© 2008 Nature Publishing Group http://www.nature.com/natureimmunology

Cancer exploiting complement: a clue or an exception?

Bruce E Loveland & Jonathan Cebon

Cancer cells are more resistant to complement-mediated lysis and use this attribute to set up a locally immunosuppressive environment. However, new findings suggest that tumor-driven complement activation can also provide the tumor a growth advantage.

Treatments aimed at boosting the immune response to cancer date back to the late nineteenth century, when New York surgeon William Coley recognized that tumor regression could follow certain suppurative infections. He developed a crude therapy based on a patient's own bacterial flora, which met with some sporadic success. Indeed, refined forms of 'Coley's toxins' continued to be offered for the systemic treatment of cancer until the advent of cytotoxic chemotherapy in the 1940s[1]. More than a century later, the mechanisms underlying Coley's observation, and the complexities of the cancer-inflammation nexus, are still being unraveled. Attempts aimed at exploiting the host response have spawned many biological therapies for cancer, from cytokines to vaccines, antibodies and the adoptive transfer of immune cells[2]. Such approaches have yielded the occasional spectacular success; however, so far, only one biological approach has induced tumor regression with sufficient consistency to be adopted into routine clinical practice: the instillation of bacille Calmette-Guerin into the urinary bladder to treat superficial tumors. The paper by Markiewski and colleagues in this issue of *Nature Immunology*[3] provides new insights and possibly new opportunities for treatment. They show evidence of tumor-induced complement activation, which in turn modulates the anticancer immune response, thereby giving the tumor a growth advantage.

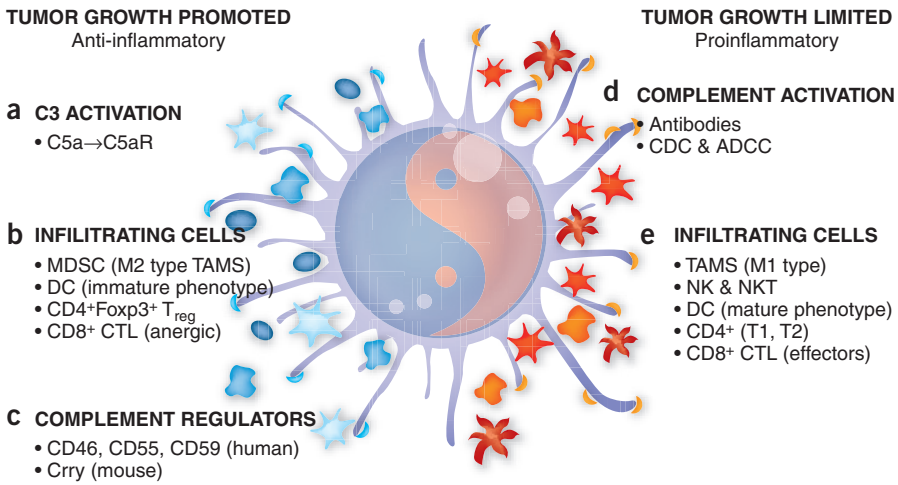

Figure 1 The 'yin and yang' of complement activation in the tumor microenvironment. Markiewski *et al.* have provided evidence that complement C3 activation can give a tumor growth advantage[3]. (**a**) C5a-mediated triggering of the C5a receptor (C5aR) signaling pathway to enhance infiltration by myeloid-derived suppressor cells (MDSC; 'M2' type macrophages) and subsequent regulation of cytotoxic $CD8^+$ T cells (CTL). (**b**) Growing tumors are known to have a measurably 'anti-inflammatory' environment, including cellular infiltration consisting of tumor-associated macrophages (TAM; including myeloid-derived suppressor cells), dendritic cells (DC) with an immature phenotype, $Foxp3^+$ regulatory T cells (T_{reg}) and suppressed or anergic $CD8^+$ T cells. (**c**) Higher expression of the cell surface complement regulators (in particular CD55, CD59 and CD46 in humans, and Crry in mice) that provide greater tumor resistance to complement-mediated cytolysis. (**d**) In a regressing tumor, a 'proinflammatory' environment can be found, which can include a greater amplitude of complement activation demonstrated by antibody-initiated complement-dependent cytolysis (CDC) and antibody-dependent cell-mediated cytolysis (ADCC). (**e**) A cellular infiltration consisting of 'M1' type cytocidal macrophages, dendritic cells with a mature phenotype, $CD4^+$ helper T cells (the so-called T_H1 and T_H2 cells), cytotoxic natural killer cells and natural killer T cells (NK & NKT) and $CD8^+$ effector T cells.

