Tumor-mediated apoptosis of cancer-specific T lymphocytes—Reversing the "kiss of death"?

Recent studies have provided evidence that some cancers can aberrantly express molecules capable of inducing apoptosis of tumor-reactive lymphocytes. Several other potential tumor escape mechanisms that can block the cytotoxic pathways activated by killer T cells have also been proposed.

Although considered highly controversial just a decade ago, there is now growing consensus among immunologists that many cancers elicit a cellular immune response that in some cases can influence tumor formation, growth, and/or metastasis (Smyth et al., 2001). Immune responses can be categorized into two general types. Cellular, or Th1, responses result in the stimulation of effector cells such as cytotoxic T lymphocytes, whose principal function is to kill virus-infected cells. Alternatively, a humoral or Th2 response is normally elicited in response to extracellular pathogens such as bacteria and to their toxins, and results in the formation of specific, circulating antibodies, secreted by B lymphocytes. A number of recent studies using gene-targeted mice have provided the first strong evidence that perforin and interferon-γ, secreted by cytotoxic T lymphocytes and recognized as key players in defense against viruses, can also be protective against carcinogenesis. Evidence for immunoprotective mechanisms dependent on these molecules extends to carcinogen-induced sarcoma formation (van den Broek et al., 1996; Kaplan et al., 1998), and the development of spontaneous lymphoma (Smyth et al., 2000) and certain epithelial malignancies (Shankaran et al., 2001; Smyth and Trapani, 2001) in a number of mouse strains. These tantalizing observations affirm the controversial hypothesis of cancer immune surveillance initially enunciated by pioneers such as Burnet and Thomas, and offer hope to researchers and clinicians that strategies to harness the immune system to combat established cancer may hold great promise. However, there is equally good evidence in cancer patients that the inherent genomic instability of cancers endows them with many opportunities to escape from immune control. Most frequently, cancer cells can become "invisible" to T lymphocytes by losing the expression of specific class I antigens (Lehmann et al., 1995), or failing to process the peptides these molecules present to T cells. Just as discouraging, cancer cells can skew or stunt an emerging cellular response by elaborating suppressive cytokines such as TGF-β or IL-10.

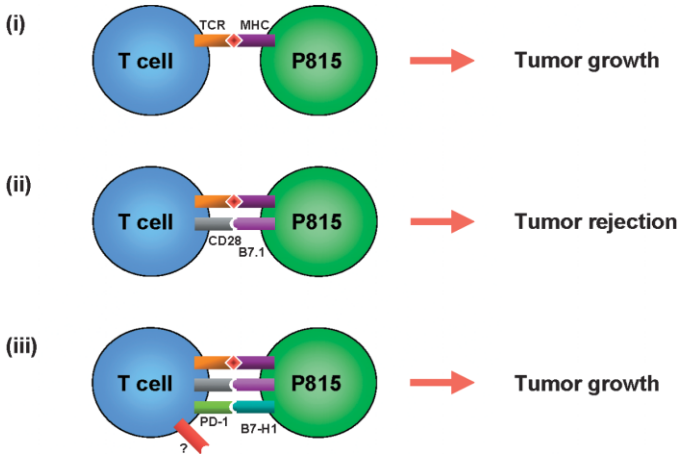

Figure 1. B7-H1 expression on P815 mastocytoma cells prevents rejection by syngeneic DBA/2 mice

(i) P815 cells (H-2^d) grow rapidly and kill their hosts when implanted subcutaneously. (ii) If the costimulatory molecule B7.1 is expressed on the tumor cell surface and can bind CD28 on T cells, an effective immune response is primed against tumor peptides presented on MHC class I molecules, resulting in tumor rejection. (iii) B7-H1 overexpression in addition to B7.1 overrides the mouse's ability to generate anti-P815 immunity, restoring the virulence of the tumor and death of the mouse. In this graphic, B7-H1 is shown hypothetically bound by its only well-defined receptor PD-1, but alternative receptors almost certainly exist.

For diverse reasons, many cancer cells are also poorly immunogenic. To be optimally activated, T cells require two distinct signals from antigen-presenting cells. First, clonotypic antigen receptors on the T cell bind to antigenic peptides presented on MHC molecules, providing specificity for the interaction. In addition, "costimulation" through the binding of receptor-ligand pairs on the T cell and antigen-presenting cell provides a second signal necessary for T cell proliferation and the development of efficient effector function. Most nonhematological malignancies do not express costimulatory molecules such as B7.1 (CD80) or B7.2 (CD86) to interact with CD28 on T cells and are therefore unable to prime an effective immune response directly. In this regard, an even greater problem may be posed by the recently published findings of Dong et al. These investigators showed that aberrant expression on cancer cells of B7-H1, a newly characterized member of the B7 costimulatory family, resulted in strong promotion of tumor growth in vivo, and could even override an otherwise effective immune response in a syngeneic cancer rejection model. While little is known about the normal physiological function of B7-H1, mice that fail to express its only known ligand, PD-1, are prone to autoimmune diseases. The same authors and other investigators have shown that the PD-1/B7-H1 interaction can inhibit proliferation and cytokine secretion by activated T cells. Collectively, these and other findings have been taken to indicate that B7-H1 is involved in regulating the activity of certain autoreactive T cells (cells that interact with self-antigens and can cause tissue damage) and may lead to the promotion of Th2 responses.

