in many settings, we should have dispensed with these notions years ago*"[3]. This echoed the sentiment in Scientific American in a previous 1994 article entitled A War Not Won "*Despite dramatic scientific gains, cancer remains an undaunted killer*"[4].

Clearly, these sorts of concerns led Laurence Baker, MD, senior US oncologist and Chairman of the Southwest Oncology Group (SWOG) to state in 2010 in the *Journal of the National Cancer Institute* (*JNCI*) "*A cure is the expectation of society, we are not taking that seriously enough; We have a system that doesn't even really try to meet that expectation; I am trying to get people to stop saying how successful the cancer research enterprise is. It is not true. It is just not true*"[5].

Even today, in the midst of a "transformative" immunotherapy era of checkpoint inhibitor therapy, only a minority of patients derive benefits and at the cost of substantial biological and financial toxicity. Despite these negative and troubling historic comments, there is room for optimism.

"Nature often gives us hints to her profoundest secrets" - William B Coley[6]
For more than a century and since William Coley and his bacterial toxins, the notion that the immune system can be harnessed to treat cancer has met with limited and sporadic clinical success and much controversy[7]. And like cancer therapy today, it has been hit and miss. Those occasional spectacular "miracles" under various "immune tweaking" treatment modalities has intrigued clinicians and scientists alike and fuelled the relentless enthusiastic pursuit to make these random minority of successes and long term survival a reality in most patients. How can we translate/duplicate/amplify the efficacy at least seen in the mouse experiments? A broad explanation of why these sporadic successes occur infrequently was articulated by Prof Lloyd Old in 1993.

"

*Why hasn't Coley's approach been forged into a widely available therapy with a predictable benefit for cancer patients? The best reason is, - the cellular and molecular language of inflammation and immunity had to be understood before the forces that Coley unleashed could be predictably translated into tumor cell destruction".
- Lloyd Old 1993*[7].

His words back then actually described the way forward, detailed aspects of which are in the process of being elucidated today for clinical utility. Clearly, the correct sequence of therapeutic events already happens, at least randomly in some patients, even with the crudest approaches such as those used by Coley. The fundamental understanding (language) of the intimate interactions of the tumour with the immune system, particularly in the tumour microenvironment (TME), is providing answers to why some very different modalities can work effectively but only occasionally.

The cellular and molecular language of inflammation and immunity
A major advance in recent years has been the realisation that the immune system is not ignorant to the presence of cancer, and in particular, the cellular interactions of the TME collectively are "shielding" the cancer from immune destruction[8]. Specifically, the immune system is suppressing itself, tolerant to the

growing tumour burden and creating substantial multilayered barriers to therapeutic success[9]. Remove or interfere with the mechanistic components of these immune circuits; these barriers can be broken down and can lead to tumour destruction, complete responses and improved survival[10]. Arguably, the most important critical lesson learnt with respect to the TME induced immune suppression is the realisation that this suppression has been caused by normal immune homeostasis. This homeostatic impasse is an intentional part of the way the immune system processes antigens and stops unwanted inflammation and damage to surrounding "normal" tissue. Importantly and recently, this impasse is being appreciated as being reversible[11]. Understanding a few simple rules of this homeostatic process has the potential to remove the "hit and miss" randomness that currently exists in cancer therapies.

So who is the real enemy here, the cancer or the immune system?

After decades of failure of tumour-centric approaches to cancer therapy, attention has gradually now turned to our immune system to do the "heavy lifting".

Irrespective of tumour morphology, patients with advanced cancer exhibit simultaneous immune activation and suppression[12]. While the TME consists of both cancerous and non-cancerous cells, most of these subpopulations cooperate synergistically to drive via positive feedback loops that are conducive to tumourigenesis. Further, the resultant local inflammatory environment appears to be a consistent component of malignant tumours and displays increasing concentrations of cytokines locally and systemically, particularly with rising disease burden[13]. Malignant tumours can significantly interfere with the patient's immune system, leading to fevers, paraneoplastic autoimmunity and sepsis[14,15]. Thus, despite a general environment of immune stimulation, evidence suggests that anti-tumour immune responses are being continuously attenuated, and this appears universal across the tumour types[16,17].