Bruce E. Loveland is with the Burnet Institute, Heidelberg 3084, Australia. Jonathan Cebon is with Austin Health and the Cancer Vaccine Laboratory at The Ludwig Institute for Cancer Research, Austin Hospital, Heidelberg 3084, Australia.
e-mail: bloveland@burnet.edu.au

It is clear that just as physicians have sought to exploit the host-cancer relationship, cancer's inherent genetic instability provides it with the potential to take advantage of the host response. Malignant transformation results from genetic and epigenetic events that affect the phenotype and function of the cell. The relationship between the emerging tumor and the stromal tissues of its host is an intimate and dynamic one, and the tumor can evolve under whatever selective pressure exists in that environment. Tumor immunity serves as an example: immune effectors may eliminate tumor cells; however, if this process is incomplete, an interval of equilibrium may ensue in which tumor progression is balanced by the host response that holds it in check. The consequence is 'editing' of

© 2008 Nature Publishing Group http://www.nature.com/natureimmunology

the tumor's phenotype that results in tumor evolution such that it can ultimately escape host control[4].

It is recognized that microenvironmental signals from the host response can be exploited by malignant cells. Stromal elements, including fibroblasts, macrophages and endothelium, can all produce signals for the proliferation of cancer cells and blood vessels, dampen immunity and facilitate tumor growth. Presumably, any element of the host response also has the potential to apply selective pressure. Because this is complex, the tumor might respond in myriad ways not just to immune selection but also to the various growth and inhibitory signals provided by the stroma and inflammatory infiltrates. Myeloid cells, particularly monocytes and macrophages, seem to be important in this and can promote or inhibit tumor growth through the generation of such signals[5,6]. As the tumor evolves, there is the potential for such growth signals to be exploited if they provide a survival advantage and for inhibitory mechanisms to be bypassed, subverted or even 'hijacked' by the tumor.

The conventional view of the complement system has focused on the tagging, clearance and lysis of microbes (complement's central function in innate immunity) and in antibody-mediated responses through complement-dependent cytotoxicity. In the cancer setting, it has served a similar function, particularly as a mediator of complement-dependent cytotoxicity after therapeutic antibody targeting[7]. Studies of clinically proven antibodies such as trastuzumab and rituximab have identified the Fc portion as being critically important to their efficacy[8], which has led to rational modifications to exploit Fc-mediated functions[9]. Consistent with an anticancer function for complement and a countermeasure to its action, tumors often have higher expression of complement-regulatory proteins such as CD46, CD55 and CD59. These demonstrably raise the tumor's threshold of resistance to complement activation[10–12].

Far from demonstrating an anticancer effect, the paper by Markiewski and colleagues[3] in this issue of *Nature Immunology* upsets conventional wisdom and shows for the first time that elements of the complement system may perversely assist tumor growth. It provides evidence that a tumor can exploit the complement system through the classical pathway of activation, generating the C5a component as the effector that can set up an immunosuppressive environment by means of myeloid-derived suppressor cells that regulate $CD8^+$ cytotoxic T cells. This study uses complement-component deficient mice to show that for the TC-1 cervical cancer, C3 activation leading to C5a receptor signaling provides the tumor with a growth advantage. The activating trigger is not investigated in this study, but classical-pathway complement activation could be driven by antibodies to tumor-specific antigens or, in experimental models, even retroviral infection of the tumor cell line.

To our knowledge, the findings of Markiewski *et al.*[3] are novel for studies of cancer, but the general ideas are entirely consistent with the growing recognition of the homeostatic function of the complement system, which includes the 'fine tuning' of both the innate and cognate immune responses[13]. In particular, the role of complement components and the complement-regulatory proteins (notably CD46 and CD55) in regulating T cell function and providing essential dampeners to inflammatory immune responses is turning dogma on its head.