Dong and collaborators now present considerable evidence for a new form of immune evasion resulting from T cell apoptosis induced by their direct contact with B7-H1-overexpressing cancer cells. P815 mastocytoma cells transplanted into syngeneic DBA/2 recipients form rapidly growing tumors, unless they are engineered to express costimulatory B7.1 on their surface (Figure 1). This

leads to the priming of an effective tumor-specific T cell response resulting in tumor rejection after initial growth of the cancer for several days. When P815 cells coexpressed B7-H1 in addition to B7.1, tumor rejection did not occur; rather, tumor growth continued unabated, and killed the recipients in a similar timeframe as wild-type or mock-transfected P815 cells. The effect was probably not due to enhanced tumor growth per se, as B7-H1-expressing P815 tumors grew at the same rate as mock-transfected cells in RAG-1$^{-/-}$ immunodeficient recipients. These gene-targeted mice do not express the recombinase necessary for rearrangement of T and B cell receptor gene segments. As the expression of a functional antigen receptor is necessary for positive selection, RAG-1$^{-/-}$ mice have virtually a complete lack of all T and B cells. Moreover, by implanting the P815 tumor variants in the peritoneal cavity of RAG-1$^{-/-}$ mice, the authors demonstrated that B7-H1 overexpression on the tumor cell surface could reduce the number of tumor-reactive T cells found in the peritoneal cavity, by inducing their apoptotic death. For these experiments, T cells from transgenic mice expressing a T cell receptor that recognized a P815 tumor peptide presented on H-2L^{d} were adoptively transferred into tumor-bearing recipients. Importantly, T cell apoptosis was inhibited, and growth of P815 cells was once more retarded when the animals were additionally preinfused with a monoclonal antibody that blocked the interaction of tumor B7-H1 with PD-1 on the T cells. The mechanism of T cell death has not been fully elucidated, but the authors postulate this may involve both FasL expression and secretion of IL-10, a cytokine previously shown to induce death of activated T cells. A number of in vitro experiments were also presented in which tumor cells that were either transfected to express B7-H1 or expressed the molecule constitutively induced apoptosis in cognate T cells with which they were mixed. T cell death was inhibitable by adding B7-H1-Ig fusion protein to the culture medium, but was not dependent on PD-1 expression on the T cells. Similarly, primary T cells incubated with B7-H1-Ig and anti-CD3 antibody initially proliferated, then underwent programmed cell death. However, neither proliferation nor apoptosis was inhibited by PD-1-Ig. It is therefore very likely that T cell apoptosis in these experiments was mediated through interaction of B7-H1 with an alternative cellular receptor(s), the identity of which remains unknown.

What is the relevance of these findings for human cancer? Firstly, Dong and coworkers found a remarkably high incidence of B7-H1 expression in freshly isolated human cancers, including 95% of histologically diverse lung cancers, 87% of ovarian cancers, 100% of melanomas, and about half of the colon cancers examined. By comparison, juxtaposed normal tissues in the same histological sections expressed no B7-H1, and expression in normal tissues was restricted to macrophages or resident tissue histiocytes such as liver Kuppfer cells. The authors postulated that the "cytokine milieu" in some cancers might be conducive to B7-H1 expression, due to local inflammation and the production of interferon-γ, as this cytokine was shown to induce B7-H1 expression on cancer cell lines in vitro. If substantiated, this latter observation would be at odds with the many beneficial effects of interferon-γ, which include upregulated antigen presentation by the tumor, enhanced MHC class II expression on professional antigen-presenting cells, chemotaxis of NK cells, and inhibition of angiogenesis. The incidence of B7-H1 expression reported on human tumors was also remarkably high, and far fewer of these tumor types typically display significant inflammatory infiltrates. As discussed above, caution particularly needs to be exercised in view of how little we know about B7-H1 ligands other than PD-1, and their potential functions. Might such receptors even be coexpressed on some cancer cells together with B7-H1? The intriguing set of data presented by Dong et al. cannot exclude the possibility that B7-H1 plays a role in cancer biology that might have little to do with modulation of immune function. Clearly, the present findings will have to be revisited and further experiments performed, as new information on the function of B7-H1 and its ligands emerges.