The immune system has evolved the ability to recognise, destroy and remember foreign or corrupted peptides and antigens that are detrimental to our survival. Estimates suggest that the somatic hypermutation/recombination mechanisms to generate antigen-specific receptors in T cells can program for as many as 10^{15} possible unique receptors[18]. This diverse repertoire suggests that a functional immune system should be able to accommodate cancer adaptability and mutational drift. In support of the aforementioned, laboratory assays can detect T and B cell responses (autoantibodies *etc.*) to tumour-associated antigens and therapy-induced neoantigens[19-21]. Also, assays can detect attenuating antigen-specific regulatory T cell responses and indicative pro-inflammatory and immune-suppressive cytokines/inflammatory markers[22,23].

The immune cells of the TME and their function

The TME can consist of a diverse immune cellular presence, including T and B lymphocytes, natural killer (NK), natural killer T (NKT) cells and regulatory T cells (Tregs). Tregs comprise diverse subsets of immunosuppressive cells that play critical roles in not only maintaining immune homeostasis and self-tolerance, but also suppressing antitumour responses of cytotoxic lymphocytes. Other important cells include tumour-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCS). All these cells can account for circa 10% of the total tumour cell population and can also be found in substantial concentrations within the tumour and in the tumour periphery. Collectively, they are major drivers of an immunosuppressive TME. TAMs exhibit both anti- and pro-tumoural effects. The high density of TAMs is a characteristic of most tumours and has been correlated to poor clinical outcomes[24-26].

Within the TME, TAMs can polarise to M1-like pro-inflammatory interferon-γ phenotype or the anti-inflammatory/immunosuppressive M2-like phenotype, which induces the secretion of IL-10 and TGF-β to limit inflammation, enhances tissue repair, and promotes vascularisation. While these two TAMs are

dominant, there is a high degree of plasticity and intermediaries. Macrophage M1/M2 polarisation appears to be transient, time and tissue-associated[27]. The M1/M2 phenotypes can shift in response to stimuli in the TME. As the "vanguard" antigen processor of the host immune response, TAMs offer the possibility to use their plasticity to modulate the underlying immune suppression, break tolerance and potentially improve clinical outcomes[28]. MDSCs can inhibit antitumour activities of T and NK cells and stimulate Treg, leading to tumour progression through the production of cytokines IL-10 and TGF-β[29].

In concert, all these aforementioned diverse immune cell types homeostatically maintain a "tumour-friendly" microenvironment both locally and systemically under a growing tumour burden. This all points to an underlying tightly controlled, dynamic "three-way conversation" between tumour cell populations, the pro-inflammatory and the immunosuppressive immune response circuits. Individually and collectively, these cells offer opportunities as therapeutic targets.

The positive and negative roles of cytokines within the immune response

Cytokines are produced by a plethora of immune other cells. Both APCs and T and B lymphocytes produce various cytokines cells in response to antigen processing and recognition. The amount of cytokine produced is dependent on the amount of antigen encountered and/or its antigenic potential. Interestingly, a number of specific cytokines play a major role paradoxically in both pro-inflammatory and immunosuppressive immune cellular pathways, either initiating or homeostatically terminating that immune response. In addition, another physiological/mechanistic insight is the transient actions of cytokine/receptor cellular interactions together with normal short half-life restrictions. This temporal aspect contributes to the ability of cytokines to have selective loco-regional/systemic effects[30-32].

Three cytokines, in particular, are now appreciated to have opposing duality or bimodal attributes and have a long history in cancer immunotherapeutics. They are interleukin-2 (IL-2), interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α). Normally, all have relatively short physiologic half-lives[33-35].

This apparent temporal duality or paradox has confounded their clinical utility of knowing how to administer these cytokines (with respect to dose and duration) for the best clinical effect. In particular, IL-2 has an extensive 30-year history in treating advanced malignant melanoma (MM) and renal cell carcinoma RCC. In both incidences, on average, ~7% of patients treated with IL-2 achieve complete responses and long-term survival after limited treatment[36].