Perhaps the key to the new paradigm is understanding the continuous 'gray scale' transition from immunological quietness, through low and chronic inflammatory responses, to full-blown acute reactions. Although the complement cascade by design is capable of creating the 'storm' of an acute antimicrobial response or hyperacute graft rejection (classical pathway activation), the 'tickover' (continuous trickle) of both the classical[14] and alternative pathways of activation allows the system to 'idle' and provide a homeostatic base[15], which is probably also important in promoting the phagocytosis of necrotic cells and allowing tissue remodeling. This spectrum of activation states must be viewed against the finely tuned balance between any stimulus that activates the complement cascade and the self-protective threshold set by various complement-regulatory proteins (in humans, C1inh, CR1 (CD35), CR2 (CD21), DAF (CD55), MCP (CD46), protectin (CD59), factor H, C4bp and so on).

The idea that cells express complement regulatory proteins that set a 'threshold of activation' is important in understanding the sensitivity of cells to complement-mediated lysis (measured *in vitro*) and the ability to survive a proinflammatory environment *in situ*. For example, cells in the salivary gland, kidney tubules, lumen of the gut and placenta have very high expression of membrane-bound CD46, CD55 and CD59 and are in an environment in which C3 activation frequently occurs. Lymphocytes and endothelial cells normally have 'low' expression of these proteins, and we surmise that these cells should be sensitive to the effects of complement activation that are triggered only by a specific and unusual (local) event. Markiewski and colleagues provide evidence that tumor cells are able to set up a more subtle complement-activating environment but are more resistant than the surrounding stroma. One effect of this would be a relative absence of C3b, C5b and the membrane attack complex on tumor cells but more complement-activation products on adjacent cells that have lower expression of the complement regulators. It seems likely that mutations in tumor cells that promote this effect will give cells a survival advantage and will be part of the evolution of cancer (**Fig. 1**).

What should be sought now is clinical evidence of cancer cells driving complement activation to the point at which sufficient C5a can be produced. This would extend the findings of Markiewski *et al.* to human pathophysiology. It would also provide consistency with the accepted view that inflammation and infection can promote and exacerbate tumor growth and studies of complement function in other pathologies and immune regulation[13].

In addressing this critical issue, it must be determined whether the phenomenon described is a frequent event during the *in vivo* evolution of human cancer, an unusual or rare occurrence, or even merely an idiosyncratic feature of this mouse tumor model. For the phenomenon to be clinically relevant, the observation now needs to be confirmed in other models and, more importantly, in the human context. Is this a paradigm shift in how the cancer environment and evolution of tumors are viewed? If it is generally applicable, then it has the potential to be exploited for therapeutic benefit.

1. Nauts, H.C. & McLaren, J.R. *Adv. Exp. Med. Biol.* **267**, 483–500 (1990).
2. Davis, I.D. *et al. J. Leukoc. Biol.* **73**, 3–29 (2003).
3. Markiewski, M.M. *et al. Nat. Immunol.* **9**, 1225–1235 (2008).
4. Dunn, G.P. *et al. Annu. Rev. Immunol.* **22**, 329–360 (2004).
5. Sica, A. & Bronte, V. *J. Clin. Invest.* **117**, 1155–1166 (2007).
6. Allavena, P. *et al. Immunol. Rev.* **222**, 155–161 (2008).
7. Dechant, M. *et al. Cancer Res.* **68**, 4998–5003 (2008).
8. Clynes, R.A. *et al. Nat. Med.* **6**, 443–446 (2000).
9. Stavenhagen, J.B. *et al. Cancer Res.* **67**, 8882–8890 (2007).
10. Watson, N.F. *et al. Cancer Immunol. Immunother.* **55**, 973–980 (2006).
11. Zell, C. *et al. Clin. Exp. Immunol.* **150**, 576–584 (2007).
12. Varela, J.C. *et al. Cancer Res.* **68**, 6734–6742 (2008).
13. Kemper, C. & Atkinson, J.P. *Nat. Rev. Immunol.* **7**, 9–18 (2007).
14. Manderson, A.P. *et al. J. Exp. Med.* **194**, 747–756 (2001).
15. Wu, X. *et al. J. Immunol.* **181**, 2732–2740 (2008).