If corroborated, the findings presented by Dong and colleagues also have important implications for cancer immunotherapy. Some immunotherapeutic approaches to cancer involve expanding a patient's tumor-reactive lymphocytes ex vivo, by exposing them to autologous tumor cells and stimulatory cytokines. Clearly, adoptive therapy using preactivated T cells might fail because of T cell deletion upon cancer cell engagement. Alternatively, strategies aiming to raise active T cell responses to tumor antigens might have to consider including a strategy to block B7-H1. However, as alluded to above, such an approach might theoretically predispose to an unacceptable risk of raising self-reactive T cells. It is premature to jump to such conclusions without further extensive in vivo studies once the basic biology of B7-H1 and its ligands is better understood. Preferably, physiologically relevant models examining spontaneous and/or carcinogen-

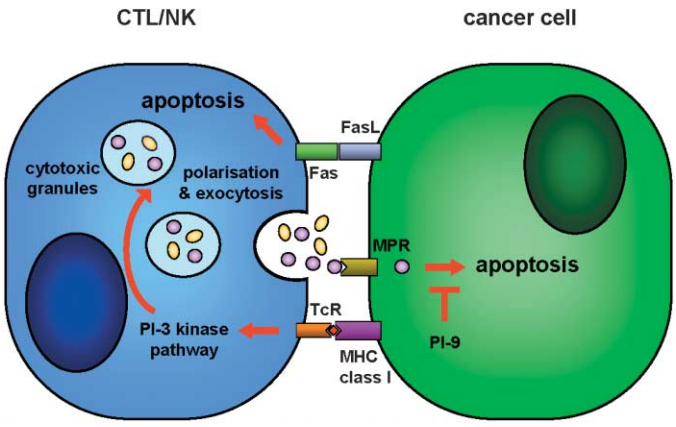

Figure 2. Putative mechanisms for inhibition of T cell-mediated apoptosis following ligation of cancer cells

Several mechanisms have recently been proposed through which tumor cells might block T cell cytolysis. FasL expression on cancer cells has been postulated to induce death of effector T cells following engagement of Fas, but this mechanism has not stood up to more rigorous testing (see text for details). In addition, it has been proposed that some cancer cells can withstand CTL or NK cell attack by expressing protease inhibitors (for example the serpin, PI-9) that inactivate granzyme B (shown in pink, binding to its putative receptor, MPR) by downregulating expression of granzyme receptors or by blocking signal transduction pathways resulting in trafficking ("polarization") of T cytotoxic granules toward the immunological synapse. Perforin molecules are shown schematically in yellow.

induced tumor formation in gene targeted or transgenic mice might provide insights relevant to human carcinogenesis. An instructive and relevant parallel to the current findings was the initial excitement generated by several papers that described FasL expression on tumors. FasL, a member of the TNF family of pro-apoptotic molecules, can induce the death of cells that express the cognate death ligand Fas. Theoretically, this could lead to T cell deletion and "immune privilege" status for the tumor. The resulting predictions that FasL overexpression on tissue allografts would delay or prevent rejection were not fulfilled. In fact, implantation of FasL-overexpressing cells in mice resulted in precisely the opposite result: even more rapid rejection and abscess formation in the transplanted tissue (reviewed in Restifo, 2000).

Along with the present report of Dong and collaborators, the literature currently contains several others postulating or purporting to show inhibition of T cell effector function as a result of interaction with cancer cells (Figure 2). These reports include models where cancer cells may (i) overexpress serpins (protease inhibitors) that block granzyme B-mediated apoptosis (Medema et al., 2001); (ii) downregulate expression of purported granzyme receptors such as the 280 kDa mannose-6-phosphate receptor (Motyka et al., 2001), also blocking apoptosis; or (iii) transmit a signal to cytotoxic T lymphocytes that blocks the PI-3 kinase pathway, leading to failure of perforin and granzyme secretion due to dysregulated granule trafficking (Radoja et al., 2001). Each of these hypotheses requires further testing in appropriate animal models, and extreme caution is essential before applying the apparently logical consequences of these "lessons" to human cancer therapy.

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salvador—The persistence of proliferation

Despite years of extensive studies on genes that regulate proliferation and cell death, two processes that must be tightly coordinated throughout development to regulate cell number, remarkably few genes have been shown to affect both processes. Using an elegant genetic screen in the fly eye, Tapon et al. (2002) have identified a gene, *salvador*, which is especially significant, because it not only regulates and coordinates both exit from the cell cycle and apoptosis, but also has a human homolog that may play a key role in tumorigenesis.

The fly eye develops from merely 30 progenitor cells into an exquisitely precise and highly ordered structure consisting of approximately 800 individual units or ommatidia and numbering more than 15,000 cells. To reach this final form, which is so beautifully regular that it has been called a "neurocrystalline lattice" (Ready et al., 1976), the signals for proliferation, patterning, exit from the cell cycle, differentiation, and cell death all must be carefully regulated and coordinated. The fly eye has provided a sensitive system for the discovery of genes and regulatory networks that control these processes, but noticeably, few genes have been shown to regulate both exit from the cell cycle and execution of the apoptotic program—two developmental events that must be tightly coordinated to regulate cell number. When these processes are uncoupled or disrupted, the host is at risk for developing a tumor.

Tumor suppressors are genes that, when inactivated, confer a proliferative advantage over normal cells. This can occur by a variety of different mechanisms and can lead to the development of tumors, disorganized masses of tissue that can cause the death of the host—whether that host is a fruit fly (Woodhouse et al., 1998) or a human being. In fact, many, if not all, human cancers involve the inactivation of tumor suppressors (Hanahan and Weinberg, 2000). These genes comprise an important and diverse group, and their discovery and characterization have helped us develop a deeper understanding of cancer cell biology. To date, a large number of tumor suppressor genes have been reported in flies, and many of them have human homologs that