Known as the "master cytokine" IL-2, when this agent works successfully, it is clearly modulating an underlying/pre-existing tumour-specific immune response. The paradox arose in the mid-1990s when it was discovered that IL-2 also stimulates Tregs and can suppress an immune response[37]. Thus, this provided an explanation for its limited and random efficacy. Similar duality opposing activity over Tregs was later elucidated for IFN-γ and TNF-α[34,35]. More recently, similar attributes have been reported for PD-1 & CTLA4 monoclonal antibodies (Mabs) as their targets are also expressed on Tregs, and blockade can cause tumour hyperprogression and self-limit efficacy[38,39].

Antigen load, recognition and tolerance induction - a critical insight

Improved understanding of the immune system's role in cancer has reinvigorated research on the interplay between antigen load and immune tolerance induction. Indeed, elegant work by Gratz *et al.*[40] and Pinheiro *et al.*[41] has shown that initial low levels of antigen promote Tregs and subsequently control the balance between T-effector lymphocytes & Tregs and thus the balance between responsiveness and tolerance. This is a critically important insight into the fundamental nature of cancer immune suppression

and antigen as a "prime mover" in the immune response. Moreover, this immune balance is mediated by an IL-2 centric feedback loop. Importantly, plasticity exists at the cytokine level between these two states[42,43]. A concept allergists are familiar with is the introduction of low dose antigens to promote tolerance to an otherwise vigorous response. There exist parallels with tumourigenesis, as presumably, cancer would start with a single cell with certain mutations and then proliferate, initiating a low tolerising dose of tumour-associated antigens (TAAs)[44]. The discontinuity theory of immunity, as articulated by Pradeu and Vivier[45], Pradeu and Cooper[46], and Matzinger's earlier danger signal hypothesis, provides a supporting theoretical framework to the experimental observations of Gratz *et al.*[40] and Pinheiro *et al.*[41]. Together, this helps contextualise the influence of complex, slow, low-dose continuous TAA recognition and how sudden (perhaps therapy-induced) changes in antigen levels within the TME may subvert immune suppression and break tolerance in certain circumstances.

Further, interesting parallels in human pregnancy and cancer have also been drawn between the early immune response and subsequent induction of immune tolerance.

A number of studies have demonstrated that tumour and placenta tissue use the same mechanisms to suppress host immunity[47]. The immune privilege offered to develop neoplasms by Tregs mirrors that of a developing embryo, representing a highly effective and evolutionarily conserved immune tolerance mechanism that is co-opted by tumours. In midtrimester pregnancy and advanced cancer, systemic alterations in immunity are also detectable, particularly with respect to a helper T cell type 2 polarisation, NK induction of tolerogenic/angiogenic dendritic cells and NK permissiveness, despite low levels of major histocompatibility complex I expression[48].

Another reproductive analogy in the context of cytokine cancer therapy is the introduction of the contraceptive pill. The "pill" came about as a result of detailed research and understanding of the menstrual cycle's orchestrated temporal dynamic interaction of reproductive hormones, their levels, cellular receptors and the various cell types in the female reproductive organ systems. Essentially, the effect of the pill (in part) was to "trick" the female physiology into thinking she was pregnant. This "trickery" was achieved by exogenously adding a little bit more of the same hormones (albeit now synthetic) into the system. This hormonal addition changed the "normal" sequence of events, modulated the reproductive homeostasis and allowed predictable and successful control of this dynamic system[49].

Tricking the system-disturbing immune homeostasis in the TME and breaking tolerance

Numerous mouse models and some translational approaches have shown that tumour immune suppression following immune recognition is a significant obstacle to breaking immune tolerance[50]. Importantly and as argued above, this suppression/tolerance induction is an intentional antigen-specific process mediated by normal aspects of immune homeostasis and not that dissimilar to pregnancy? Consequently, taking advantage of our "new" understanding of immune homeostasis and its role in tumour establishment, maintenance, and progression, reveals promising therapeutic opportunities. Some of which are already available, and may require minor protocol modifications in order to substantially improve efficacy. From the immune modulation experience of the past 30 years, it has become apparent that there are several positions in the tumour immune circuitry that provide opportunities for selective therapeutic intervention points. These include: Treg ablation with chemo and radiotherapy, bolus cytokine therapy, checkpoint inhibitor monoclonal antibodies, intratumoural agents, or antigen load modification with various modalities[50-53]. The latter is discussed below.

Transmutability of antigen to cytokine conversion within and the adjacent TME

Perhaps the simplest approach to immune modulation in order to break tumour tolerance appears to be using the TME's (and surrounding tissue's) own resident APCs to generate endogenous INF dominant cytokine production[54]. Causing sufficient tumour cell destruction and complex antigen production, at the same time preserving the nearby TME's APCs, has the potential via STING/ DAMP signalling pathways to break tumour tolerance locally and systemically[55]. A number of intratumoural strategies do the same thing; they supply a complex "soup" of antigen as a "burst" to the TME, which then is rapidly converted via APCs to INFs[56,57]. Modalities such as radiotherapy, high intensity focused ultrasound, radio wave ablation, cryotherapy, intratumoural food dye rose bengal, diterpene esters, all cause localised tumour cell destruction for immune processing and TME remodelling[58-61]. Similarly, it is now appreciated that the principal local and systemic mechanism of action of oncolytic viruses is caused by the lytic induced immune response against viral-infected cells[62].

Ground-breaking clinical work by Tubin *et al.*[63]. and confirmed by Markovsky *et al.*[64]. in mouse experiments, demonstrated that systemic immune modulation and complete abscopal responses could be achieved by single/double dose partial tumour irradiation, particularly incorporating the hypoxic segment in the radiation field. Partial tumour hypofractionated (1-3 doses) irradiation preserves the TME's cellular immune signalling capabilities in the un-irradiated segment to do the "heavy lifting" locally and systemically via an ensuing antigen/cytokine-induced antitumour immune response[63,64].

CONCLUSION

Finally, our relatively recent understanding of the immune cellular components and interactions within the TME and their role in tumourigenesis and maintenance provides evidence on how to better use existing modalities to improve therapeutic outcomes. Further, these insights explain why earlier crude attempts at cancer immunotherapy worked occasionally/randomly and really not better than today's much more sophisticated varied attempts.

We now know the immune system is homeostatically suppressed, with the immune system tightly protecting the tumour burden from immune destruction. Normal inflammatory controls by loco-regional TAMs, Tregs, MDSCs, together with orchestrating cytokines such as IL2, INF-γ and TNF-α involved in physiologic feedback loops, maintain the tumour friendly TME.

The prime mover in this suppression is the antigen load coming from the tumour. An apparent "normal" tissue in the eyes of the immune system, the tumour is not that dissimilar to the developing embryo.

We also know that introducing extra therapeutic cytokine into the intratumoural system can destabilise the status quo and thus interrupt "a natural course of events". Rather than attempting to immune modulate systemically and "deep" into the immune circuitry with toxic, expensive and inconsistent drugs, the simplest emerging and comprehensive solution may be to use therapeutic modalities that utilise the local TME APC machinery. Complex antigen loading via the STING/PAMPS pathways can induce endogenous cytokine production at sufficient levels to "throw a spanner in the works" of the underlying homeostatic suppression and break tolerance locally and systemically.

Further, evidence this can happen with various agents and modalities is appearing in the abscopal/bystander literature[65]. Clearly, the spatio-temporal influences of tumour-specific antigen load, the resultant cytokine production and the presence of opposing cell populations homeostatically balanced, are waiting to be used. In the face of a growing tumour burden, the system can be easily destabilised and thus break the

homeostatic nature of tolerance. Put simply. It appears possible to use the TME's underlying immunology to be our best tumour-specific rational drug design factory.

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